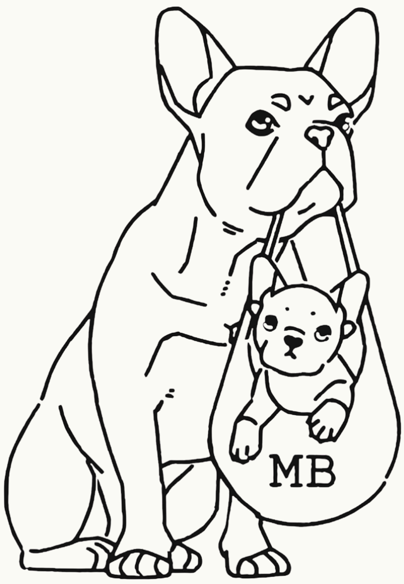
FROM GESTATION TO NEONATAL LIFE IN THE CANINE SPECIES: MATERNAL AND FETAL PHYSIOLOGY AS PREDICTORS OF PERINATAL OUTCOME

DE LA GESTACIÓN AL NACIMIENTO EN LA PERRA: ASPECTOS FISIOLÓGICOS MATERNOS Y FETALES COMO INDICADORES DEL PRONÓSTICO PERINATAL

RAQUEL RODRÍGUEZ TRUJILLO

DOCTORADO DE INVESTIGACIÓN EN BIOMEDICINA

OCTUBRE 2025



ULPGC
Instituto Universitario de
Investigaciones Biomédicas y Sanitarias

ULPGC
Universidad de
Las Palmas de
Gran Canaria

vet
FACULTAD DE
VETERINARIA

ULPGC
Universidad de
Las Palmas de
Gran Canaria
Escuela de
Doctorado

PROGRAMA DE DOCTORADO DE INVESTIGACIÓN EN BIOMEDICINA

DE LA GESTACIÓN AL NACIMIENTO EN LA PERRA: ASPECTOS FISIOLÓGICOS MATERNOS Y FETALES COMO INDICADORES DEL PRONÓSTICO PERINATAL

FROM GESTATION TO NEONATAL LIFE IN THE CANINE SPECIES: MATERNAL AND FETAL PHYSIOLOGY AS PREDICTORS OF PERINATAL OUTCOME

DOCTORANDA**:**

RAQUEL DEL CARMEN RODRÍGUEZ TRUJILLO

DIRECTORES:

MIGUEL JESÚS BATISTA ARTEAGA

CRISTIAN SUÁREZ SANTANA

TUTOR:

JOSÉ MANUEL VILAR GUEREÑO

LAS PALMAS DE GRAN CANARIA,

OCTUBRE 2025

PROGRAMA DE DOCTORADO DE INVESTIGACIÓN EN BIOMEDICINA

DE LA GESTACIÓN AL NACIMIENTO EN LA PERRA: ASPECTOS FISIOLÓGICOS MATERNOS Y FETALES COMO INDICADORES DEL PRONÓSTICO PERINATAL

FROM GESTATION TO NEONATAL LIFE IN THE CANINE SPECIES: MATERNAL AND FETAL PHYSIOLOGY AS PREDICTORS OF PERINATAL OUTCOME

DOCTORANDA: **EL DIRECTOR:** **EL CODIRECTOR:**

LAS PALMAS DE GRAN CANARIA,

OCTUBRE 2025

AGRADECIMIENTOS

¡Qué bonito empezar este apartado, porque, aunque pueda parecer el menos relevante para la tesis, sin duda es en el que más sentimientos me invaden!

Creo que el primer párrafo tengo clarísimo que es para **mis padres, Pablo y Mari**. Mi mayor apoyo y mis pilares fundamentales. Es irónico porque ellos siempre me dicen "Nuestra mayor suerte ha sido tenerte", pero lo que ellos no saben es que la suerte es mía de tenerlos como padres. Me han secado mil lágrimas, me han obligado a levantarme cuando he caído y me han enseñado a volar a pesar de no tener alas. Porque cuando he tenido miedo o he sentido la necesidad de rendirme, mi primer pensamiento ha sido siempre ellos. Nunca se han rendido, ni cuando la marea se ha puesto revuelta, ni cuando la tormenta aparecía ni mucho menos cuando se ahogaban en el mar sin nadie saberlo. ¿Cómo iba a ser yo menos? Creo que la frase más dicha por mi madre en el último año ha sido "No tenía suficiente con tu padre que me tuviste que salir igual", pero lo que no te imaginas es el orgullo que me invade cuando lo escucho, porque en la vida mi único objetivo y mi principal meta es ser como ustedes. Corazón de oro puro combinado con fuerza de titanes, con un toque de malhumor, de autoexigente y sobre todo, con esas ganas tan bonitas de querer comerme el mundo. Simplemente gracias mami y papi.

Mi segundo párrafo tiene que ser para alguien que aun que nunca podrá leerlo, me niego a pensar que no quedará aquí escrito: mi pequeña Gissel, la que siempre digo que fue, es y será el amor de mi vida. Por acompañarme durante toda la carrera, incluso en la preparación de la PAU cuando mi único sueño era entrar a Veterinaria. Me acuerdo la cantidad de veces que me viste llorar y sin entender nada, simplemente me secaste las lágrimas con mis besitos. Como siempre digo: inmortal hasta que se demuestre lo contrario, porque niego a pensar que no será así. Y ahora mencionar mis tres estrellitas: **Luna, Katia y Pita**. No he aprendido a vivir sin ustedes, pero una persona que me ha enseñado mucho (casualmente el director de esta tesis) siempre me ha dicho: nunca muere al que no se olvida. Y cuánta razón, porque estarán siempre en mis corazones. Y obviamente mis pequeños motorcitos: **Lara, Perla, Olaf, Zeus y Keysi**. Mis pequeños terremotos que espero que me duren toda la vida.

Mi acompañante, él que ha sido fundamental para este camino, **Axelito**. ¿Cómo te explico que sin ti no hubiese sido posible? Me acuerdo de que una de nuestras primeras conversaciones fue sobre el tiempo que le dedico a mi profesión y recuerdo decirte "mi vida se basa en ir a urgencias, vivir en el hospital y tener que estar 24/7 pendiente de Repro" y me dijiste "por eso no voy a tener problema nunca, siempre te apoyaré" y así ha sido hasta día de hoy. Después de 3 años juntos, nunca te han faltado palabras para motivarme, donde al menos un millón de veces me has repetido lo orgulloso que estás de mí y donde siempre has buscado como sacarme una sonrisa hasta cuando me iba llorando de madrugada con ansiedad a una cesárea. Creo que los agradecimientos nunca serán suficientes, pero te estaré eternamente agradecida. Creo que hay una frase que es completamente nuestra que dice "siempre en mi equipo" y es que no me imagino un compañero mejor. Por ser mejor amigo, por ser aún mejor pareja y por ser ese toque de tranquilidad que le falta a mi cuerpo lleno de ansiedad y nervios. Sin duda creo que somos el Ying Yang perfecto. Nos complementamos de una manera tan bonita que solo puedo darte las gracias. Gracias por enseñarme el significado verdadero del amor, espero compartirlo toda una vida.

A mi familia: mis abuelos **Carmen y Mentrín**, **Tata Abenchara y Antonio**, Mil gracias. Gracias por siempre recordarme cuanto valgo, por nunca dejar que me rinda, por siempre estar ahí para mí y sobre todo por siempre cuidarme. Les quiero muchísimo.

Y a mi otra familia: **Carlos y Mariel, Pablo y Débora, Mateo y Laura, Ale y Alba**, que sin duda siempre han estado ahí. Siempre han tenido buenas y bonitas palabras para animarme, siempre me han apoyado y han enseñado a que, sin duda, nada más bonito que la familia que me ha tocado.

Y no, no me he olvidado de ti **Santi**, pero necesitaba hacerte una pequeña mención aparte, porque sin duda has sido un apoyo increíble este tiempo. Aunque no lo sepas, me has hecho reír muchos días donde no me aguantaba ni a mí misma, también me has hecho enfadar mil veces (lo de limpiar es un tema que seguimos teniendo pendiente), pero sin duda has sido ese hermano pequeño que nunca he tenido. Mil conversaciones en la cocina, mil veces me has escuchado desahogarme y un millón me has dado el abrazo que necesitaba sin yo pedirlo. Gracias de verdad.

Y obviamente como buena pepito grillo que soy, no puede faltarme quien ha hecho esta Tesis posible. Señor **Batista**, es su turno. Recuerdo la primera vez que te conocí donde me dirigí a ti como "Batista" y tu tan simpático como siempre me dijiste "a mi padre lo quiero mucho, pero mi nombre es Miguel". Cómo iba a imaginarme que el cascarrabias al que todo el mundo le tenía respeto y algunos hasta miedo, iba a convertirse no solo en mi director de tesis sino en un gran amigo. A pesar de habértelo dicho muchas veces, creo que nunca es suficiente, pero sabes que te estoy y siempre estaré enormemente agradecida por esta oportunidad, por todo lo aprendido y sobre todo, por impulsarme en este camino. No tengo duda que me quedan muchos años dando caña en el reproteam, pero mami y papi siempre me han dicho "es de bien nacido ser agradecido" y sin duda, nunca olvidaré todas las oportunidades, todo el tiempo dedicado y todas las veces que a pesar de irte llorando por no saber cómo seguir el camino, me has apoyado y ayudado para continuar.

En este agradecimiento no puede faltar mi team, pero ojo, no un team cualquier, sino el mejor team que podrá existir: **REPROTEAM**. Desde los inicios donde no pueden faltarme **Ale, Lara, Carla, Rocio y Raquel**, hasta las siguientes generaciones con **Pablo, Uko, Cristian y Alba**, hasta la actualidad donde comparto quirófano con los mejores: **Sara, Jamaica, Alba, Javi, Alexia, Evelyn, Laura, Samu, Bea, Seyma**... Y hasta los próximos que vengan, ¡Queda mucho reproteam para dar y regalar! Gracias por hacerme las cesáreas de las 2 de la mañana más fáciles, por las risas, las lágrimas y los sin fin de recuerdos que tengo gracias a todos y cada uno de ustedes. Y como no, mi super compi de equipo, **Kseniia**. Gracias por estar siempre y por animarme, por sacarme una sonrisa cuando me dices "Caarmenn" y por permitirme compartir este camino contigo.

Mi chupipandi, era imposible que no ocuparan un pedacito: **Pauli, Malena y Christelle**. Que decirles a ustedes mis niñas. Han sido sin duda esas hermanas que toda la vida quise tener. Gracias por aparecer en mi vida durante la carrera, por hacerla más amena y por ser un apoyo incondicional durante ella. Pero aún más, gracias por ser apoyo incondicional durante el doctorado, por las mil veces que me han escuchado mis batallas, mis problemas y me han aconsejado. Por las mil palabras bonitas que siempre han tenido para mí, porque sin duda sois magia pura amigas. Bendito regalo de la vida, que os puso en mi camino. Y aquí no pueden faltarme **Kili y Ale** (y aunque a **Micka** lo conozco menos, obvio es uno más de nosotros), quienes sin duda forman parte también de esta pequeña familia que hemos creado y de la que me siento tan afortunada. Dicen que los amigos son la familia que uno elige y cuánta razón. Gracias a todos por siempre estar. Les quiero mucho.

Mi pequeña American Bully o como nos llaman algunos, mi media naranja, **Noe**. Empezamos este camino juntas y prometimos terminarlo juntas. Y mirarnos, cumpliendo sueños, callando bocas y brillando más bonito que nunca. Solo puedo agradecerte por el apoyo incondicional, por siempre tener clínex para secarme las lágrimas, por ser compañera de sonrisas, por nunca dejarme sola y por nunca dejar que me rindiera. Nunca podrá olvidar el día en que llegué llorando al despacho y te dije "se acabó, no puedo más, voy a abandonar" y me dijiste "si si, tu llora todo lo que quieras que de aquí no te vas a ir". Me abrazaste, me apoyaste y me dijiste "en unos meses nos reiremos de este momento y te darás cuenta que esto es lo tuyo. Y así fue amiga. Sin mencionar la llamada Barcelona-Las Palmas donde llorando me contaste que te perdiste en un bosque de jabalís y que no sabías como seguías viva, mientras me decías que no podías más, que el doctorado no era lo tuyo y que querías dejarlo. Recuerdo que te dije "¿después de sobrevivir a los jabalís vas a dejarlo de verdad?" y aun que en ese momento fue una broma para hacerte olvidar los problemas, lo decía totalmente en serio: ¿De verdad crees que podemos abandonar después de todo lo conseguido? Somos oro puro amigas y esto es lo nuestro. Gracias por darle luz al camino.

Mis gemelas lindas, **Sori y Yai**. Estaba claro que así como me han conquistado el corazón, también iban a conquistar un pequeño espacio de estos agradecimientos. Mis pequeños fosforillos pero también las niñas más cariñosas, amorosas y buenas que he conocido. Me acuerdo que con ustedes todo comenzó mucho antes, cuando estaba en segundo de carrera y curaron a Luna y Katia, desde ese momento se ganaron mi corazón. Pero aún más cuando se convirtieron en dos pilares fundamentales. Mi pequeña Sori, gracias por cada risa, gracias por permitirme crecer a tu lado, por cuidarme y muchas veces guiarme, por las mil llamadas infinitas, por las horas de Excel y por permitirme formar parte de tu vida. Mi Yai, mil gracias por las llamadas a kilómetros de distancia, por recordarme siempre mi potencial y por siempre recordarme que estoy en el camino correcto, que no puedo rendirme. Gracias a las dos, por siempre confiar en mi más que yo misma. Sois magia.

¿Qué pensabas, que no me iba a acordar de mi compi favorita de hospital, pero también de vida? Mi **Leti**. Te conocí cuando estaba en tercero de carrera y no ha llovido ni nada. Gracias por demostrarme el significado de amistad. Por cada consejo, por cada palabra bonita y por siempre estar. Nunca has dudado de mí y todavía mejor, cuando he tocado fondo, has bajado hasta la oscuridad, te has sentado a mi lado y me has dicho "de esta salimos juntas, tomate tu tiempo, pero me quedo aquí a tu ladito". Quiero hacer una pequeña mención a alguien que quiero mucho y que siento que también me une un poquito a ti, mi pequeño Bobby, otra estrellita que nos alumbra cada noche y que se que está feliz de vernos unidas y luchando con todo y contra todos. Gracias por ser amiga, pero todavía más, gracias por ser hermana.

Y necesito mencionar al otro team que me adoptó desde el minuto cero: mi cardioteam. Aquí necesito hacer especial mención a **Elena y Alberto**, quienes nunca han dudado en echarme una mano. Quienes siempre me han recordado que, aunque los corazones no son lo mío, soy una más de la familia tan bonita que tienen. Quienes me han adoptado en el famoso "despacho de becarios" y quienes siempre han tenido siempre palabras bonitas para mí. Y aquí incluyo al resto de miembros de este pedazo equipo, que sin duda me siento muy afortunada de poder estar con ustedes y compartir momentos.

Estoy eternamente agradecida con muchísimas personas que han formado parte de mi vida durante todo este período y por eso doy las gracias. Pero aquí quiero agradecer al hospital clínico veterinario: En este caso quiero agradecer por regalarme tantas cesáreas, tantos propietarios bonitos, tantos llantos y tantas sonrisas, tantos compañeros e incluso amigos, tantas risas y tanta felicidad. Quiero agradecer a todos los compañeros del resto de servicios, así como a muchos profesores que han sido un apoyo incondicional estos años y que, además, siempre han tenido palabras bonitas para mí, especialmente en esta última etapa que me han apoyado mil veces más. A **Soraya Déniz** por compartir ese amor por los animales tan especial y confiar en mi para todas las cirugías; **Eligia** (tanto mami como hija) que siempre han tenido palabras bonitas para mí y tienen un corazón enorme; **Pedro** por ser compi de quirófano y convertirme en una mini-anestesista básica pero que siempre me ha seguido animando a crecer, recordándome que puedo con todo; al super equipo de hospi: **Tati, Miriam, Maria, Paula, Saray, Isa, Claudia, Sara, Pepe, Marina**... (si me dejo alguno obvio también va incluido). Por otro lado, agradecer también a **Oliver** por los ánimos, el apoyo y las bromas que hacen más amenas las horas en el hospital y por las mil veces que a pesar de tener el tiempo justo, lo he necesitado y ha estado. Se que me queda muchísima gente que mencionar y con la que estoy muy agradecida: **Marta**, **Tania, Débora, Eli, Anais, Juanmi, Otilia, José Vilar**...

Además, agradecer a muchos de nuestros criadores por todas las palabras bonitas durante este tiempo, por confiar en nosotros y por seguir permitiéndonos vivir las gestaciones y las cesáreas. Especialmente agradecer a **Montse** quien en el último tiempo me ha animado y me ha apoyado muchísimo recordándome siempre que puedo llegar muy lejos. Animándome a seguir y ofreciéndome siempre una mano si la necesito para cualquier cosa. Es muy bonito que la gente confié en ti incluso más que tú misma en muchas ocasiones.

Como no, agradecer el super equipo de Veterinaria de la Universidad de Murcia (**Xiomara y Alberto**) por acogerme como una más durante las estancias, por todos los conocimientos, por la motivación, por siempre tener palabras bonitas y por animarme tanto. Es bonito compartir especialidad con personas que la viven tanto como uno mismo y que te impulsan a querer ser mejor cada día.

Y, para terminar, me gustaría agradecer también al equipo del IUSA, especialmente a **Cristian** que me ha aguantado con las mil locuras que se me pasaban por la cabeza y sé que quedan muchos artículos por delante. Gracias además a **Toño y a Óscar** por siempre echar una mano cuando lo he necesitado.

Quiero pensar que no me he olvidado a nadie y si lo he hecho, solo pedir disculpas porque les prometo que he intentado tenerles a todos presente. Para terminar, solo quiero decirles que este es el final de una etapa, pero queda mucho por delante y así como ahora mismo me siento muy afortunada con todos y los que me rodean, espero que estén mucho tiempo más en mi vida, porque no puedo sentirme más afortunada.

TABLE OF CONTENTS

AGRADECIMIENTOS 6
TABLE OF CONTENTS 11
SUMMARY ABSTRACT 14
REVISIÓN BIBLIOGRÁFICA 17
1. THE MOTHER AND GESTATION 18
1.1 Physiology of Gestation 18
1.1.1 Progesterone 20
1.2 Physiological changes in the pregnant dog 21
1.2.1 Placentation in the Bitch 22
1.3 Pregnancy diagnosis 23
1.4 Childbirth 27
1.4.1 Dystocia 29
1.4.2 Cesarean section 31
1.4.3 Non-surgical treatment 32
1.5 Lactation 34
1.6 Pathologies of the pregnant dog 36
2. NEONATOLOGY 38
2.1 Neonatal physiology 38
2.1.1 Cardiovascular system 39
2.1.2 Respiratory system 40
2.1.3 Hematopoietic system 42
2.1.4 Hypothalamic and Nervous system 43
2.1.5 Gastrointestinal and hepatic tract 43
2.1.6 Urinary system 44
2.2 Apgar score 45
2.3 Neonatal resuscitation 47
2.4 Importance of weight 48
3. NEONATAL PATHOLOGIES: DIAGNOSIS AND TREATMENT 50
3.1 Hypoglycemia 50
3.2 Hypothermia 52
3.3 Hypoxia 53
3.4 Dehydration 54
3.5 Aspiration pneumonia 54
3.6 Fading Puppy Syndrome 55
3.7 Malformations 56
.... 57

3.7 Septicemia ... 57
4. NEONATAL BIOMARKERS ... 59
4.1 Glucose ... 59
4.2 Lactate ... 60
4.3 Cortisol ... 61
4.4 pH and blood gases ... 62
4.5 Measurements in amniotic fluid ... 63
REFERENCES ... 64
5. RESUMEN ... 74
6. OBJECTIVES ... 87
7. ARTÍCULOS ... 91
8. CONCLUSIONES ... 140
9. CONTRIBUCIONES CIENTÍFICAS ... 154

SUMMARY
ABSTRACT

The objective of this work is to deepen and generate an integrated vision of the physiological and pathological aspects that may appear during the gestation of, childbirth or neonatal adoption, with special emphasis on the determination of risk factors through the use of biomarkers, which allow clinical decisions to be made.

Progesterone is primarily responsible for maintaining gestation, which is secreted by the corpus luteums. The sudden drop in the hair is one of the first indicators that labor is beginning. However, other hormones with estrogen do not appear to play a major role during this process. Recent studies support the theory that fetal maturation and increased fetal cortisol may play a key role in the onset of labor. In addition, prolactin has been determined to be an important luteotropic hormone in maintaining progesterone levels.

Birth planning is very important, especially in those pregnancies where there is a high risk of dystocia, as well as in case you want to schedule a cesarean section. That is why different parameters are used, such as: plasma progesterone levels, heart rate monitoring, control of other measurements such as biparietal diameter, gastrointestinal motility or echogenicity of the kidneys.

The neonatal period is defined as the first three to four weeks of the animal's life, which represent a critical stage. During this time, neonates require rapid adaptation in terms of thermoregulation, respiratory and metabolic functions, and immunity. These adaptations require maternal care, colostrum intake, optimal temperature control and adequate environmental hygiene.

The most common causes of neonatal morbidity and mortality include hypothermia, hypoglycemia, hypoxia, dehydration, aspiration pneumonia, congenital malformations, and sepsis. Hypoglycemia is particularly frequent in low birth weight puppies due to limited glycogen reserves and immature hepatic gluconeogenesis. Blood glucose levels below 50 mg/dL are associated with neurological depression, seizures, and death. Similarly, hypothermia can impair metabolic enzyme function, reduce gastrointestinal motility, and exacerbate hypoglycemia and immune suppression. Neonates are highly vulnerable to heat loss due to their high surface area-to-volume ratio and lack of brown adipose tissue.

Perinatal hypoxia can be affected by prolonged labour or even the use of anaesthetic agents during a caesarean section, which can increase the likelihood of neonatal death. This hypoxia in turn favors the appearance of acidosis, bradycardia, multi-organ alterations... During the delivery process or in caesarean sections, aspiration of meconium may occur, which causes aspiration pneumonia, which can in turn promote respiratory distress and an increase in neonatal mortality due to unknown cause. Congenital malformations such as hydrocephalus, anasarca or skeletal malformations can also contribute to neonatal losses. Many of these causes are usually racially biased.

That is why the objective is to determine quantifiable biomarkers that allow neonatal viability to be assessed, such as: blood glucose, lactate, pH or cortisol levels. It has now been observed that high lactate values are related to anaerobic metabolism and poorer perfusion; while pH values below 7.1 suggest metabolic acidosis with a poor prognosis. On the other hand, high levels of cortisol in the amniotic fluid or in the neonatal serum reflect intrauterine stress that is also correlated with low Apgar scores.

Therefore, this review tries to bring together the most relevant literature, as well as different clinical strategies in canine reproduction and neonatology, emphasizing early detection, preventive care or intervention based on objective evidence. That is why a set of parameters such as hormonal measurements, fetal monitoring, use of neonatal care tools or the use of biomarkers are used, with the aim of reducing neonatal mortality and improvising the health of mothers and newborns.

REVISIÓN BIBLIOGRÁFICA

1. THE MOTHER AND GESTATION

Canine reproductive physiology differs significantly from that of other domestic mammals. Bitches are monoestrous, non-seasonal breeders and exhibit polyovulatory cycles (Miranda *et al*, 2018). Gestation lasts approximately 65 ± 1 days from the preovulatory luteinizing hormone (LH) surge, which is designated as day 0 of gestation (Lopate, 2012).

The peripartum stage is characterized by a series of physiological and behavioral changes, including hormonal fluctuations, the onset of lactation, uterine involution, and the onset of maternal behaviors (Arlt, 2020). These changes are critical, especially considering that neonates are born in immature physiological conditions. Therefore, their survival depends exclusively on adequate maternal care and colostrum intake. Therefore, the mother's health status is key to proper neonatal viability and survival (Arlt, 2020).

For correct management and understanding of neonatal, a detailed clinical history must begin with information on the parents, the progress of pregnancy, the conditions of delivery, environmental influence, characteristics of the litter and individual neonatal parameters (Pereira *et al*., 2024). A complete approach is essential since neonatal health does not depend exclusively on one factor, but involves multiple aspects such as genetic, maternal, environmental and perinatal (Pereira *et al*., 2024).

Given that many neonatal disorders may originate from maternal dysfunctions, a thorough evaluation of the dam is warranted. This includes appropriate diagnostic to detect nutritional imbalances, lactation disorders such as agalactia or hypogalactia, mastitis, abnormal maternal behavior, or pathological changes in vaginal or uterine secretions (Pereira *et al*., 2024).

1.1 Physiology of Gestation

The reproductive physiology of the bitch is unique among domestic mammals. Nonetheless, the luteal phase exhibits remarkable similarities in both pregnant and non-pregnant bitches, lasting approximately two months (Verstegen-Onclin *et al*., 2008). In pregnant animals, plasma progesterone levels tend to be lower and decline more abruptly toward the end of the luteal phase (Verstegen-Onclin *et al*., 2008).

Despite ongoing research, the exact mechanisms triggering parturition in the bitch remain incompletely understood (Arlt, 2020). Current evidence suggests that the maturation of the fetal hypothalamic-pituitary-adrenal (HPA) axis plays a central role, with both fetal and maternal cortisol acting as key initiators (Johnson, 2008;

Artl, 2020). This endocrine activation sets off a hormonal cascade involving estrogens, oxytocin, and relaxin, which together stimulate the production of prostaglandins, particularly prostaglandin F2α. This prostaglandin surge, occurring approximately 36 hours prior to labor, induces luteolysis and a sharp drop in circulating progesterone, a property event in the onset of parturition (Arlt, 2020). However, the precise hormonal interactions and regulatory pathways involved in this process remain to be fully investigated (Fusi *et al*., 2022).

In several domestic species, estrogens play a critical role in the initiation of labor, favoring dilation of the cervix and increasing uterine sensitivity to oxytocin (Fusi & Veronesi, 2022). However, in the bitch, the concentrations of circulating estrogens in the plasma do not present significant values at the time of delivery. Although the concentration of15-β-estradiol remains in range during delivery, it begins to decline shortly after delivery (Baan *et al*., 2008; Milani *et al*., 2017). Studies have shown that neither estron nor estradiol levels increase during delivery in this species, which supports the hypothesis that estrogens do not play an important role in the initiation of labor in the bitch (Onclin *et al*., 2022; Fusi & Veronesi, 2022).

Among the various hormones involved in the physiology of childbirth, relaxin plays a critical role. It is produced by the placenta and contributes to the relaxation of the pelvic ligament and cervix, as well as acting indirectly on luteal maintenance by stimulating the production of prolactin (Verstegen-Onclin *et al*., 2008; Lopate, 2012). Relaxin begins to be detectable in maternal plasma between days 20-22 after the LH peak, reaching its peak between days 20-25 of placentation (Johnson, 2008). Because of its specificity, relaxin is used as a diagnostic method in pregnancies, although false positives can occur in the case of miscarriages (Verstegen-Onclin *et al*., 2008). Levels usually drop sharply during delivery, although they remain detectable in the blood during the following nine weeks postpartum due to the persistence of trophoblastic activity (Johnson, 2008).

Prolactin, a pituitary hormone, is the primary luteotrophic factor in the bitch. From approximately day 25 of gestation onward, corpus luteum maintenance becomes dependent on both prolactin and luteinizing hormone (LH) (Verstegen-Onclin *et al*., 2008; Fusi *et al*., 2022). During the early stages of pregnancy, luteal support may occur independently of pituitary input, potentially mediated by local prostaglandins (Fusi *et al*., 2022). Prolactin levels rise progressively during gestation and reach peak concentrations shortly before parturition (Fusi *et al*., 2022). Although no direct correlation has been observed between luteinizing hormone (LH) concentrations and progesterone levels during pregnancy in the bitch, the inhibition of prolactin during this period has been shown to induce a rapid decline in circulating progesterone concentrations, thereby confirming the luteotropic role of prolactin in canine gestation (Onclin *et al*., 2000).

Additional hormonal players in the peripartum period include corticosteroids, vasopressin, parathyroid hormone, and thyroid hormones (Fusi *et al.*, 2022). Among glucocorticoids, cortisol is the most significantly elevated prior to delivery, likely reflecting both maternal and fetal stress responses rather than serving as a direct trigger of labor (Fusi & Veronesi., 2022). Although concentrations of other corticosteroids, such as cortisone, corticosterone, 11-deoxycorticosterone, and 11-deoxycortisol, have also been reported to increase, only cortisol shows a statistically significant rise (Fusi & Veronesi., 2022).

1.1.1 Progesterone

Progesterone is the primary hormone responsible for maintaining pregnancy in the bitch (Lopate, 2012; Kowalewski, 2023). Unlike other domestic species, where the placenta contributes significantly to progesterone synthesis, in dogs, the corpus luteum remains the sole source of this hormone throughout gestation (Hinderer, 2021; Lopate, 2012). Following ovulation, progesterone concentrations rise to support implantation and placental development. However, circulating levels during the luteal phase are similar in both pregnant and non-pregnant bitches, due to sustained luteal activity regardless of pregnancy outcome (Lopate, 2012).

In the veterinary clinic, the measurement of plasma progesterone is used as a useful tool to estimate the date of ovulation and gestation time (Uchansla *et al.*, 2022). When the progesterone concentration is around 1.5 ng/mL, it corresponds to the LH peak, considering this moment as day 0 of gestation and allowing predicting the due date, which usually occurs between days 62 and 64 (Uchansla *et al.*, 2022).

For labor to occur, there is a drop in progesterone. Most studies establish a value of around 2 ng/mL (approximately 6.4 nmol/L) in the 36-38 hours prior to the start of labor (Siena and Chiara, 2021; Fusi et al., 2022; Daniela *et al.*, 2025). To be more precise, values lower than 5-8 nmol/L or 8.7 nmol/L, estimate an onset of labor at 48 and 96 hours respectively with 99% accuracy. On the other hand, values below 3.18 nmol/L predict the onset of labor in the following 24 hours (Fusi *et al.*, 2022).

The main drop during the prepartum is associated with the action of prostaglandin F2α (PGFα), which induces luteolysis about 36 hours before the start of labour (Siena and Chiara, 2021). In the case of pregnancies with singleton puppies, the onset of luteolysis may be insufficient, causing progesterone levels to remain high at term and as a consequence, a delay in delivery (Siena and Chiara, 2021; Cuts *et al.*, 2025; by Cramer *et al.*, 2025).

From a clinical perspective, progesterone also exerts thermogenic effects. As a result, the prepartum decline in serum progesterone is often accompanied by a drop in rectal temperature, typically up to 0.84°C, within 12–24 hours prior to labor (Milani *et al.*, 2020; Fusi *et al.*, 2022). This correlation has led the use of daily temperature monitoring as an indirect predictor of parturition (Fusi *et al.*, 2022). However, not all bitches exhibit this temperature drop, and some authors caution against relying on this parameter as a stand-alone indicator (Artl, 2020).

In the case of scheduled caesarean sections, the measurement of progesterone has become a fundamental tool. A progesterone concentration of less than 2 ng/mL is considered a safe value for surgery, regardless of breed or litter size (Crummer *et al.,* 2025).

Another reason why progesterone is considered essential in the maintenance of pregnancy is due to pathologies such as hypoluteinism, a condition that is characterized by insufficient secretion of progesterone by the corpus lutei (Hinderer *et al.*, 2023). This insufficient release can lead to embryological loss or miscarriages if not properly diagnosed and treated. Although the etiology is unknown, some authors suggest that it may be related to inadequate production by luteotropic agents such as prolactin or relaxin (Hinderer *et al.,* 2023). These agents are responsible for maintaining luteal function and maintaining progesterone secretion during pregnancy (Hinderer *et al.,* 2023). To confirm cases where there is suspicion, exogenous progesterone is administered to prevent gestational loss, although dosage protocols, route of administration, and therapy vary among the different authors (Hinderer *et al.*, 2023)

1.2 Physiological changes in the pregnant dog

Beyond hormonal fluctuations, gestation in the bitch is associated with several hematological and inflammatory changes. A progressive, normocytic and normochromic anemia often develops from mid-gestation, frequently accompanied by mild thrombocytopenia (Lopate, 2012; Uchanska *et al.*, 2022). While some authors consider this anemia physiological, others recommend close monitoring to rule out underlying pathology (Arlt, 2020). Notably, hematocrit appears to inversely correlate with litter size, decreasing approximately 0.14% per additional fetus (Artl, 2020).

Inflammatory markers such as fibrinogen and C-reactive protein also increase between days 30 and 50 of gestation (Holst *et al.*, 2018), reflecting placental development and trophoblastic invasion (Sebastián, 2020; Lopate, 2012). This process is also associated with mild leukocytosis, interpreted as a non-specific inflammatory response (Lopate, 2012).

During pregnancy, several components of the acute-phase response become activated, reflecting a complex interplay between hormonal modulation and immunological adaptation. This physiological response is characterized by a series of defense mechanisms, including alterations in leukocyte profiles, vascular permeability changes, and metabolic shifts (Holst *et al.*, 2018). Although the exact triggers for this activation remain unclear, it is hypothesized that ovarian hormones, may influence the maternal immune system and contribute to these changes (Holst *et al.*, 2018).. An important marker of the acute-phase response is fibrinogen, a positive acute-phase protein whose plasma concentration increases significantly during gestation. This rise has been associated with elevated progesterone levels, suggesting a hormonal regulation of hepatic synthesis of acute-phase proteins during pregnancy (Holst *et al.*, 2018).

1.2.1 Placentation in the Bitch

Placental development in the bitch follows a distinct ontogeny, beginning with the transient formation of a choriovitelline placenta derived from mesodermal trophoblasts (Aralla *et al.*, 2013). This early structure is eventually replaced by the definitive chorioallantoic placenta, which supports fetal development throughout gestation (Aralla *et al.*, 2013). Furthermore, the canine placenta is thought to be a significant source of luteolytic prostaglandin F2α (PGF2α) production during parturition, contributing to the cascade of hormonal events that lead to luteolysis and expulsion of the fetus (Gram *et al.*, 2014).

Placentation plays a fundamental role in embryonic and fetal development. Any abnormality in the placental structure or its function can interfere with gestation and increase the risk of fetal compromise or lead to miscarriages (Sarli *et al.,* 2021). The placenta is not only an organ that connects the fetus and the mother, but is also responsible for certain essential physiological processes such as the transport of nutrients, gas exchange or the elimination of metabolic waste products, all of which are essential for fetal development (Farias *et al.*, 2023). Recent studies highlight the implication of incorrect placental function or intrauterine nutritional restrictions, associated with an increase in chronic pathologies or even neoplastic conditions in adult life (Gloria *et al.*, 2024).

The mature canine placenta is classified as endotheliochorial, zonary, and allantochorionic (Tesi *et al.*, 2021). This type of placentation is characterized by a band-shaped (zonary) area of contact between maternal and fetal tissues (Gloria *et al.*, 2024). These regions are characterized by the accumulation of green pigment, attributed to biliverdin from localized maternal hemorrhage during placental development (Aralla *et al.*, 2013) (Figure 1).

Figure 1. Fetal membranes and placenta from a puppy delivered by cesarean section at the Veterinary Clinical Hospital (HCV).

Histologically, the canine placenta can be divided into three distinct regions: the labyrinth zone, the junctional zone, and the glandular zone (Premanandan & Runcan, 2019; Farias *et al.,* 2023; Gloria *et al.,* 2024). The labyrinth serves as the primary fetomaternal interface, where nutrient and gas exchange occurs (Premanandan & Runcan, 2019). The glandular zone, derived from maternal endometrial tissue, contributes secretory products that are essential for fetal development. Situated between these regions is the junctional zone, composed of vascularized maternal connective tissue projections covered by trophoblasts (Gloria *et al.,* 2024). Notably, this zone plays a key role during parturition, as it is the anatomical site where placental separation takes place (Premanandan & Runcan, 2019).

From a clinical perspective, the placenta has been recognized as a diagnostic tool, since microscopic and macroscopic examinations have been identified with the diagnosis of intrauterine infections or exposure to toxins (Tesi et al., 2021). For example, necrosis in the labyrinthine area has been linked to herpesvirus infections, leishmaniasis, brucellosis, or drug administration (Tesi *et al.*, 2021).

1.3 Pregnancy diagnosis

Gestational monitoring and accurate estimation of the expected date of parturition are essential components of canine reproductive management (Siena *et al.*, 2021; Fernandes *et al.*, 2025). Precise prediction of whelping not only facilitates appropriate obstetric planning, but also supports timely decision-making regarding elective cesarean sections, thereby minimizing both neonatal and maternal morbidity and mortality (Siena *et al.*, 2021). Given the variability in gestational length among breeds (ranging from 57 to 72 days), multiple diagnostic modalities and predictive algorithms have been developed to improve accuracy. These often rely on ultrasonographic parameters and are frequently adapt to specific breed sizes (Siena *et al.*, 2021; Gil *et al.*, 2014).

Initial pregnancy diagnosis may be performed via abdominal palpation between days 25 and 35 post-LH surge, during which time gestational sacs typically measure 1–3 cm in diameter (Lopate, 2012). However, this technique may be unreliable in obese bitches or those with tense abdominal musculature, and becomes increasingly difficult after day 35, as the sacs elongate (Lopate., 2012).

In addition to imaging techniques, endocrine assays can provide valuable information during pregnancy monitoring. In this context, relaxin, a hormone produced specifically by the placenta in the bitch, can be detected around day 19 after the LH surge, with peak levels typically observed between days 30 and 35 of gestation (Evci *et al.,* 2022). Although this test is commonly used in clinical practice to confirm pregnancy, it is important to acknowledge its limitations. For instance, in cases of embryonic loss or pregnancy resorption, relaxin levels may remain elevated for some time, potentially leading to false-positive results (Verstegen-Onclin *et al.*, 2008).

Ultrasonography remains the most versatile and widely used tool for both pregnancy confirmation and fetal assessment. Some authors have reported that uterine wall thickening can be detected via ultrasonography as early as day 7 post-mating (Fernandes *et al.*, 2025). Accurate diagnosis of gestation should rely on more specific ultrasonographic findings, such as the identification of gestational vesicles, (Fernandes *et al.*, 2025). Gestational sacs can be visualized as early as day 19, with embryos consistently observed by days 21–22. Cardiac activity, indicating viability, is typically detectable by day 24 (Lopate, 2012; Evci *et al.*, 2022) (Figure 2).

Guidelines have outlined key developmental organs detectable via ultrasonography, including: visualization of the urinary bladder and stomach between days 35–39, kidneys from days 39–47, and intestines from days 57–63 (Lopate, 2008; Milani *et al.*, 2020). Fetal kidneys initially display a well-differentiated cortex and medulla with a small, non-dilated pelvis. These structures become more defined as gestation advances (Lopate, 2008). Intestinal peristalsis typically begins around days 62–64, although some authors report earlier observations between days 39–48. Detection requires careful, prolonged scanning of multiple intestinal segments (Milani *et al.*, 2020). The intestinal wall becomes more defined near term, with a hypoechoic lumen surrounded by a hyperechoic muscular layer visible from days 58–60 (Lopate, 2008).

Fetal heart rate (FHR) monitoring is a critical tool for identifying fetal distress and determining the need for emergency cesarean section. FHR should be approximately 2–3 times higher than the maternal heart rate, typically ranging between 220–240 bpm (Lopate, 2008). Monitoring is most informative during the last 10 days of gestation. A FHR below 180 bpm is considered concerning, while values under 140–160 bpm are strongly associated with fetal hypoxia (Gil *et al.*, 2014; Lopate, 2008). In addition to FHR, fetal fluid echogenicity may provide valuable insight into intrauterine conditions. Increased echogenicity, possibly due to meconium or intra-amniotic hemorrhage, may suggest premature placental separation (Lopate, 2008). Notably, a pattern of transient bradycardia followed by rebound tachycardia has been observed within 72 hours of parturition, serving as a potential predictor of imminent labor and assisting in the optimal timing of elective cesarean delivery (Milani *et al.*, 2020).

Abnormal vascular development at either the maternal or fetal level has been linked to poor pregnancy outcomes, such as intrauterine growth restriction (IUGR), fetal distress, and even gestational loss through abortion (Fernandes *et al.*, 2025). Among the diagnostic approaches used to monitor fetal health and estimate the timing of delivery, Doppler velocimetry has gained increasing relevance in clinical settings. One parameter of particular interest is the resistance index (RI) of uterine and umbilical arteries, which serves as an indirect indicator of placental perfusion. Fernandes et al. (2025) reported that RI values below 0.7 were associated with reduced vascular resistance and often preceded parturition by less than 72 hours.

A number of ultrasonographic measurements have been validated to estimate gestational age and assist in the prediction of delivery. These include inner chorionic cavity (ICC) diameter, biparietal diameter (BPD), and fetal body diameter (Lopate, 2008). ICC is most accurate when measured before day 40 of gestation and should ideally be assessed in at least two fetuses. Formulas based on ICC offer an accuracy range of 64–91% in small to medium-sized breeds, and 85–88% in larger breeds when applied before day 37–40 (Lopate, 2008; Siena *et al.*, 2022). Later in gestation, BPD becomes the most reliable parameter. For example, in English Bulldogs, a BPD of 29.5 mm or greater is considered an appropriate threshold for scheduling elective cesarean section (Cramer et al., 2018; Batista et al., 2015).

Beyond traditional parameters such as biometric data, fetal heart rate, and placental characteristics, recent research has highlighted gastrointestinal (GI) motility as a novel ultrasonographic marker of fetal maturity (Siena *et al.*, 2022). The presence of GI motility signals the completion of organogenesis and may be valuable in clinical decision-making during the final days of gestation. Authors observed that GI motility was detectable in only 17.1% of fetuses during the last 10 days of gestation; however, this figure increased markedly to 63.3% within the final

5 days prior to delivery (Siena *et al.,* 2022). These findings suggest a strong correlation between increased GI motility and advanced fetal development.

Figure 2. Ultrasound image of an embryonic vesicle in a bitch at 26 days of gestation, obtained at the Reproduction Service of HCV.

Figura 3: Ultrasound image showing biparietal diameter measurement in a pregnant bitch at 46 days of gestation, performed at the Reproduction Service of HCV.

Radiography becomes a reliable diagnostic tool after day 45, coinciding with fetal skeletal mineralization. Prior to this stage, only soft-tissue densities are visible, which may be indistinguishable from uterine pathologies such as pyometra or hydrometra (Lopate, 2012). A single lateral radiograph (Figure 5) is generally adequate to confirm pregnancy and estimate litter size, while a ventrodorsal projection (Figure 4) can provide additional information about pelvic dimensions in relation to fetal head size (Lopate, 2008). The presence of intra- or perinatal gas within or around fetuses is suggestive of fetal death (Lopate, 2008).

Figure 4. Ventro-dorsal radiograph of a pregnant bitch with three fetuses at 56 days of gestation at the Reproduction Service of the HCV.

Figure 5. Latero-lateral radiograph of a pregnant bitch with three fetuses at 56 days of gestation at the Reproduction Service of the HCV.

1.4 Childbirth

Canine gestation generally lasts between 57 and 72 days post-mating, though this range can vary based on breed, litter size, and individual physiological differences (Uchanska *et al.*, 2022). As the expected date of parturition approaches, veterinarians may observe a combination of physiological and behavioral signs. One of the most clinically consistent indicators is a drop in serum progesterone levels below 2 ng/mL, typically occurring within 24 to 48 hours prior to labor (Cortes *et al.,* 2025). This hormonal shift is often accompanied by a decrease in rectal temperature and behavioral changes such as restlessness, nesting behavior, digging, panting, and withdrawal from social interaction (Cortes *et al.,* 2025).

In practice, several monitoring tools are used to anticipate the onset of labor. These include serial progesterone assays, temperature tracking (via rectal or vaginal readings), and fetal heart rate (FHR) monitoring (Milani *et al.,* 2020). Ultrasonography also plays a valuable role, particularly when evaluating fetal kidney echogenicity and the presence of gastrointestinal motility, both of which can suggest fetal maturation. Notably, significant fluctuations in FHR (exceeding 30% in a 12-hour window) have been associated with imminent labor, with a reported predictive sensitivity of approximately 88% (Cortes *et al.*, 2025).

Prolonged gestation may result in fetal compromise or death, especially if the metabolic demands of the fetuses surpass placental capacity (Lopate, 2008). Therefore, careful monitoring is warranted, particularly as the pregnancy nears its physiological limit.

Labor in the bitch is classically divided into three different stages:

- **Stage I:** The preparatory phase involves cervical relaxation and the onset of uterine contractions. It is hormonally regulated by relaxin and influenced by cytokines and the activation of the autonomic nervous system (Lopate, 2012; Artl, 2020). In primiparous or anxious dogs, this phase can last up to 36 hours, although it is normal for it to last 6-12 hours (Lopate, 2012).

- **Stage II**: During this stage, circulating progesterone levels have dropped sharply, facilitating cervical softening and dilation, which are essential for the 27uration27 of the fetuses (Fusi *et al.*, 2022; Siena y Chiara, 2021). In addition, the sensitivity of myometrial oxytocin receptors increases significantly, enhancing uterine contractility and promoting effective fetal 27uration27 (Gram *et al.*, 2014). The active phase of fetal expulsion, often overlapping with Stage III, typically lasts 6 to 8 hours. However, longer durations are occasionally observed (Lopate, 2012). Fetal delivery results from coordinated uterine contractions and voluntary abdominal straining

(Uchanska *et al.*, 2022). As the fetus enters the birth canal, sensory stimulation activates spinal reflexes that enhance abdominal muscle contraction (Artl, 2020). A negative correlation has been reported between the duration of Stage II and neonatal mortality, with shorter 28urationns (around 5.5 hours) associated with higher neonatal survival (Fusi *et al.*, 2021).

- **Stage III**: The final stage involves the expulsion of fetal membranes, usually occurring immediately or within 15 minutes after each puppy is born (Uchanska *et al.*, 2022). Complete expulsion of all membranes may take up to 1–2 hours following the last delivery (Lopate, 2012).

The total duration of parturition may range from 6 to 24 hours. In nervous or primiparous bitches, labor may extend up to 36 hours without necessarily being pathological (Uchanska *et al.*, 2022).

As parturition begins, the fetus undergoes a series of rapid physiological changes aimed at ensuring adaptation to life outside the uterus. These catecholamines play a critical role in the redistribution of fetal blood flow, prioritizing perfusion to vital organs such as the brain, heart, and adrenal glands (Kredatusova *et al.,* 2011). This redistribution is considered critical for immediate postnatal survival, especially in species with precocial development, like the dog (Kredatusova *et al.,* 2011).

On the maternal side, labor is physiologically demanding. The dam usually experiences an increase in respiratory rate as a result of pain, anxiety, and muscular effort (Kredatusova *et al.*, 2011). This hyperventilation often induces a state of respiratory alkalosis, which can interfere with efficient placental oxygen exchange. As contractions intensify and labor proceeds, a mild metabolic acidosis may develop. When prolonged or severe, this imbalance can compromise fetal acid-base stability (Kredatusova *et al.,* 2011). Moreover, episodes of maternal hypoventilation between contractions can lead to transient desaturation of maternal hemoglobin, further compromising fetal oxygenation. These dynamic maternal-fetal interactions underscore the complexity of perinatal physiology and the fine regulatory mechanisms at play during labor (Kredatusova *et al.,* 2011).

Elective cesarean section is recommended in specific clinical scenarios where spontaneous delivery is unlikely or poses a risk. These include singleton pregnancies, giant breeds with small litters (resulting in fetal oversize), bitches with extremely large litters (predisposing to uterine inertia), or those with a history of dystocia or primary uterine inertia (Lopate, 2008). In singleton pregnancies, limited fetal cortisol production may fail to sufficiently stimulate prostaglandin F2α synthesis in the endometrium, impairing luteolysis and delaying labor onset

(Lopate, 2008). Cortisol plays a fundamental role in the regulation of the physiological mechanisms that initiate parturition. Its concentration increases in late gestation as a result of enhanced production of adrenocorticotropic hormone (ACTH) by the fetal pituitary gland (de Araújo *et al.*, 2025). This rise in fetal cortisol is a key endocrine signal that triggers a cascade of hormonal events culminating In the onset of labor. One of the primary effects of elevated fetal cortisol is the stimulation of enzymatic pathways that convert progesterone into estrogens, producing a notable decline in circulating progesterone levels, an essential condition for the initiation of myometrial contractions and cervical dilation (de Araújo *et al.*, 2025).

1.4.1 Dystocia

Dystocia, defined as difficulty during parturition, is a relatively frequent complication in the bitch, with an overall incidence of up to 5%-16% of all canine births (Conze *et al.*, 2022; Long *et al.*, 2022). However, it occurs considerably more often in certain breeds, reaching up to 59.4% in miniature and toy breeds (Bolis *et al.*, 2017). Both maternal and fetal factors can be responsible, and in many cases, the etiology is multifactorial (Lopate, 2012; Cornelius *et al.*, 2019). Accurate diagnosis of dystocia is essential to improve neonatal survival and guide timely intervention (Uchanska *et al.*, 2022).

Figure 6. Obstructive dystocia due to fetomaternal disproportion in a bitch presented as an emergency at the Reproduction Service of the Veterinary Clinical Hospital (HCV).

Breed predisposition is a well-documented factor in the occurrence of dystocia in canine species (de Araújo *et al.*, 2025; Long *et al.*, 2022). Certain breeds, such as Scottish Terriers and Boston Terriers, have been reported to inherit a dorsoventral pelvic flattening associated with a reduced vertical pelvic diameter, which increases the risk of obstructive dystocia due to fetal–maternal disproportion (Cornelius *et al.*, 2019) (Figure 6). This anatomical conformation often leads to secondary uterine inertia as a result of prolonged or failed labor efforts. Additionally, brachycephalic breeds such as Bulldogs are at significantly increased risk of dystocia, primarily due to a mismatch between the broad fetal head and the maternal pelvic canal (Cornelius *et al.*, 2019). In these cases, elective cesarean section is frequently recommended as a preventive measure to reduce perinatal morbidity and mortality, both in the dam and the neonates.

There are factors that can predispose to dystotic processes, such as maternal age (over 6 years), extreme breed sizes (miniature or giant), single puppy syndrome in small breeds or very large litters (more than 11 fetuses) (Uchanska *et al.,* 2022).

The most common maternal cause of dystocia is uterine inertia, which may be classified as primary or secondary (de Araújo *et al.*, 2025; Lopate, 2012). Other maternal contributors include pelvic or vaginal anatomical abnormalities (e.g., narrow pelvic canal, vaginal septum), excessive perivaginal fat, inguinal hernia, uterine torsion, uterine rupture, or behavioral disturbances such as pain-induced anxiety or fear, which may interfere with labor progression (Lopate, 2012).

Primary uterine inertia is characterized by the complete failure of the uterus to initiate effective contractions, preventing the expulsion of the first fetus. A partial form may also occur, in which labor begins normally but ceases after delivery of one or more puppies (Lopate, 2012). Contributing factors include malnutrition, systemic diseases, hypocalcemia, and uterine pathology such as endometritis, metritis, or premature labor (Lopate, 2012). This condition is particularly common in bitches with large litters, where uterine overstretching impairs contractility, or in very small litters, where hormonal stimulation may be insufficient to trigger labor (Lopate, 2012). Bitches carrying more than 12 fetuses may develop weak or ineffective contractions due to uterine fatigue, which can also lead to secondary inertia (Uchanska *et al.*, 2022; Cornelius *et al.*, 2019). In some nervous or fearful dogs, stress may suppress uterine contractility altogether (Lopate, 2012).

Secondary uterine inertia refers to the cessation of uterine contractions after the delivery of one or more neonates. Although it may result from the same underlying factors as primary inertia, the most common causes include hypocalcemia, uterine muscle fatigue, and, less frequently, hypoglycemia (Lopate, 2012). Hypoglycemia is particularly relevant in toy breeds and is often linked to prolonged cortisol elevation (Lopate, 2012).

Secondary inertia may also follow fetal obstruction, where excessive uterine effort leads to exhaustion (Lopate, 2012). Maternal age is another influential factor: bitches under one year or over six years of age tend to have smaller litters, which in turn increases the risk of dystocia, compared to females aged between 2 and 5 years (Uchanska *et al.*, 2022).

Fetal causes include abnormal fetal presentation, position, or posture (Lopate, 2012). Structural abnormalities such as anasarca, hydrocephalus, or abdominal wall defects (e.g., hernias) may also lead to obstructive dystocia (Lopate, 2012). Macrosomic (oversized) fetuses are another common cause. In the case of dead fetuses, they may not be able to activate muscle tone on their own, preventing passage through the birth canal and favoring the appearance of obstructive dystocia (Lopate, 2012).

1.4.2 Cesarean section

During gestation, the bitch experiences profound physiological changes that must be carefully considered when planning a cesarean section. These include a significant increase in blood volume and cardiac output, as well as elevated intra-abdominal pressure due to uterine enlargement. The gravid uterus can compress the aorta and caudal vena cava, reducing venous return and compromising uterine perfusion (Lopate, 2012). Concurrently, respiratory adaptations occur, including increased tidal volume, respiratory rate, and alveolar ventilation (Lopate, 2012). For optimal maternal and neonatal outcomes, cesarean sections should be timed to ensure fetal maturity while avoiding the onset of labor or fetal compromise (Crummer *et al*., 2025). Cesareans are typically classified into three categories:

1. Elective prepartum cesarean (no labor, mature fetuses)
2. Cesarean during Stage I labor (no fetal distress)
3. Emergency cesarean, including fetal distress or dystocia (Crummer *et al*., 2025)

Emergency cesarean sections carry significantly higher risks, with maternal mortality reported around 1%, and neonatal mortality reaching up to 20% (Uchanska *et al*., 2022). Brachycephalic breeds such as French Bulldogs, Boston Terriers, Chihuahuas, and Pugs are frequently overrepresented in emergency cases (Uchanska *et al*., 2022). Although brachycephalic breeds historically show a higher incidence of cesarean delivery, certain non-brachycephalic breeds, such as Golden Retrievers, Labrador Retrievers, Miniature Dachshunds, and Poodles, also exhibit predisposition (Crummer *et al*., 2025).

Scheduled cesarean sections become safe 48 hours after the start of phase I of labor, thus confirming fetal maturity (Crummer *et al.,* 2015). Regarding fetal heart rate, this is used as a prognostic indicator, since values below 180 bpm are indicative of immediate intervention; while values below 120 bpm correlate with poor neonatal survival (Uchanska *et al.,* 2022) (Figure 7).

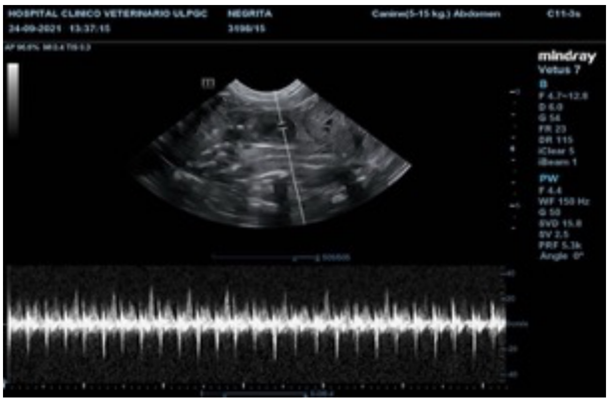


Figure 7. Fetal heart rate assessment by ultrasound in a bitch at 45 days of gestation, performed at the Reproduction Service of HCV.

From an anesthetic point of view, we must minimize the induction-delivery interval, preserve uterine perfusion, and use those anesthetic agents that minimize fetal impact and allow them to be reversed if necessary (Lopate, 2012; Di Cesare *et al.,* 2025). The placenta plays an important role in regulating the maternal-fetal transfer of anesthetic agents, so it should be taken into consideration to avoid neonatal depression and other adverse effects (Di Cesare *et al.,* 2025; Navarro-Altuna *et al.,* 2025).

Several maternal conditions may necessitate a scheduled cesarean due to elevated perinatal risk. These include bitches with diabetes mellitus, toxemia, or those receiving progesterone supplementation due to luteal insufficiency. The latter may result from chronic endometritis, maternal stress, partial abortion, or idiopathic luteal failure (Lopate, 2008). However, certain obstetrical conditions may warrant the consideration of elective cesarean section as a proactive strategy to minimize perinatal complications and improve neonatal outcomes. These include scenarios such as singleton pregnancy (singleton puppy syndrome), which is associated with a higher incidence of dystocia due to insufficient uterine stimulation, as well as very small or excessively large litter sizes, both of which can be linked to uterine inertia or fetal–maternal disproportion (Di Cesare *et al.*, 2025).

Regardless of the surgical process, it is necessary to perform a correct neonatal resuscitation protocol to improvise neonatal viability. This includes: immediate drying of the neonates, airway release, breathing stimulation, heart rate assessment and, if necessary, administration of drugs (Urchanska *et al.,* 2022).

1.4.3 Non-surgical treatment

Cesarean section is not the only therapeutic option in cases of dystocia. Various pharmacological treatments are available, with oxytocin being the most used agent (Oliwia *et al.*, 2022). In addition, administration of calcium gluconate and glucose is frequently recommended as part of medical management (Oliwia *et al.*, 2022).

Prior to initiating medical treatment, it is essential to perform an ultrasonographic evaluation to assess for fetal distress—specifically, a fetal heart rate below 180 bpm. In such cases, immediate cesarean section is indicated (Runcan *et al.*, 2018). Medical management is only appropriate under specific conditions: the bitch must be systemically stable, should not have experienced prolonged labor, and there must be no evidence of obstructive dystocia or fetal distress (Runcan *et al.*, 2018). If there is no response to medical treatment, emergency cesarean section becomes mandatory to avoid fetal compromise (Oliwia *et al.*, 2022).

The therapeutic protocol includes the combination use of calcium and oxytocin. Calcium improves the frequency of uterine contractions, while oxytocin increases their intensity by acting directly on myometrial oxytocin receptors (Runcan *et al.*, 2018; Münnich and Küchenmeister, 2009). Calcium gluconate should be administered slowly via intravenous bolus at 10–20 mg/kg, with cardiac monitoring to avoid arrhythmias. Other option is the aplication subcutaneously at 22 mg/kg, diluted 1:1 with saline to prevent local tissue irritation and granuloma formation (Runcan *et al.*, 2018).

Oxytocin must never be administered in cases of obstructive dystocia, as it may lead to uterine rupture (Runcan *et al.*, 2018). The recommended dose is 0.25–1 IU, administered intravenously, intramuscularly, or subcutaneously. The cumulative dose should not exceed 5 IU per bitch (Runcan *et al.*, 2018). If no neonate is expelled after 2–3 doses, despite strong abdominal contractions, surgical intervention is required. However, if a neonate is delivered successfully, the oxytocin protocol may be repeated, with a minimum interval of 30 minutes between applications (Runcan *et al.*, 2018).

Figure 8. Uterine rupture following double administration of oxytocin in a bitch with obstructive dystocia, treated as an emergency at the Reproduction Service of HCV.

The response to oxytocin is influenced by multiple factors, including maternal stress, receptor availability, and receptor desensitization (Münnich and Küchenmeister, 2009). High doses or repeated administrations of oxytocin (particularly intravenous) are associated with increased risk of fetal hypoxia and death (Münnich and Küchenmeister, 2009). Recent evidence suggests that in cases where fewer than two fetuses are present in the birth canal, smaller, repeated doses (0.25–0.5 IU) may yield better outcomes (Münnich and Küchenmeister, 2009). Although adverse effects are relatively uncommon, excessive dosing increases the risk of uterine rupture, especially in the presence of mechanical obstruction (Karen *et al.*, 2010) (Figure 8).

It has been observed that uterine sensitivity to oxytocin increases in the period preceding parturition, which explains the clinical use of this hormone for the treatment of non-obstructive dystocia and uterine inertia (Gram *et al.*, 2014). Plasma oxytocin concentrations are significantly higher and more variable during the expulsive stage of labor compared to late gestation. Interestingly, no correlation has been found between circulating oxytocin levels and either litter size or individual fetal weight (Gram *et al.*, 2014).

Analytical monitoring is also essential during medical management. In cases of hypoglycemia, glucose should be administered intravenously, along with fluid therapy to correct electrolyte and acid-base imbalances (Bergström *et al.*, 2006). Although hypocalcemia is not commonly observed in uterine inertia, glucose and calcium supplementation may still improve uterine contractility (Bergström *et al.*, 2006). Interestingly, some studies have reported hyperglycemia at the time of dystocia, likely associated with elevated cortisol levels during labor (Bergström *et al.*, 2006).

The Ferguson reflex activated by fetuses during passage through the birth canal promotes the endogenous release of oxytocin, favoring uterine contractions (Bergström *et al.,* 2006). In human medicine, oxytocin has been shown to be able to mobilize intracellular calcium stores and facilitate the passage of extracellular calcium into myometrial cells. This mechanism would explain why the administration of calcium prior to oxytocin improves the outcome during dystocia in the bitch (Bergström *et al.,* 2006).

1.5 Lactation

Lactation in the bitch is regulated by two key pituitary hormones: prolactin and oxytocin, with no direct contribution from ovarian hormones (Trass, 2008). Prolactin promotes milk synthesis, while oxytocin is responsible for milk let-down via contraction of the myoepithelial cells in the mammary gland (Trass, 2008). A decrease in milk production may occur due to perioperative hypotension, inadequate pain management following cesarean section, suboptimal maternal nutrition, or insufficient water intake (Trass, 2008).

Figure 9. One-day-old neonate suckling maternal milk. Image taken by the Reproduction Service of the Veterinary Clinical Hospital (HCV).

Neonatal survival is influenced by two main factors: the quality of passive immune transfer and early postnatal weight gain (Chastant-Maillard *et al.*, 2016). An Adequate transfer of colostral immunoglobulins, particularly IgG, determines circulating antibody levels in the neonate. Weight loss exceeding 4% of birth weight during the first 48 hours is associated with increased mortality (Chastant-Maillard *et al.*, 2016).

Colostrum is the initial secretion from the mammary gland after parturition, although it can be produced antenatally in some cases (Chastant-Maillard *et al*., 2016). Mammary development during gestation is driven by estrogen and progesterone, while the initiation of lactogenesis is dependent on prolactin stimulation following the decline in circulating progesterone levels near parturition (Chastant-Maillard *et al*., 2016). Milk synthesis may begin as early as two weeks before whelping, but onset of lactation can be delayed by 2–3 days in some bitches (Chastant-Maillard, 2023).

The colostral phase typically lasts up to 48 hours postpartum, with IgG concentrations declining markedly around 24 hours after birth (Chastant-Maillard, 2023) (Figure 9). Beyond its immunological role, colostrum provides essential energy substrates required for organ development and cellular differentiation (Chastant-Maillard, 2023).

During peak lactation (2–4 weeks postpartum), milk production increases progressively—by approximately 1.3% and 5.9% daily, respectively (Chastant-Maillard, 2023). Litter size significantly affects milk output; bitches nursing large litters (>6 puppies) produce up to three times more milk compared to those with smaller litters (<4 pups) (Chastant-Maillard, 2023).

Unlike some other species, dogs have minimal transplacental transfer of immunoglobulins during gestation (5–10%) due to their endotheliochorial placental structure, which consists of four tissue layers separating maternal and fetal circulation (Pereira *et al*., 2023). This limitation underscores the vital role of colostrum in conferring passive immunity.

Colostrum is a key component in gastrointestinal protection. It contains immunoglobulin A (IgA), macrophages, neutrophils, lymphocytes, and various non-specific antimicrobial factors that support mucosal immunity (Pereira *et al*., 2023). Furthermore, colostrum modulates the development of the intestinal microbiota and promotes maturation of Peyer's patches and the intestinal epithelium (Pereira *et al*., 2023). In addition to immunological and nutritional components, colostrum is rich in endocrine factors such as cortisol, insulin, thyroxine, and growth hormone. These hormones contribute to the functional development of multiple neonatal organs, including the gastrointestinal tract, liver, pancreas, and thyroid gland (Pereira *et al*., 2023).

It is important to consider that the administration of pharmacological agents in the mother during or after parturation will cause its excretion through the milk, exposing the fetuses to these agents (Ferrari *et al.,* 2022). This consideration is relevant due to the limitation in the number of existing studies so far. Neonatal physiology is characterized by an immature liver and kidney system, so the metabolism of these drugs can be altered, increasing the risk of toxicity (Ferrari *et al.,* 2022).

Despite these concerns, the use of certain medications remains essential for ensuring maternal welfare, particularly in the management of postoperative pain, such as after cesarean section; or in the treatment of specific postpartum complications (Ferrari *et al.*, 2022). Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most used analgesics in these scenarios. Recent studies have quantified the concentration of selected NSAIDs in maternal milk and have demonstrated that their transfer is minimal, with levels considered safe for suckling neonates (Ferrari *et al.*, 2022).

1.6 Pathologies of the pregnant dog

Several pathological conditions can occur in pregnant bitches at term or during the postpartum period, and their early recognition is critical to safeguard both maternal and neonatal health. The most tipical disorders:

- Mastitis: Mastitis is an inflammatory and infectious condition of one or more mammary glands, which may lead to contamination of the milk with bacterial toxins (Wiebe and Howard, 2009; Uchanska *et al.*, 2022) (Figure 10). It represents a significant risk to neonates, specially during the first two weeks of life, and is frequently associated with metritis (Uchanska *et al.*, 2022). Affected neonates normally present: intense vocalization, poor weight gain, diarrhea, and abdominal distension due to gas accumulation (Uchanska *et al.*, 2022).

Figure 10. Queen with mastitis at 18 days postpartum and surgical resolution by unilateral radical mastectomy.

- **Eclampsia (Hypocalcemia):** Eclampsia is caused by low concentrations of ionized calcium in the extracellular space and is most common during the early postpartum period (Shukla, 2011; Gonzalez, 2018). This hypocalcemia alters the excitability of cell membranes, triggering spontaneous discharges in peripheral nerves, which in turn induce tonic or tonoclonic muscular contractions (Gonzalez, 2018). The increased calcium demand is associated with fetal skeletal mineralization and lactation. Contributing factors include inadequate calcium absorption, excessive supplementation during pregnancy (leading to parathyroid suppression), or poor-quality diets (e.g low blood levels of albumen) (Pathan *et al.*, 2011; Gonzalez, 2018).

- **Postpartum metritis**: Postpartum metritis is an infection of the endometrium and/or myometrium, typically occurring within 1 to 7 days after whelping (Gonzalez, 2018), affecting the capacity to nurse and care from puppies (Lection *et al.*, 2021). The condition arises due to the ascending migration of bacteria during delivery when the cervix is dilated, facilitating uterine contamination (Gonzalez, 2018). Prompt diagnosis and treatment are essential to prevent systemic illness and poor neonatal outcomes.

- **Agalactia**: Agalactia refers to inadequate milk production. It may be primary, rare and often due to congenital or physiological defects in the mammary–pituitary–ovarian axis; or secondary, resulting from insufficient galactopoiesis or milk let-down (Wiebe and Howard, 2009; Gonzalez, 2018). Mammary development typically begins around day 45 of gestation, with milk production and let-down starting at parturition or shortly thereafter (Gonzalez, 2018). Failure to produce or deliver milk can rapidly compromise neonatal survival.

- **Inappropriate behaviors of the mother**: Maternal behavior is modulated primarily by oxytocin, released from the posterior pituitary in response to neuroendocrine signals such as nipple stimulation and the Ferguson reflex (Gonzalez, 2018). It is critical to closely monitor the dam during the first 24–72 hours postpartum, especially after cesarean section, to detect behavioral issues such as neglect, aggression, or failure to stimulate the neonates' excretion reflexes—actions essential to neonatal viability (Gonzalez, 2018).

2. NEONATOLOGY

Neonatology is a specialized branch of veterinary medicine that focuses on the physiology, pathology, and clinical care of newborn animals (Pereira *et al.*, 2024). In canine and feline species, the neonatal period extends from birth until the onset of weaning. After this stage, the animal is considered pediatric until reaching sexual maturity, typically around six months of age, depending on breed and species (Boller *et al.*, 2025).

Childbirth involves a rapid and critical transition from the intrauterine to the extrauterine environment. This process is known as "neonatal adaptation" which includes complex physiological changes for neonatal survival (Veronesi and Fusi, 2022; Plavec *et al.*, 2022; Bolis *et al.*, 2017). It is important that there is a correct adaptation due to factors that hinder it such as: fetal immaturity, neonatal viability or due to the inefficient physiological response of the newborns to the external environment (Veronesi and Fusi, 2022; Brenda *et al.*, 2022).

Neonatal mortality may occur at different stages: in utero, during delivery, immediately postpartum, or within the first hours of life (Brenda *et al.*, 2021). Most neonatal deaths occur during parturition or within the first seven days after birth. The perinatal asphyxia, often associated with dystocia, is responsible for approximately 70% of these losses (Kusse *et al.*, 2018; Reyes-Sotelo *et al.*, 2021). Therefore, prepartum assessment of fetal maturity is important and should be based on the clinical history, hormonal markers, and ultrasonographic findings (Riva *et al.*, 2023).

In neonatal medicine, two key timeframes must be distinguished: the peripartum period and the neonatal period itself. Recent studies have emphasized the perinatal period, which includes the final stages of intrauterine life and the early postnatal phase (Veronesi and Fusi, 2022). This part is considered the most critical for neonatal survival due to the differences in physiological, anatomical, and biochemical neonatal conditions, occurring during this transition. Hormonal mechanisms control essential processes such as the initiation of respiration, cardiovascular adaptation, and the activation of energy metabolism (Reyes-Sotelo *et al.*, 2021).

2.1 Neonatal physiology

It is important to know the neonatal particularities in order to correctly interpret the parameters, which differ mostly from adults and vary depending on the week of life of the newborn (Pereira *et al.*, 2024).

2.1.1 Cardiovascular system

The cardiovascular system of canine and feline neonates is functionally immature at birth, requiring rapid physiological adaptations to ensure survival in the extrauterine environment (Veronesi and Fusi, 2022). This transition is referred to as the cardiorespiratory adaptation process, which encompasses critical respiratory and circulatory changes occurring immediately after birth (Boller *et al.*, 2025).

Neonates exhibit lower systemic blood pressure and reduced peripheral vascular resistance compared to adults, with mean arterial pressures ranging from 50 to 70 mmHg in both species (Pereira *et al.*, 2024). Systolic blood pressure in puppies ranges from 61–70 mmHg at birth and increases progressively to approximately 139 mmHg by the fourth week of life (Pereira *et al.*, 2024). In kittens, systolic pressure starts at around 50 mmHg and reaches 120 mmHg by six weeks of age (Pereira *et al.*, 2024). To maintain adequate tissue perfusion in this low-pressure, low-volume system, neonates rely on elevated heart rates (often exceeding 200 bpm), which gradually decline over the first four weeks of life (Da Cunha, 2024). Cardiac output is primarily heart rate–dependent, as stroke volume is limited by small ventricular chamber size and immature myocardial contractility (Da Cunha, 2015).

Before birth, fetal circulation is characterized by a parallel configuration of the right and left ventricles (Boller *et al.*, 2025). After delivery, the circulatory system undergoes a change, resulting in a serial circulation, where the right ventricle supplies the pulmonary system and the left ventricle supplies the systemic circuit (Boller *et al.*, 2025). This change is due to the onset of pulmonary ventilation, causing the closure of the ductus arteriosus and foramen ovale and increasing pulmonary blood flow (Boller *et al.*, 2025).

Bradycardia in neonates is most often a consequence of hypoxemia, not vagal stimulation (Da Cunha, 2015). In fact, baroreceptor reflexes are immature until approximately 12 weeks of age, which limits the capacity for reflex vasoconstriction (Da Cunha, 2015).

At birth, puppies born vaginally present with an initial heart rate of approximately 180 bpm, whereas those delivered via cesarean section may show lower initial rates, around 165 bpm (Boller *et al.*, 2025). However, by five minutes (vaginal delivery) or four hours (cesarean), heart rates typically normalize to 200–220 bpm (Boller *et al.*, 2025). Bradycardia is an adaptive response to hypoxia designed to preserve myocardial oxygen consumption. While atropine may increase heart rate, it does not improve oxygenation and may enhance ischemia under severe hypoxic conditions (Boller *et al.*, 2025). Therefore, the primary therapeutic approach should focus on correcting hypoxia through appropriate respiratory support.

Cardiac troponin I (cTnI) is a regulatory protein located in the contractile apparatus of cardiomyocytes and serves as a sensitive biomarker for myocardial injury (Pereira *et al.*, 2022). It modulates the interaction of actin, myosin, and tropomyosin in response to intracellular calcium. Elevated cTnI levels at birth have been associated with myocardial damage secondary to prolonged hypoxia, as observed in neonatal lambs and foals with sepsis or muscular dystrophy (Pereira *et al.*, 2022). In veterinary neonatology, the measurement of cTnI may be used in conjunction with blood gas analysis and Apgar scoring systems to assess the severity of perinatal asphyxia (Pereira *et al.*, 2022).

2.1.2 Respiratory system

Neonates have immature lungs with less expansive capacity in terms of chest walls, immature carotid receptors, and a higher metabolism that predisposes them to the appearance of hypoxia (Pereira *et al.*, 2024). At the time of birth, respiratory changes must occur, since in the intrauterine environment the newborn depends exclusively on the placenta, and gaseous exchange is carried out through it, transitioning at birth to breathing through the lungs (Boller *et al.*, 2025). The synthesis and secretion of pulmonary surfactant are essential for adequate respiratory adaptation during the immediate postnatal period (Silva *et al.*, 2015). This substance is primarily composed of 90–95% phospholipids, 5–10% proteins, and trace amounts of carbohydrates. The predominant phospholipids involved in surfactant function are lecithin (phosphatidylcholine) and sphingomyelin (Silva *et al.*, 2015).

Pulmonary development during gestation has been classified into four stages: pseudoglandular (between days 35–48 of gestation), canalicular (between days 49–56), saccular (between days 57–60), and the alveolar phase, which occurs early in the postnatal period (Benzato *et al.*, 2017). The canalicular phase is where pneumocytes begin to develop and mature to produce surfactant fluid. It is currently suggested that endocrine factors, including glucocorticoids, play an important role in the regulation of lung development and, therefore, in the transition to extrauterine life (Vannucchi *et al.*, 2012).

Despite the fact that pulmonary volume and ventilation are lower, the respiratory rate is higher than in adults. Feline neonates have a higher heart and respiratory rate compared to canine neonates (Pereira *et al.*, 2024). At the time of birth, inefficient breathing causes a cascade of physiological changes that allow adaptation to the extrauterine environment (Veronesi and Fusi, 2022). Contractions and pressure on the pelvic area result in an acidotic environment and fetal hypoxia (Vannucchi *et al*., 2012). This transition is usually reflected in different degrees of hypoxia, which are generally well tolerated by newborns (Veronesi and Fusi, 2022). However, some authors have reported that the absence of thoracic compression during passage through the birth canal may reduce the stimulation of the respiratory reflex, particularly in neonates delivered via cesarean section (Silva *et al*., 2015). This mechanical compression during vaginal delivery is believed to contribute to the expulsion of fetal lung fluid and to promote the onset of effective spontaneous respiration (Reid and Donelly, 2024). Additionally, the lack of this physiological stimulus may impair pulmonary fluid clearance, predisposing the neonate to respiratory distress (Silva *et al*., 2015). Consequently, puppies born through natural vaginal delivery generally exhibit higher vitality scores and better respiratory adaptation compared to those delivered surgically (Silva *et al*., 2015).

Different studies have observed that transient asphyxia occurs during childbirth, favoring the appearance of hypercapnia and transient acidosis (Reyes-Sotelo *et al*., 2021). However, if these conditions are prolonged, metabolic acidosis may develop in the newborn, which can depress respiratory function (Reyes-Sotelo *et al*., 2021). Prolonged or intermittent asphyxiation in utero or during labor may reduce the chance of adaptation to the extrauterine environment (Vannucchi *et al*., 2012). Studies indicate that neonates born via dystocic cesarean section require greater obstetric assistance and exhibit more pronounced depression at birth, demonstrating the effects of dystocia on neonatal viability (Vannucchi *et al*., 2012).

At the time of birth, fetuses may experience asphyxia due to inadequate oxygenation, characterized by hypercapnia and hypoxemia, which can be fatal and predispose to other neonatal pathologies (Veronesi and Fusi, 2022). It has been described that neonates experience transient acidosis at birth, which persists during the first 60 minutes of life; however, if this asphyxia is prolonged, respiratory acidosis may develop, leading to the accumulation of organic acids and resulting in metabolic acidosis, hypoglycemia, and bradycardia (Veronesi and Fusi, 2022).

In humans, respiratory distress syndrome has been studied and is attributed to insufficient production of surfactant fluid due to immaturity of type II pneumocytes (Banzato *et al*., 2017); this impairs alveolar maturation, which then continues during the postnatal stage. This syndrome is not well characterized in puppies, although recent studies have described a correlation between obstetric and respiratory conditions (Banzato *et al*., 2017).

At the ultrasound level, the appearance of fetal lungs has been described, with clear differentiation between abdominal and thoracic cavities observed on days 34–36 of gestation, along with increased pulmonary echogenicity (Banzato *et al*., 2017). In human medicine, lung and liver ultrasound have been used to assess fetal maturity and to predict the possible presence of respiratory distress syndrome (Banzato *et al*., 2017).

The most important and critical adaptive change for neonatal survival is respiratory (Abreu *et al*., 2024). In utero, fetal lungs are filled with fluid secreted by the pulmonary epithelium, which is essential for lung structural development and must be eliminated at the time of delivery (Azevedo *et al*., 2024). This process, known as lung clearance, is necessary to initiate breathing (Azevedo *et al*., 2024). Respiratory and cardiovascular changes that occur at birth are completed within the first day of neonatal life (Azevedo *et al*., 2024).

The workload and effort required to maintain tidal breathing are increased in neonates, and respiratory chemoreceptors are immature and less sensitive to increases in CO_2 and decreases in O_2 (Da Cunha, 2015). With each inspiration, only a portion of the alveolus inflates, highlighting the importance of adequate alveolar expansion. During this phase, the lungs contain amniotic fluid, resulting in a lower concentration of soluble oxygen (Vannucchi *et al*., 2012).

Neonates born via cesarean section have lower concentrations of lecithin/sphingomyelin, which are phospholipid components of the surfactant fluid (Vannucchi *et al*., 2012). However, the exact mechanisms of lung maturation during labor remain unknown (Vannucchi *et al*., 2012). A significant percentage of airway and pulmonary fluid is expelled during labor due to uterine contractions, postural changes, maternal abdominal contractions, and passage of the fetus through the birth canal (Manuel Boller *et al*., 2025).

Lung aeration is an essential process at the time of delivery, as it reduces pulmonary vascular resistance and initiates the circulatory transition (Boller *et al*., 2025). Administration of positive pressure ventilation is the most important step to ensure pulmonary aeration in newborns, especially those with apnea or reduced vigor (Boller *et al*., 2025). These neonates are at increased risk of severe bradycardia, apnea, or respiratory distress (Boller *et al*., 2025).

2.1.3 Hematopoietic system

Hematological results in neonates require special care in terms of interpretation due to the differences they present with adults (Münnich *et al*., 2014). In neonates, leukositosis is primarily caused by a white blood cell count of no more than 30 G/l (Münnich *et al.*, 2014).

2.1.4 Hypothalamic and Nervous system

Neurological development in neonatal dogs is characterized by the progressive emergence of essential reflexes and functional sensory responses critical for survival. Among the earliest reflexes to appear is the sucking reflex, which is vital for effective nursing and postnatal energy intake (Fitzgerald and Newquist, 2010).

Although the eyelids and ear canals remain anatomically closed during the first weeks of life, neonates are able of responding to auditory stimuli and may exhibit a blinking reflex in response to light projected through the closed eyelids (Fitzgerald and Newquist, 2010). These early responses denote partial sensory functionality despite the apparent immaturity of the visual and auditory systems (Fitzgerald and Newquist, 2010).

In neonatal puppies, the blood-brain barrier (BBB) has not yet reached full functional maturity. This developmental stage allows greater permeability to certain molecules, including lactic acid (Fitzgerald and Newquist, 2010). Under conditions such as fasting or hypoglycemia, lactate may serve as an alternative energy source for the neonatal brain (Fitzgerald and Newquist, 2010). While this increased permeability can be physiologically beneficial in some cases, it also presents clinical challenges. Immature BBB function makes the central nervous system more susceptible to the effects of drugs and other circulating agents. For this reason, particular caution is advised when administering medications during the early neonatal period, especially those with known central nervous system activity (Fitzgerald and Newquist, 2010).

2.1.5 Gastrointestinal and hepatic tract

In neonates, the cytochrome P-450 system is immature at birth and develops primarily during the postnatal stage (Da Cunha, 2015). Glucose levels are generally maintained within normal limits in healthy neonates; however, hypoglycemia may occur in stressful situations due to relatively low glycogen stores and limited gluconeogenic capacity (Da Cunha, 2015).

The gastrointestinal system of the newborn undergoes functional changes, considering that digestive processes are initially carried out via the placenta (Beretta *et al.*, 2025). The postnatal period is characterized by rapid bacterial colonization of the gastrointestinal tract (Beretta *et al.*, 2025). Initially, it was believed that the mammalian gastrointestinal tract was sterile during intrauterine life, and that microbial colonization began postnatally through contact with the mother's vaginal canal, skin, or milk (Beretta *et al*., 2025). However, recent molecular studies have identified bacteria in the placenta, uterus, and amniotic fluid of several mammalian species, suggesting the possibility of intrauterine microbial transmission (Beretta *et al*., 2025).

Liver enzymes in neonates are significantly elevated compared to adult animals, a finding likely associated with colostrum intake (Münnich *et al*., 2014). However, enzymatic activity is not considered a reliable diagnostic tool in neonates (Münnich *et al*., 2014). The development of the mycobiota is influenced by environmental factors, maternal health status, and the absence or insufficiency of colostrum intake (Nagendra Singh *et al*., 2025). Colostrum intake is critical for neonatal survival and health (Nagendra Singh *et al*., 2025).

A stable and diverse gut microbiota plays a critical role in neonatal development, particularly in promoting healthy weight gain and supporting the overall vitality of the litter (Nagendra Singh *et al*., 2025). When this microbial ecosystem is disrupted, puppies may become more vulnerable to various conditions, including Fading Puppy Syndrome (Nagendra Singh *et al*., 2025). Maternal health, mode of delivery (especially cesarean section), colostrum quality, perinatal antibiotic exposure, and prematurity have all been linked to alterations in gut flora composition. These disruptions may have long-term consequences, contributing to immune-mediated disorders such as asthma, allergies, and even metabolic issues like obesity (Tal *et al*., 2021).

In clinical settings, paying attention to neonatal feces can offer valuable clues about gastrointestinal function and general well-being. Direct observation can be challenging, as dams often consume the feces instinctively as part of their maternal behavior (Fitzgerald and Newquist, 2010). When feces are observed, the presence of diarrhea should prompt further evaluation, as it may indicate several underlying conditions, including dysbiosis, excessive milk intake, hyperosmolar feeding formulas, viral enteritis, or parasitic infections (Fitzgerald and Newquist, 2010).

2.1.6 Urinary system

In the canine species, nephrogenesis is not completed until the third week of life, and this period is characterized by renal function with reduced clearance rate, lower glomerular filtration rate, decreased renal plasma flow, reduced filtration fraction, diminished amino acid and phosphate reabsorption, and limited urine concentration capacity (Fávera, 2015).

Renal function in neonatal dogs is immature during the first weeks of life, which is reflected in the physical and biochemical characteristics of urine. Urine specific gravity tends to be lower compared to adult values, typically ranging between 1.006 and 1.017 during the first 8 weeks after birth (Fitzgerald and Newquist, 2010). In addition to its lower concentration capacity, neonatal urine often contains elevated levels of protein, glucose, and amino acids. These findings are attributed to the functional immaturity of the renal tubules, particularly the incomplete development of the proximal tubular reabsorption mechanisms (Fitzgerald and Newquist, 2010).

In neonates, phosphorus concentrations are typically elevated, whereas creatinine and urea levels are lower compared to adult values (Fávera, 2015). In neonatal patients, blood urea nitrogen (BUN) and total plasma protein levels are also reduced in comparison to adult animals (Münnich *et al.*, 2014). Glycosuria may be observed during the first three weeks of life (Münnich *et al.*, 2014).

At birth, the kidneys are immature both structurally and functionally, resulting in decreased glomerular filtrate production, renal plasma flow, and filtration fraction (Molina *et al.*, 2020). Renal glucose reabsorption does not normalize until approximately three weeks of age in young puppies (Molina *et al.*, 2020). Renal immaturity constitutes a significant physiological limitation in neonatal puppies, increasing their susceptibility to fluid and electrolyte imbalances, particularly dehydration (Kusse *et al.*, 2018). In addition to reduced renal concentrating ability, the neonate's body composition further predisposes them to fluid loss. At birth, approximately 82% of a puppy's body weight is comprised of water, with a notably higher proportion of this water residing in the extracellular compartment compared to adult animals (Kusse *et al.*, 2018).

2.2 Apgar score

The APGAR score system is a standardized method used to assess neonatal vitality, neurological depression, and viability at the time of birth. Its primary objective is to determine the health status of the newborn and to identify those critical patients who require increased veterinary support (Pereira *et al.*, 2024). The use of the Apgar score to detect the level of asphyxia at birth requires further investigation, as some authors suggest it can serve as a predictor of neurological disorders and mortality, while others point out limitations in its application (Oliva *et al.*, 2018).

This method was originally developed by Dr. Virginia Apgar after three years of observing newborn infants in human medicine (Pereira *et al.*, 2024). In 2009, a modified version of the Apgar scoring system was adapted for use in canine neonates (Plavec *et al.*, 2022). This technique involves evaluating mucous membrane color, heart rate, respiratory effort, muscle tone, and reflex irritability, assigning a score of 0, 1, or 2 depending on the observed values (Table 1) (Uchanska *et al.*, 2022) (Figure 11). Interpretation of the final score is species-specific and must consider breed-related size and physiological differences (Pereira *et al.*, 2024).

However, the Apgar score is often used in conjunction with other parameters, such as blood gas analysis, to provide additional information on the newborn's condition (Plavec *et al.*, 2022).

Based on the final score, neonates can be classified into three groups: a score of 10–7 points indicates unstressed neonates with good viability; a score of 6–4 points corresponds to moderately stressed but viable neonates; and a score of 3–0 points identifies critically ill neonates (Uchanska *et al.,* 2022). This classification system has been adapted, especially in brachycephalic breeds, where certain parameters have been modified to reflect the specific characteristics observed at birth (Uchanska *et al.,* 2022). Over time, other scoring systems have been developed to assess neonatal viability, focusing on the evaluation of the basic reflexes that a newborn should exhibit during the postnatal period (Table 2) (Uchanska *et al.,* 2022).

Table 1. Apgar score assessment

Paramether	***Values***	***Interpretation***
Heart rate	>220 bpm 220 – 180 bpm < 180 bpm	2 points 1 point 0 point
Respiratory effort	>15 rr 6 - 15 rr < 6 rr	2 points 1 point 0 point
Reflex irritability	Vigorous Grimace alone No response	2 points 1 point 0 point
Motility	Active motion Some reflextions Flacced movements	2 points 1 point 0 point
Mucus color	Dark Pink Pale Cyanotic	2 points 1 point 0 point

Figure 11. Comparison of mucosal color in two neonates born via cesarean section at the Reproduction Service of the Veterinary Clinical Hospital (HCV). The neonate on the left exhibits a pink mucosal tone, consistent with a score of 2 in the Apgar assessment. The neonate on the right shows cyanotic mucosa, corresponding to a score of 0.

Table 2. Apgar score interpretation.

Paramether	***Values***	***Interpretation***
Reflex Suckling	Strong (> 5 suckles/min)	2 points
	Weak (> 3 suckles/min)	1 point
	Absent	0 point
Rooting reflex	Inmmediate	2 points
	Slow	1 point
	Absent	0 point

To evaluate the sucking reflex, a plastic nipple or a gloved finger can be gently inserted into the neonate’s mouth to assess the strength and presence of the sucking response. For the turning (righting) reflex, the neonate is placed in dorsal recumbency on a soft surface, and its ability to reposition itself into sternal recumbency is observed. Neonates exhibiting low scores in these reflexes are often associated with a higher degree of hypoxia (Uchanska *et al*., 2022).

2.3 Neonatal resuscitation

Resuscitation comprises interventions performed at the time of delivery and during the immediate postnatal period to support respiration and circulatory function in neonates (Trass, 2008; Boller *et al*., 2025). Neonates born via natural parturition generally do not require human intervention, as maternal care is usually sufficient to facilitate neonatal adaptation (Boller *et al*., 2025). However, neonates delivered by cesarean section often require resuscitation measures, particularly those born via emergency procedures (Davidson, 2015). These neonates tend to exhibit lower vitality at birth, and maternal instability during the postoperative period frequently limits the dam’s ability to provide adequate neonatal care (Boller *et al*., 2025).

Resuscitation must be implemented without compromising temperature regulation. During this period, neonates should be dried with clean towels to prevent heat loss and provide tactile stimulation (Reid and Donelly, 2024; Boller *et al.*, 2025). After cesarean section, it is essential to clean and dry the neonates, removing fluids from nostrils and oral cavity to facilitate an effective respiration (Trass, 2008; Boller *et al.*, 2025).

In cases where the neonate is non-vigorous, fails to vocalize, or presents with abundant oropharyngeal secretions, airway suctioning is indicated to remove meconium or fluid obstructing the airways (Davidson, 2015; Boller *et al.*, 2025). This can be achieved using a mucus aspirator (e.g., DeLee catheter), suction bulb, syringe, or similar devices (Boller *et al.*, 2025). The practice of "swinging" or "balancing" the neonate (previously recommended) should be strictly avoided, as it has been associated with serious risks such as intracranial hemorrhage, traumatic injury, and aspiration of gastric contents (Reid and Donelly, 2024; Boller *et al.*, 2025). In neonates born via cesarean section from dams that received alpha-2 adrenergic agonists or benzodiazepines, it may be necessary to administer pharmacologic antagonists such as naloxone, atipamezole, or flumazenil to reverse neonatal depression caused by transplacental transfer of these agents (Boller *et al.*, 2025).

At birth, neonates experience a physiological state of hypoxemia due to intermittent uterine contractions, which may be higher in cases of dystocia or cesarean delivery (Pereira *et al.*, 2019). Moreover, hypoxemia may be further intensified during cesarean sections due to the depressing effects of anesthetic agents that suppress the neonatal respiratory rate (Vilar *et al.*, 2018; Pereira Helena *et al.*, 2019).

In utero, the placenta facilitates gas exchange by allowing deoxygenated blood to return via the umbilical arteries and oxygenated blood to reach the fetus through the umbilical vein (Pereira *et al.*, 2019). Early clamping of the umbilical cord can reduce central venous return, increase peripheral vascular resistance, and ultimately decrease cardiac preload (Pereira *et al.*, 2019). For this reason, in human medicine, delayed cord clamping has been recommended to preserve placental circulation during the first minutes of life, providing cardiovascular and hematologic benefits to the newborn (Pereira *et al.*, 2019).

2.4 Importance of weight

In many species, birth weight has been identified as one of the most significant risk factors associated with neonatal mortality (Mugnier *et al.*, 2019). In canine neonates, low birth weight has been linked to a twelve-fold increase in mortality compared to neonates of normal weight (Mugnier *et al.*, 2019; Schrank *et al.*, 2020).

Neonates weighing less than 25% of the breed-specific average birth weight are at significantly higher risk of mortality (Uchanska *et al*., 2022). Low birth weight is often associated with intrauterine growth restriction, the etiology of which remains largely unknown (Mugnier *et al*., 2023). It is recommended to perform weight monitoring every 12 hours, with an expected daily weight gain of approximately 10% (Boller *et al*., 2025) (Figure 12).

Figure 12. Neonatal weighing immediately after cesarean section at the HCV.

Low birth weight is correlated with an increased risk of hypoglycemia and hypothermia, which may contribute to higher mortality rates (Groppetti *et al*., 2017; Mugnier *et al*., 2019), as these neonates often present with greater fetal immaturity and reduced capacity for postnatal adaptation (Uchanska *et al*., 2022). These individuals have decreased energy reserves, lower vigor, and consequently greater difficulty in suckling, which impairs proper colostrum intake and predisposes them to hypoglycemia and dehydration (Uchanska *et al*., 2022). Furthermore, these neonates are at increased risk for developing neurocognitive deficits, cerebral palsy, intracranial hemorrhage, sepsis, apnea, and other complications (Uchanska *et al*., 2022).

Birth weight is influenced by multiple factors, including maternal, fetal, and placental components (Uchanska *et al*., 2022) (Figure 13 and 14). Maternal size, body weight, body condition score, age, and breed all contribute, in addition to environmental factors and litter size (Uchanska *et al*., 2022). Since birth weight reflects intrauterine nutritional status, placental function is considered a key determinant of neonatal condition (Uchanska *et al*., 2022). Although the precise causes of low birth weight remain unclear, they are commonly associated with intrauterine growth restriction. However, the exact role of the placenta in this process has yet to be fully elucidated (Gloria *et al*., 2024).

Figure 13. Litter of Chihuahuas delivered by cesarean section showing size variation at birth.

Figure 14. Litter of Bull Terriers delivered by cesarean section showing size variation at birth.

3. NEONATAL PATHOLOGIES: DIAGNOSIS AND TREATMENT

Neonatal care encompasses multiple components, including thorough evaluation of the newborn, the dam, and the entire litter (Pereira *et al.*, 2024). Neonatology is the branch of veterinary medicine responsible for the care, clinical evaluation, and physiological understanding of neonates, as well as the diagnosis and management of neonatal conditions (Beretta *et al.*, 2025).

The neonatal period included the first 3 to 4 weeks of life and represents a particularly vulnerable period for puppies, causing significant challenges for both breeders and veterinarians due to the elevated risk of morbidity and mortality (Pereira, 2021). During this critical phase, mortality rates can reach up to 20%, being the most common causes: hypoxia, infections, congenital malformations, hypothermia, and hypoglycemia (Fuchs *et al.*, 2023).

3.1 Hypoglycemia

Transient hypoglycemia is a common condition in neonates, primarily due to insufficient glycogen storage at the time of birth, particularly in low-birth-weight individuals (Plavec *et al.*, 2022). This may be attributed to immaturity in several organ systems (Fuchs *et al.*, 2023), especially the liver, which presents limited glycogen reserves during the first 24 hours of life (Uchanska *et al.*, 2022). It has been reported that full hepatic maturity in dogs is not reached until approximately 4–5 months of age (Fuchs *et al.*, 2023).

Canine neonates are considered altricial, meaning they are born in a highly dependent and underdeveloped state, making them particularly vulnerable to conditions such as hypoglycemia, and thus requiring constant maternal care (Fuchs *et al.*, 2023). Maternal malnutrition or inadequate nutrition during gestation has been associated with reduced neonatal glycogen stores (Uchanska *et al.*, 2022). In contrast, neonates born to healthy, well-nourished dams are better equipped to maintain euglycemia for several hours post-ingestion, highlighting the direct relationship between maternal nutrition during pregnancy and the neonate's capacity for glycemic regulation (Fuchs *et al.*, 2023).

During the first 8–12 hours postpartum, colostrum intake is critical for maintaining blood glucose levels. Failure to ingest colostrum can lead to a rapid depletion of glycogen stores, potentially resulting in severe hypoglycemia, characterized by clinical signs such as nervousness, vocalization, irritability, lethargy, mental depression, seizures, tremors, and, ultimately, death (Uchanska *et al.*, 2022). Glucose is essential for neonatal brain function, as it is the sole energy substrate utilized by the central nervous system (Fuchs *et al.*, 2023). Neonates have high energy demands, estimated at 20–26 kcal/100 g body weight, due to their elevated surface area-to-body mass ratio, thermoregulatory immaturity, and increased metabolic requirements (Fuchs *et al.*, 2023).

Blood glucose concentration has been proposed as a prognostic indicator of neonatal mortality. Concentrations below 92 mg/dL within the first 24 hours of life have been associated with increased mortality risk during the first three weeks of life (Fuchs *et al.*, 2023). However, these thresholds remain controversial, because other studies have proposed differences values such as 76 mg/dL or even 65 mg/dL as indicative of normoglycemia (Fuchs *et al.*, 2023).

Before birth, glucose is transported across the placenta, facilitated by glucose transporter proteins such as GLUT-1 (Fuchs *et al.*, 2023). Low birth weight neonates, defined as <75 g in small breeds, <200 g in medium breeds, and <400 g in large breeds, as well as oversized neonates, have greater hormonal and metabolic immaturity, with reduce hepatic glycogen reserves, and therefore a worse regulation of glycemia (Fuchs *et al.*, 2023).

Hypoglycemia is often correlated with other conditions such as hypothermia. Both contribute to neonatal depression, apathy, and reduced suckling reflex, causing a decrease in milk intake and, consequently, intensifying hypoglycemia (Fuchs *et al.*, 2023). Congenital malformations such as cleft palate, cleft lip, or macroglossia can also make intake difficult, leading to inadequate colostrum intake and subsequent hypoglycemia (Fuchs *et al.*, 2023).

A significant decrease in Apgar scores and neonatal reflexes, as well as the occurrence of seizures or comatose states, have been associated with severe hypoglycemia, particularly when blood glucose concentrations fall below 40 mg/dL (Fuchs *et al.*, 2023).

Regarding treatment, various therapeutic strategies have been described. These include the administration of 10% or 12.5% glucose solutions at a volume of 0.2–0.5 mL per 100 g of body weight, delivered intravenously or intraosseously (Fuchs *et al.*, 2023). In patients with persistent hypoglycemia, continuous glucose infusion at 5% can be used, adjusted to a total volume of 6–18 mL per 100 g over 24 hours (Fuchs *et al.*, 2023). Oral glucose administration is generally the least effective method in neonates with severe hypoglycemia. If used, it can be delivered via syringe, but in neonates lacking a suckling reflex, orogastric tube administration is required, an option reserved exclusively for normothermic neonates (Fuchs *et al.*, 2023).

Recent studies suggest that oral supplementation with high-calorie nutritional products (including vitamins, carbohydrates, fatty acids, and amino acids) may serve as a glucose alternative, offering metabolic and nutritional support. It is recommended that these supplements contain approximately 43% carbohydrates and 35% fatty acids (Fuchs *et al.*, 2023).

Finally, urinary glucose loss has been associated with catabolism of muscle and adipose tissue, resulting in reduced availability of fatty acids for energy production and thus contributing to the development of hypoglycemia (Molina *et al.*, 2020).

3.2 Hypothermia

Immediately after birth, neonates are exposed to an environment that is significantly colder than intrauterine conditions (Uchanska *et al.*, 2022). This abrupt thermal transition can result in hypothermia, which, in turn, may lead to bradycardia, reduced respiratory rate, loss of the suckling reflex, and subsequent complications such as dehydration and gastrointestinal disturbances (Uchanska *et al.*, 2022).

In newborn dogs and cats, thermoregulatory mechanisms do not fully develop until at least 7 days of age, so they cannot properly maintain body temperature. This physiological immaturity predisposes them to rapid heat loss and increases the risk of hypothermia (Uchanska *et al.*, 2022). Although specific data in dogs and cats remain limited, studies in human neonates have demonstrated a strong correlation between hypothermia and increased morbidity, as well as mortality (Boller *et al.*, 2025).

A transient drop in body temperature immediately after birth is considered a physiological and normal protective response. However, if hypothermia persists, it becomes potentially harmful (Veronesi *et al.*, 2021). In cesarean delivered puppies, initial body temperatures of approximately 34ºC are common, decreasing to around 32ºC within the first 20 minutes postpartum. In contrast, neonates born via vaginal delivery typically exhibit an initial temperature of approximately 36.2ºC, which drops to about 34ºC within the same period (Boller *et al.*, 2025).

Currently, specific reference values have been suggested to identify hypothermia risk. A rectal temperature below 33.9 ± 1.2ºC in neonates under 7 days of age has been significantly associated with increased mortality (Vassalo *et al.*, 2015). In neonates older than 1 hour, a normal body temperature of approximately 35ºC has been reported (Veronesi *et al.*, 2021). Smaller neonates are at higher risk of hypothermia, likely due to their greater surface area-to-body mass ratio, which accelerates heat loss (Veronesi *et al.*, 2021). In general, a rectal temperature below 35.5ºC during the neonatal period is considered a clinical threshold for hypothermia.

Prolonged hypothermia may lead to a cascade of clinical sign, including central nervous system depression, abscense suckling reflex, bradycardia, hypoglycemia, necrotizing enterocolitis, aspiration pneumonia, and poor immune responses (Veronesi *et al.*, 2021).

3.3 Hypoxia

Neonates are highly susceptible to hypoxemia due to their elevated metabolic oxygen demand, pulmonary immaturity, and the incomplete development of peripheral chemoreceptors such as the carotid bodies (Pereira *et al.*, 2024). At birth, a transient state of hypoxia is physiologically expected and typically results in mild hypercapnia and acidosis. However, in complicated deliveries (such as those involving distocia) this hypoxic state may be exacerbated, leading to severe respiratory compromise that hinders postnatal adaptation and often necessitates immediate veterinary intervention (Pereira *et al.*, 2024).

Perinatal asphyxia has been implicated in up to 60% of neonatal deaths in canines (Veronesi, 2016; Uchanska *et al.*, 2022). Puppies born in anterior presentations, especially when delivered prematurely or via prolonged labor, appear to have a higher risk of developing respiratory and metabolic acidosis (Uchanska *et al.*, 2022).

Several factors have been identified as predisposing agents for neonatal hypoxia, including the administration of drugs such as oxytocin, anesthetic agents, and those used during surgical procedures like cesarean section (Vilar *et al.*, 2018; Uchanska *et al.*, 2022). These interventions may depress the neonate's respiratory center or interfere with placental oxygen exchange prior to delivery. Neonates affected by severe hypoxia often present with bradycardia, which reduces tissue perfusion, worsening oxygen delivery and promoting ischemia. (Trass, 2008). This cascade of events can progress to multiorgan dysfunction and ultimately, death (Uchanska *et al.*, 2022).

3.4 Dehydration

Renal immaturity at birth is a common physiological characteristic in neonates and is often associated with inefficient excretory function (Uchanska *et al.*, 2022). This condition may also be exacerbated by inadequate milk intake during the early postnatal period (Uchanska *et al.*, 2022). At birth, the kidneys are not fully developed, and nephrogenesis continues postnatally, requiring approximately 2–3 weeks to complete before renal function becomes fully established (Uchanska *et al.*, 2022). As a result, neonates exhibit a heightened susceptibility to dehydration.

When fluid therapy is indicated, the recommended maintenance dosage is 80–120 mL/kg/day for canine neonates and 60–80 mL/kg/day for feline neonates (Boller *et al.*, 2025).

3.5 Aspiration pneumonia

Meconium aspiration can lead to a cascade of pathological events due to its interference with effective gas exchange. Obstruction of the airways, chemical injury to pulmonary tissues, surfactant inactivation, pulmonary inflammation, and systemic hypotension are among the most commonly reported consequences (Van Ierland & Beaufort, 2009). Airways that are partially or completely obstructed become hypoxic, triggering an inflammatory response and contributing to further pulmonary damage (Mokra *et al.*, 2013). Chronic hypoxia may induce an increase in pulmonary vascular smooth muscle tone, resulting in persistent pulmonary hypertension. This condition can eventually lead to severe respiratory and circulatory failure if not promptly recognized and managed (Van Ierland & Beaufort, 2009).

In human neonatology, studies have demonstrated that newborns who die within the first three days of life often present with aspirated amniotic fluid, asphyxia, and signs consistent with intrauterine or perinatal infection (Bernstein & Wang, 1961). In veterinary neonates, studies are limited, but similar pathophysiological mechanisms are suspected, specially in cases of dystocia or prolonged labor.

Aspiration pneumonia can also result from improper feeding techniques, particularly when orogastric or nasogastric tube feeding is performed by inexperienced personnel. In neonatal puppies, the absence of a developed gag reflex, typically not present until around 10 days of age, greatly increases the risk of tracheal misplacement during tube insertion (Fitzgerald & Newquist, 2010). Tube feeding may also result in gastric overdistension, which predisposes neonates to regurgitation and subsequent aspiration pneumonia. Additionally, feeding neonates with hypothermia is strongly discouraged, as a low body temperature can impair gastrointestinal motility and increase the likelihood of regurgitation and aspiration (Fitzgerald & Newquist, 2010).

3.6 Fading Puppy Syndrome

This syndrome is common in neonatology, causing significant neonatal loss. It frequently occurs in the first 2 weeks of life and is characterized by a sudden death of infants that appear healthy (Nagendra Singh *et al.*, 2025). It can affect up to 30% of litters up to 3 weeks of age, although most deaths have been recorded in the first 7 days (Smadar Tal *et al.*, 2021). Breeders commonly use this term to refer to neonates that appear clinically healthy at birth but die suddenly within the first weeks of life (Kusse et al., 2018).

This syndrome can appear in an individual or in a litter, presenting a complex and multifactorial etiology (Nagendra Singh *et al.*, 2025). It can be caused by infections, environmental, genetic, or maternal conditions (Nagendra Singh *et al.*, 2025). The etiology of sudden neonatal death syndrome (SNDS) in puppies remains largely unknown and is considered multifactorial. Potential contributing factors include hypothermia, inadequate colostrum intake, birth trauma, congenital malformations, low birth weight, and bacterial or viral infections (Kusse *et al.*, 2018).

Histological examinations often do not specifically detail lesions or consistent evidence of infectious etiology. In a study by Blunden (2012), affected neonates did not show pathognomonic signs of viral or bacterial involvement. Observed changes included lymphocyte depletion in the thymus and an increased number of macrophages. These alterations, however, were interpreted as nonspecific and possibly related to neonatal stress rather than infection (Blunden, 2012).

Brown adipose tissue (BAT), which plays a critical thermogenic role in neonates, was found to be markedly reduced in affected puppies. Histologically, this was accompanied by loss of cytoplasmic vacuolization and atrophy of BAT lobules (Blunden, 2012). This may predispose neonates to hypothermia, although is not always the direct cause of death. In puppies that had eat prior to death, BAT vacuolization appeared preserved, suggesting a possible protective effect of early energy intake (Blunden, 2012).

Pulmonary changes have also been reported. Affected neonates showed lower concentrations of lecithin, a major component of pulmonary surfactant, potentially compromising alveolar stability and gas exchange (Blunden *et al.*, 1987; Blunden, 2012). These findings may reflect a maturational delay of the pulmonary system or impaired surfactant synthesis.

3.7 Malformations

Congenital defects can result in structural or functional abnormalities affecting multiple organ systems, which may compromise neonatal viability and, in severe cases, lead to neonatal death (Pereira *et al.*, 2019). The reported incidence of congenital malformations in newborns ranges from 1% to 3%; however, due to the limited number of current studies, this figure may be underestimated (Pereira *et al.*, 2019). A breed predisposition has been identified, with brachycephalic breeds showing a higher incidence of certain malformations (Uchanska *et al.*, 2022).

Congenital anomalies may arise from genetic mutations or from teratogenic influences during gestation (Pereira *et al.*, 2019). Teratogenic agents can include maternal medication, nutritional deficiencies (such as inadequate intake of proteins or vitamins A and D), exposure to radiation, toxins, chemical substances, or infectious diseases (Pereira *et al.*, 2019).

Among the most frequently reported malformations is cleft palate, which may have either a genetic or environmental origin. This condition is notably more prevalent in brachycephalic breeds (Pereira *et al.*, 2019). Neonates affected by cleft palate often experience difficulty suckling, which commonly leads to aspiration pneumonia and mortality within the first four weeks of life (Pereira *et al.*, 2019).

The prevalence of specific congenital anomalies has been reported as follows: cleft palate (2.8%), hydrocephalus (1.5%), anasarca (0.7%), cleft lip (0.6%), polydactyly (0.5%), segmental intestinal aplasia (0.4%), and anal atresia (0.4%), among others (Pereira *et al.*, 2019; Uchanska *et al.*, 2022).

Figure 15. Neonate with complete Pentalogy of Cantrell born by dystocic cesarean section at the Veterinary Clinical Hospital.

Figure 16. Cleft lip in an American Bully neonate born by cesarean section at the Veterinary Clinical Hospital.

Figure 17. Chihuahua neonate with acrania and exophthalmos born by cesarean section at the

3.7 Septicemia

Among the primary causes of neonatal mortality, both environmental and infectious factors play a critical role. Infections, particularly those of bacterial origin, represent the second most frequent cause of death in neonatal litter losses (Pereira, 2021; Lima Gorza *et al*., 2020). In canine neonates, the degree of infectious involvement can vary widely, ranging from localized infections to systemic dissemination, which may progress to sepsis and culminate in septic shock (Pereira, 2021).

Given that neonates are immunologically immature and are exposed to a high concentration of environmental pathogens immediately after birth, the establishment of an effective immune response is vital for survival (Pereira *et al*., 2023). The transition from a sterile intrauterine environment to an external environment rich in microorganisms represents a significant immunological challenge.

Neonatal infections are a significant cause of morbidity and mortality in puppies, and may result in abortions, stillbirths, or early neonatal death. Although in utero transmission is possible, most infectious mortality occurs during the neonatal period (Kusse *et al*., 2018). Multiple routes of infection have been described, including oral, umbilical, transplacental, inhalational, and through ingestion of contaminated vaginal secretions during parturition. Environmental contamination and poor hygiene are also critical risk factors (Kusse *et al*., 2018).

Common clinical signs of bacterial infection in neonates include cold extremities, decreased urination frequency, loss of the sucking reflex, and persistent vocalization or crying (Lima Gorza *et al*., 2020). Infectious processes can affect neonatal growth and development, often leading to secondary complications like: respiratory distress, gastrointestinal dysfunction, and systemic infections like bacteremia or sepsis (Nagendra Singh *et al*., 2025). These complications are more frequently observed in neonates with inadequate passive immune transfer, inadequate maternal health , or poor environmental hygiene.

Post-mortem findings may be limited or nonspecific. In many cases, no gross lesions are observed, highlighting the critical role of histopathology for having a diagnosis (Lima Gorza *et al*., 2020). Confirmation of the infectious agent may require microbiological culture or molecular diagnostics such as PCR of tissue samples (Lima Gorza *et al*., 2020).

4. NEONATAL BIOMARKERS

Taking into account the different factors that can affect neonatal viability, knowing different parameters that allow us to make a clinical prognosis at the time of birth is essential in the veterinary clinic (Valeria *et al.*, 2018).

4.1 Glucose

Hypoglycemia is a common and clinically significant condition in neonates, primarily due to hepatic immaturity, limited energy reserves, and underdevelopment of the endocrine and enzymatic systems responsible for glucose homeostasis (Veronesi and Fusi, 2022). Several factors may predispose neonates to hypoglycemia, including maternal metabolic disorders, hypothermia, neonatal sepsis, hepatic dysfunction, enzymatic deficiencies affecting glycogen metabolism, low birth weight, and infectious diseases (Veronesi and Fusi, 2022).

Glucose homeostasis in the newborn is maintained primarily through regular intake of colostrum or milk, which provides essential substrates for energy production. Without adequate intake, glucose depletion can progress rapidly, causing neurological signs such as tremors, seizures, stupor, and, ultimately, death (Veronesi and Fusi, 2022).

In a study that evaluated glucose concentrations using capillary blood samples obtained from the pad, lower glucose levels were observed in neonates considered at higher risk of mortality (Plavec *et al.*, 2022). Furthermore, assessment of amniotic fluid glucose concentration revealed significantly lower values in neonates who did not survive compared to those who did. However, further studies are needed to validate the usefulness of amniotic glucose as a prognostic biomarker. (Plavec *et al.*, 2022). Interestingly, some studies have reported hyperglycemia in neonates born via cesarean section or in cases of fetal dystocia, likely due to stress related catecholamine release during delivery (Lucio *et al.*, 2021; Plavec *et al.*, 2022).

For the management of hypoglycemia, dextrose supplementation is recommended. Intravenous administration is recommended, using boluses of 0.25 g/kg (equivalent to 0.5 mL/100 g of 5% dextrose) every 5 minutes until clinical stabilization is get. Oral or, less preferably, intraperitoneal administration may be considered if intravenous access is not available (Boller *et al.*, 2025).

Reference glucose ranges have been proposed for different stages of neonatal development: 76–155 mg/dL during days 1–3 of life; 101–161 mg/dL during days 8–10; and 121–158 mg/dL at 4–5 weeks of age (Veronesi and Fusi, 2022).

Hypoglycemia is generally defined as a blood glucose concentration below 50 mg/dL (Veronesi and Fusi, 2022). However, no definitive cut-off value has yet been established to reliably predict survival outcomes in neonates based solely on glycemia (Veronesi and Fusi, 2022).

4.2 Lactate

Lactic acid is produced during anaerobic metabolism, which is activated when tissue oxygen demand exceeds supply or when pulmonary function is compromised (Veronesi and Fusi, 2022). Elevated lactate levels are frequently associated with abnormalities in cardiovascular parameters such as heart rate and blood pressure (Plavec *et al.*, 2022). In neonates born from dystocic deliveries, high lactate concentrations have been linked to increased mortality rates and a higher incidence of neurological disorders (Oliva *et al.*, 2018; Mila *et al.*, 2017). An increased lactate concentration was positively correlated with the severity of perinatal asphyxia (Oliva *et al.*, 2018). Several authors have concluded that the measurement of venous lactate, when used in conjunction with other clinical parameters such as the Apgar score, can significantly enhance the identification of neonates requiring immediate medical intervention (Di Mauro and Schoeffler, 2016). Although maternal lactate levels may increase during labor, only a small fraction of maternal lactate can cross the placental barrier. Consequently, the majority of lactate detected in neonatal blood is of fetal origin (Kuttan *et al.*, 2016).

An increase in blood lactate indicates metabolic acidosis and has been recognized as a valuable tool for assessing neonatal distress. Its elevation has shown an important correlation with low Apgar scores (Veronesi and Fusi, 2022). Recent studies have demonstrated that lactate concentrations in umbilical venous blood provide information about neonatal viability within the first 48 hours postpartum (Plavec *et al.*, 2022; Mila *et al.*, 2017). Although a single measurement may not be a reliable prognostic indicator, serial lactate determinations can offer valuable insight into the neonate's clinical evolution and response to treatment (Castagnetti *et al.*, 2017).

Umbilical venous lactate has been proposed as a prognostic marker for neonatal survival in canine neonatology, with a suggested cut-off value of 5 mmol/L (Plavec *et al.*, 2022; Groppeti *et al.*, 2010; Veronesi and Fusi, 2022). In other species such as equines, lactate measurement at birth is routinely used to evaluate neonatal morbidity, with serial monitoring every 14–24 hours serving as a reliable indicator of disease progression and prognosis (Castagnetti *et al.*, 2017).

Additional studies have reported that values above 8 mmol/L may be associated with increased risk of death or neurological complications, suggesting the appearance of previous hypoxemia (Veronesi and Fusi, 2022). However, various thresholds have been proposed. Concentrations of 13 mmol/L have been observed in puppies that died within the first 24 hours of life, while values as high as 15.3 and 23.3 mmol/L have been proposed in neonates that did not survive (Veronesi and Fusi, 2022). Despite its clinical relevance, the number of studies evaluating lactate concentrations in newborn puppies remains limited. In a study conducted by Di Mauro and Schoeffler (2016), it was reported that neonatal puppies with venous lactate concentrations of 12.2 ± 6.7 mmol/L had a significantly higher risk of mortality compared to those with lower concentrations, averaging 6.55 ± 3.3 mmol/L (Di Mauro and Schoeffler, 2016).

The mode of delivery also appears to influence neonatal metabolic parameters, especially in blood lactate levels, which are recognized as indicators of perinatal hypoxia and anaerobic metabolism (Groppetti *et al.*, 2010). Neonates delivered via elective cesarean section have been observed to present lower blood lactate concentrations when they are compared to those born through vaginal delivery (Groppetti *et al.*, 2010). This may reflect the reduced exposure to physiological stress situations such as uterine contractions and prolonged labor, which contribute to transient hypoxic conditions during natural parturition.

The most critical metabolic profiles have been reported in neonates born via emergency cesarean section (Groppetti *et al.*, 2010). These individuals had significantly higher lactate levels, suggesting more marked hypoxic ischemic damage. The causes may include prolonged dystocia, repeated ineffective uterine contractions, impaired uteroplacental perfusion, anesthetic-induced maternal hypotension, or maternal hypovolemia (Groppetti *et al.*, 2010).

4.3 Cortisol

Recent studies have demonstrated that elevated cortisol concentrations in amniotic fluid may serve as a biomarker for identifying neonates that require enhanced clinical monitoring during the first 24 hours of life (Plavec *et al.*, 2022). An inadequate maternal environment can trigger stress responses, leading to activation of the hypothalamic–pituitary–adrenal (HPA) axis and subsequent elevation of maternal cortisol levels (Groppetti *et al.*, 2021). Given the ability of cortisol to cross the placental barrier, prolonged maternal hypercortisolemia may have detrimental effects on the fetus, including intrauterine growth restriction, low birth weight, and impaired neurological, behavioral, and immune development in the offspring (Groppetti *et al.*, 2021).

Basal cortisol concentrations have been found to be significantly higher in neonates born from dystocic parturitions compared to those born via eutocic deliveries or elective cesarean section (Plavec *et al*., 2022).

In human neonatology, amniotic fluid cortisol levels have been linked to fetal lung maturity, reinforcing its role in perinatal transition (Bolis *et al*., 2017). Furthermore, slightly increased cortisol levels have been observed in neonates who died within the first 24 hours after birth, suggesting that elevated perinatal cortisol may reflect an acute stress response associated with a poor prognosis. (Bolis *et al*., 2017).

The potential utility of cortisol as a prognostic biomarker has been supported by its correlation with Apgar scores at birth. A statistically significant association between elevated cortisol and lower Apgar scores suggests that simultaneous evaluation of both parameters could allow to identify neonates with higher risk, allowing them to benefit from critical veterinary care and close monitoring (Bolis *et al*., 2017). However, some studies have reported no significant differences in cortisol levels based on delivery type, litter size, birth weight, or neonatal sex (Bolis *et al*., 2017). These findings highlight the need for further investigation to clarify the role of cortisol as a useful predictor of neonatal viability.

4.4 pH and blood gases

Blood pH is another important parameter used to evaluate the degree of acidosis in neonatal patients. In recent years, it has gained attention as a prognostic marker to identify neonates at greater risk of mortality (Veronesi and Fusi, 2022). In veterinary neonatology, umbilical cord blood pH values ranging between 7.1 and 7.2 have been described at birth, values that would be considered acidotic in adult animals (Veronesi and Fusi, 2022).

Currently, there is limited information available regarding normal blood gas parameters in neonatal animals. However, as in human medicine, specific biochemical markers obtained from umbilical cord blood or amniotic fluid, such as lactate, cortisol, and glucosa, are being investigated to help differentiate between healthy neonates and those requiring medical intervention (Plavec *et al*., 2022).

Several authors have evaluated the utility of blood gas analysis from umbilical cord samples. Parameters such as blood glucose, lactate concentration, partial pressure of oxygen (PaO_2), partial pressure of carbon dioxide ($PaCO_2$), and blood pH have shown potential as early indicators of perinatal distress and neonatal viability.

These measurements may allow to identify newborns at risk and facilitate their clinical management (Plavec *et al*., 2022).

4.5 Measurements in amniotic fluid

In human medicine, several biochemical markers have been established to assess fetal maturation, particularly in relation to pulmonary development. Among the most used indicators are the lecithin/sphingomyelin (L/S) ratio and the concentration of surfactant-associated protein A (SP-A), both being useful for determining lung maturity (Riva *et al*., 2023).

Surfactant protein A plays a fundamental role in the synthesis, secretion, and homeostasis of pulmonary surfactant. It is a substance essential for reducing alveolar surface tension and enabling efficient gas exchange in the newborn. In humans, surfactant A concentrations increase significantly during the last third of gestation, reflecting the maturation of the fetal lungs and the preparation of the newborn for extrauterine life (Riva *et al*., 2023).

REFERENCES

Abreu, R. A. de, Almeida, L. L. de, Rosa Filho, R. R. da, Angrimani, D. de S. R., Brito, M. M., Flores, R. B., & Vannucchi, C. I. (2024). Canine pulmonary clearance during feto-neonatal transition according to the type of delivery. *Theriogenology*, *224*, 156–162. https://doi.org/10.1016/j.theriogenology.2024.05.022

Arlt, S. P. (2020). The bitch around parturition. *Theriogenology*, *150*, 452–457. https://doi.org/10.1016/j.theriogenology.2020.02.046

Arlt, S. P., Ottka, C., Lohi, H., Hinderer, J., Lüdeke, J., Müller, E., Weber, C., Kohn, B., & Bartel, A. (2023). Metabolomics during canine pregnancy and lactation. *PLoS ONE*, *18*(5 May). https://doi.org/10.1371/journal.pone.0284570

Baan, M., Taverne, M. A. M., De Gier, J., Kooistra, H. S., Kindahl, H., Dieleman, S. J., & Okkens, A. C. (2008). Hormonal changes in spontaneous and aglepristone-induced parturition in dogs. *Theriogenology*, *69*(4), 399-407. https://doi.org/10.1016/j.theriogenology.2007.10.008

Batista, M., Moreno, C., Vilar, J., Golding, M., Brito, C., Santana, M., & Alamo, D. (2014). Neonatal viability evaluation by Apgar score in puppies delivered by cesarean section in two brachycephalic breeds (English and French bulldog). *Animal reproduction science*, *146*(3-4), 218-226. https://doi.org/10.1016/j.anireprosci.2014.03.003

Bernstein, J., & Wang, J. (1961). *The Pathology of Neonatal Pneumonia*. http://archpedi.jamanetwork.com/

Blunden, A. S., Hill, M., Brown, B. D., & Morley, C. J. (1987). *Lung surfactant composition in puppies dying of fading puppy complex*.

Blunden, T. (2012). Fading puppies - Reality or myth? *In Practice*, *34*(6), 314–321. https://doi.org/10.1136/inp.e4125

Bolis, B., Prandi, A., Rota, A., Faustini, M., & Veronesi, M. C. (2017). Cortisol fetal fluid concentrations in term pregnancy of small-sized purebred dogs and its preliminary relation to first 24 hours survival of newborns. *Theriogenology*, *88*, 264–269. https://doi.org/10.1016/j.theriogenology.2016.09.037

Bolis, B., Scarpa, P., Rota, A., Vitiello, T., & Veronesi, M. C. (2018). Association of amniotic uric acid, glucose, lactate and creatinine concentrations and lactate/creatinine ratio with newborn survival in small-sized dogs - Preliminary results. *Acta Veterinaria Hungarica*, *66*(1), 125–136. https://doi.org/10.1556/004.2018.012

Castagnetti, C., Cunto, M., Bini, C., Mariella, J., Capolongo, S., & Zambelli, D. (2017). Time-dependent changes and prognostic value of lactatemia during the first 24 h of life in brachycephalic newborn dogs. *Theriogenology*, *94*, 100–104. https://doi.org/10.1016/j.theriogenology.2017.01.040

Conze, T., Büttner, K., & Wehrend, A. (2022). Parameters in Canines After Cesarean Sections. *Frontiers in Veterinary Science*, *9*. https://doi.org/10.3389/fvets.2022.886691

Cornelius, A. J., Moxon, R., Russenberger, J., Havlena, B., & Cheong, S. H. (2019). Identifying risk factors for canine dystocia and stillbirths. *Theriogenology*, *128*, 201–206. https://doi.org/10.1016/j.theriogenology.2019.02.009

Cortes, D., Ellerbrock, R., & Ferrer, M. (2025). Is the pre-partum serum progesterone decline associated with litter size in dogs? A retrospective study. *Theriogenology*, *248*. https://doi.org/10.1016/j.theriogenology.2025.117593

da Cunha, A. F. (2014). Neonatal, pediatric, and geriatric concerns. In *Canine and Feline Anesthesia and Co-Existing Disease* (pp. 310–319). Wiley Blackwell. https://doi.org/10.1002/9781118834305.ch17

Davidson, A. (2015). Neonatal resuscitation: techniques to improve outcome.

de Araújo, R. A., Rocha-Júnior, J. D., Bonavina, J. T., Bianchini, M. de O., Beretta, S., Jorge Coutinho Armani, D., Estevam, M. V., Toniollo, G. H., Mogollón García, H. D., Oba, E., & Apparício, M. (2025). Impact of Delivery Method on Serum Cortisol Levels and Neonatal Outcomes in Canine Cesarean Sections. *Animals*, *15*(12). https://doi.org/10.3390/ani15121739

de Cramer, K. G. M., & Joonè, C. J. (2025). Validation of a point-of-care serum progesterone cut-off for caesarean section timing in the bitch and its effect on neonatal outcomes. *Theriogenology*, *248*. https://doi.org/10.1016/j.theriogenology.2025.117630

de Cramer, K. G. M., & Nöthling, J. O. (2018). Is the biparietal diameter of fetuses in late gestation too variable to predict readiness for cesarean section in dogs? *Theriogenology*, *113*,50–55. https://doi.org/10.1016/j.theriogenology.2018.02.005

di Cesare, F., Brioschi, F. A., Ravasio, G., Groppetti, D., Amari, M., Giussani, E., Draghi, S., Sala, G., Pecile, A., & Cagnardi, P. (2025). Dexmedetomidine and alfaxalone intravenous co-administration for the induction of general anaesthesia in bitches undergoing C-section: impact on newborn viability and pharmacokinetics. *Theriogenology*, *247*. https://doi.org/10.1016/j.theriogenology.2025.117578

di Mauro, F. M., & Schoeffler, G. L. (2016). Point of Care Measurement of Lactate. In *Topics in Companion Animal Medicine* (Vol. 31, Issue 1, pp. 35–43). W.B. Saunders. https://doi.org/10.1053/j.tcam.2016.05.004

Evci, E. C., Aslan, S., Schäfer-Somi, S., Ergene, O., Sayıner, S., Darbaz, I., Seyrek-İntaş, K., & Wehrend, A. (2023). Monitoring of canine pregnancy by considering Anti-Mullerian hormone, C-reactive protein, progesterone and complete blood count in pregnant and non-pregnant dogs. *Theriogenology*, *195*, 69–76. https://doi.org/10.1016/j.theriogenology.2022.10.020

Farias, S. F. S. T., Barbosa, H. T. S., Pinheiro, B. Q., Xavier Júnior, F. A. F., Morais, G. B. de, Evangelista, J. S. A. M., & Silva, L. D. M. da. (2023). Macro and microscopic characteristics of the placenta and its relationship with the weight and the Apgar score of canine neonates. *Theriogenology*, *202*, 21–27. https://doi.org/10.1016/j.theriogenology.2023.02.018

Fernandes, M. P., Xavier, G. M., Baptista, M. F. B., Loiola, M. V. G., & Filho, A. de L. R. (2025). Use of ultrasonography for fetal circulation assessment in canine pregnancy: A review. In *Revista Brasileira de Saude e Producao Animal* (Vol. 26). Universidade Federal da Bahia. https://doi.org/10.1590/20240032

Ferrari, D., Lundgren, S., Holmberg, J., Edner, A., Ekstrand, C., Nyman, G., Bondesson, U., & Hagman, R. (2022). Concentration of carprofen in the milk of lactating bitches after cesarean section and during inflammatory conditions. *Theriogenology*, *181*, 59–68. https://doi.org/10.1016/j.theriogenology.2022.01.016

Fitzgerald, K. T., & Newquist, K. L. (2010). Husbandry of the neonate. *Small Animal Pediatrics*, 44.

Fuchs, K. da M., Pereira, K. H. N. P., Xavier, G. M., Mendonça, J. C., Barreto, R. O., Silva, R. C., de Souza, F. F., & Lourenço, M. L. G. (2024). Neonatal hypoglycemia in dogs—pathophysiology, risk factors, diagnosis and treatment. In *Frontiers in Veterinary Science* (Vol. 11). Frontiers Media SA. https://doi.org/10.3389/fvets.2024.1345933

Fusi, J., Carluccio, A., Peric, T., Faustini, M., Prandi, A., & Veronesi, M. C. (2021). Effect of delivery by emergency or elective cesarean section on nitric oxide metabolites and cortisol amniotic concentrations in at term normal newborn dogs: Preliminary results. *Animals*, *11*(3), 1–10. https://doi.org/10.3390/ani11030713

Fusi, J., & Veronesi, M. C. (2022). Canine parturition: what is known about the hormonal setting? *Domestic Animal Endocrinology*, *78*. https://doi.org/10.1016/j.domaniend.2021.106687

Gil, E. M. U., Garcia, D. A. A., Giannico, A. T., & Froes, T. R. (2014). Canine fetal heart rate: Do accelerations or decelerations predict the parturition day in bitches? *Theriogenology*, *82*(7), 933–941. https://doi.org/10.1016/j.theriogenology.2014.04.025

Gram, A., Boos, A., & Kowalewski, M. P. (2014). Uterine and placental expression of canine oxytocin receptor during pregnancy and normal and induced parturition. *Reproduction in Domestic Animals*, *49*, 41-49.

Groppetti, D., Meazzi, S., Filipe, J. F. S., Colombani, C., Panseri, S., Zanzani, S. A., Palestrini, C., Cannas, S., Giordano, A., & Pecile, A. (2021). Maternal and neonatal canine cortisol measurement in multiple matrices during the perinatal period: A pilot study. *PLoS ONE*, *16*(7 July). https://doi.org/10.1371/journal.pone.0254842

Groppetti, D., Pecile, A., del Carro, A. P., Copley, K., Minero, M., & Cremonesi, F. (2010). Evaluation of newborn canine viability by means of umbilical vein lactate measurement, apgar score and uterine tocodynamometry. *Theriogenology*, *74*(7), 1187–1196. https://doi.org/10.1016/j.theriogenology.2010.05.020

Groppetti, D., Pecile, A., Palestrini, C., Marelli, S. P., & Boracchi, P. (2017). A national census of birthweight in purebred dogs in Italy. *Animals*, *7*(6). https://doi.org/10.3390/ani7060043

Hinderer, J., Lüdeke, J., Riege, L., Haimerl, P., Bartel, A., Kohn, B., Weber, C., Müller, E., & Arlt, S. P. (2021). Progesterone concentrations during canine pregnancy. *Animals*, *11*(12). https://doi.org/10.3390/ani11123369

Holst, B. S., Gustavsson, M. H., Johannisson, A., Hillström, A., Strage, E., Olsson, U., Axnér, E., & Lilliehöök, I. (2019). Inflammatory changes during canine pregnancy. *Theriogenology*, *125*, 285–292. https://doi.org/10.1016/j.theriogenology.2018.11.016

Johnson, C. A. (2008). Pregnancy management in the bitch. *Theriogenology*, *70*(9), 1412–1417. https://doi.org/10.1016/j.theriogenology.2008.09.009

Kowalewski, M. P. (2023). Advances in understanding canine pregnancy: Endocrine and morpho-functional regulation. In *Reproduction in Domestic Animals* (Vol. 58, Issue S2, pp. 163–175). John Wiley and Sons Inc. https://doi.org/10.1111/rda.14443

Kredatusova, G., Hajurka, J., Szakallova, I., Valencakova, A., & Vojtek, B. (2011). Physiological events during parturition and possibilities for improving puppy survival: a review. In *Veterinarni Medicina* (Vol. 56, Issue 12).

Kusse, G., Birhanu, M., & Ayele, T. (2018). *Review on Dog Neonatal Mortality* (Vol. 65). Online. www.iiste.org

Kuttan, K. v., Joseph, M., Simon, S., Ghosh, K. N. A., & Rajan, A. (2016). Effect of intrapartum fetal stress associated with obstetrical interventions on viability and survivability of canine neonates. *Veterinary World*, *9*(12), 1485–1488. https://doi.org/10.14202/vetworld.2016.1485-1488

Lection, J., Cornelius, A. J., Moxon, R., Russenberger, J., de Amorim, M. D., & Cheong, S. H. (2021). Incidence and risk factors for canine mastitis and metritis in two guide dog populations. *Animal Reproduction Science*, *231*, 106802. https://doi.org/10.1016/j.anireprosci.2021.106802

Lima Gorza, L., Henrique Bastos dos Santos, G., Martinelli Casagrande, V., Luiz de Souza, K., Alves da Cruz Azevedo, L., Cunha Flecher, M., Camargo Granadeiro Farias, P., Domingues de Souza, T., & em Patologia Animal, M. (2020). Onfalite associada a pneumonia embólica em neonatos caninos Omphalitis associated with embolic pneumonia in canine neonates. In *Rev. Bras. Reprod. Anim., v* (Vol. 44, Issue 2).

Long, S. T., Hien, N. T. T., Hang, P. T., Hoai, N. T., & Bach, P. X. (2022). Canine Dystocia: The Risk Factors and Treatment Methods in Dogs of Hanoi, Vietnam. *World's Veterinary Journal*, *12*(3), 290–295. https://doi.org/10.54203/scil.2022.wvj36

Lopate, C. (2008). Estimation of gestational age and assessment of canine fetal maturation using radiology and ultrasonography: A review. *Theriogenology*, *70*(3), 397–402. https://doi.org/10.1016/j.theriogenology.2008.05.034

Lopate, C. (2014). Reproductive physiology of canine pregnancy and parturition and conditions of the periparturient period. In *Management of Pregnant and Neonatal Dogs, Cats, and Exotic Pets* (pp. 25–41). wiley. https://doi.org/10.1002/9781118997215.ch3

Lúcio, C. F., Silva, L. C. G. D., & Vannucchi, C. I. (2021). Perinatal cortisol and blood glucose concentrations in bitches and neonatal puppies: effects of mode of whelping. *Domestic Animal Endocrinology*, *74*, 106483. https://doi.org/10.1016/j.domaniend.2020.106483

Mila, H., Grellet, A., Delebarre, M., Mariani, C., Feugier, A., & Chastant-Maillard, S. (2017). Monitoring of the newborn dog and prediction of neonatal mortality. *Preventive Veterinary Medicine*, *143*, 11–20. https://doi.org/10.1016/j.prevetmed.2017.05.005

Milani, C., Rota, A., Olsson, U., Paganotto, A., & Holst, B. S. (2021). Serum concentration of mineralocorticoids, glucocorticoids, and sex steroids in peripartum bitches. *Domestic Animal Endocrinology*, *74*, 106558. https://doi.org/10.1016/j.domaniend.2020.106558

Miranda, S., Carolino, N., Vilhena, H., Payan-Carreira, R., & Pereira, R. M. L. N. (2018). Early embryo development, number, quality, and location and the relationship with plasma progesterone in dogs. *Animal Reproduction Science*, *198*, 238–245. https://doi.org/10.1016/j.anireprosci.2018.10.001

Mokra, D., Mokry, J., & Tonhajzerova, I. (2013). Anti-inflammatory treatment of meconium aspiration syndrome: Benefits and risks. *Respiratory Physiology and Neurobiology*, *187*(1), 52–57. https://doi.org/10.1016/j.resp.2013.02.025

Mugnier, A., Chastant, S., Saegerman, C., Gaillard, V., Grellet, A., & Mila, H. (2021). Management of low birth weight in canine and feline species: Breeder profiling. *Animals*, *11*(10). https://doi.org/10.3390/ani11102953

Mugnier, A., Gaillard, V., & Chastant, S. (2023). Relative Impact of Birth Weight and Early Growth on Neonatal Mortality in Puppies. *Animals*, *13*(12). https://doi.org/10.3390/ani13121928

Mugnier, A., Mila, H., Guiraud, F., Brévaux, J., Lecarpentier, M., Martinez, C., Mariani, C., Adib-Lesaux, A., Chastant-Maillard, S., Saegerman, C., & Grellet, A. (2019). Birth weight as a risk factor for neonatal mortality: Breed-specific approach to identify at-risk puppies. *Preventive Veterinary Medicine*, *171*. https://doi.org/10.1016/j.prevetmed.2019.104746

Münnich, A., & Küchenmeister, U. (2014). Causes, diagnosis and therapy of common diseases in neonatal puppies in the first days of life: Cornerstones of practical approach. *Reproduction in Domestic Animals*, *49*(SUPPL.2), 64–74. https://doi.org/10.1111/rda.12329

Navarro-Altuna, V., Purwin, S., & Ranninger, E. (2025). The influence of anaesthesia drugs on puppy vitality after caesarean section: a systematic review. In *Veterinary Anaesthesia and Analgesia* (Vol. 52, Issue 2, pp. 129–144). Elsevier B.V. https://doi.org/10.1016/j.vaa.2024.11.007

Nobre Pacifico Pereira, K. H., Cruz dos Santos Correia, L. E., Ritir Oliveira, E. L., Bernardo, R. B., Nagib Jorge, M. L., Mezzena Gobato, M. L., Ferreira de Souza, F., Rocha, N. S., Chiacchio, S. B., & Gomes Lourenço, M. L. (2019). Incidence of congenital malformations and impact on the mortality of neonatal canines. *Theriogenology*, *140*, 52–57. https://doi.org/10.1016/j.theriogenology.2019.07.027

Nobre Pacifico Pereira, K. H., Fuchs, K. da M., Hibaru, V. Y., Cruz dos Santos Correia, L. E., Ferreira, J. C. P., Ferreira de Souza, F., Machado, L. H. de A., Chiacchio, S. B., & Gomes Lourenço, M. L. (2022). Neonatal sepsis in dogs: Incidence, clinical aspects and mortality. *Theriogenology*, *177*, 103–115. https://doi.org/10.1016/j.theriogenology.2021.10.015

Nobre Pacífico Pereira, K. H., Hibaru, V. Y., Fuchs, K. da M., Cruz dos Santos Correia, L. E., Lopes, M. D., Ferreira, J. C. P., Ferreira de Souza, F., Machado, L. H. de A., Chiacchio, S. B., & Gomes Lourenço, M. L. (2022). Use of cardiac troponin I (cTnI) levels to diagnose severe hypoxia and myocardial injury induced by perinatal asphyxia in neonatal dogs. *Theriogenology*, *180*, 146–153. https://doi.org/10.1016/j.theriogenology.2021.12.027

Oliva, V. N. L. S., Queiroz, M. C. V. G., Albuquerque, V. B., Carreira, J. T., Souza, T. F. B., Ferreira, G. T. N. M., Abimussi, C. J. X., & Vides, J. P. (2018). Vitality evaluation methods for newborn puppies after cesarean section performed under general inhalation anesthesia. *Pesquisa Veterinaria Brasileira*, *38*(6), 1172–1177. https://doi.org/10.1590/1678-5150-PVB-5350

Onclin, K., Murphy, B., & Verstegen, J. P. (2002). Comparisons of estradiol, LH and FSH patterns in pregnant and nonpregnant beagle bitches. *Theriogenology*, *57*(8), 1957-1972. https://doi.org/10.1016/S0093-691X(02)00644-1

Pathan, M. M., Siddiquee, G. M., Latif, A., Das, H., Khan, M. J., & Shukla, M. K. (2011). Eclampsia in the dog: An overview. *Veterinary World*, *4*(1), 45-47. 10.5455/vetworld.2011.45-47

Pereira, K. H. N. P., Fuchs, K. da M., Corrêa, J. V., Chiacchio, S. B., & Lourenço, M. L. G. (2022). Neonatology: Topics on Puppies and Kittens Neonatal Management to Improve Neonatal Outcome. In *Animals* (Vol. 12, Issue 23). MDPI. https://doi.org/10.3390/ani12233426

Pereira, K. H. N. P., Fuchs, K. da M., Mendonça, J. C., Xavier, G. M., Câmara, D. R., Cruz, R. K. S., & Lourenço, M. L. G. (2024). Neonatal Clinical Assessment of the Puppy and Kitten: How to Identify Newborns at Risk? In *Animals* (Vol. 14, Issue 23). Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/ani14233417

Plavec, T., Knific, T., Slapšak, A., Raspor, S., Lukanc, B., & Pipan, M. Z. (2022). Canine Neonatal Assessment by Vitality Score, Amniotic Fluid, Urine, and Umbilical Cord Blood Analysis of Glucose, Lactate, and Cortisol: Possible Influence of Parturition Type? *Animals*, *12*(10). https://doi.org/10.3390/ani12101247

Premanandan, C., & Runcan, E. (2019). Canine placentation: normal gross and histologic structure, and confounding features of evaluation. *Clinical Theriogenology*, *11*(4), 623-625.

Reid, A., & Donnelly, E. (2024). Improving early neonatal survival in puppies and kittens: resuscitation and beyond. *In Practice*, *46*(3), 108–117. https://doi.org/10.1002/inpr.415

Reyes-Sotelo, B., Mota-Rojas, D., Mora-Medina, P., Ogi, A., Mariti, C., Olmos-Hernández, A., Martínez-Burnes, J., Hernández-ávalos, I., Sánchez-Millán, J., & Gazzano, A. (2021). Blood biomarker profile alterations in newborn canines: Effect of the mother's weight. *Animals*, *11*(8). https://doi.org/10.3390/ani11082307

Reyes-Sotelo, B., Ogi, A., Mora-Medina, P., Mariti, C., Olmos-Hernández, A., Hernández-ávalos, I., Domínguez-Oliva, A., Rosas, M. E., Verduzco-Mendoza, A., & Gazzano, A. (2022). Early Blood Analysis and Gas Exchange Monitoring in the

Canine Neonate: Effect of Dam's Size and Birth Order. *Animals*, *12*(12). https://doi.org/10.3390/ani12121508

Riva, F., Filipe, J., Pavlovic, R., Luciano, A. M., Dall'Ara, P., Arioli, F., Pecile, A., & Groppetti, D. (2023). Canine amniotic fluid at birth: From a discarded sample to a potential diagnostic of neonatal maturity. *Animal Reproduction Science*, *248*. https://doi.org/10.1016/j.anireprosci.2022.107184

Runcan, E. E., & Coutinho da Silva, M. A. (2018). Whelping and Dystocia: Maximizing Success of Medical Management. In *Topics in Companion Animal Medicine* (Vol. 33, Issue 1, pp. 12–16). W.B. Saunders. https://doi.org/10.1053/j.tcam.2018.03.003

Sarli, G., Castagnetti, C., Bianco, C., Ballotta, G., Tura, G., Caporaletti, M., Cunto, M., Avallone, G., Benazzi, C., Ostanello, F., & Zambelli, D. (2021). Canine placenta histological findings and microvascular density: The histological basis of a negative neonatal outcome? *Animals*, *11*(5). https://doi.org/10.3390/ani11051418

Schrank, M., Mollo, A., Contiero, B., & Romagnoli, S. (2020). Bodyweight at birth and growth rate during the neonatal period in three canine breeds. *Animals*, *10*(1). https://doi.org/10.3390/ani10010008

Schrank, M., Pereira, M., & Mollo, A. (2025). Diagnostic imaging in the pregnant bitch: risks, advantages, and disadvantages. In *Frontiers in Veterinary Science* (Vol. 12). Frontiers Media SA. https://doi.org/10.3389/fvets.2025.1588847

Shukla, M. (2011). Eclampsia in the Dog: An Overview. 10.5455/vetworld.2011.45-47

Siena, G., & Milani, C. (2021). Usefulness of maternal and fetal parameters for the prediction of parturition date in dogs. In *Animals* (Vol. 11, Issue 3, pp. 1–17). MDPI AG. https://doi.org/10.3390/ani11030878

Siena, G., Romagnoli, S., Drigo, M., Contiero, B., Di Nardo, F., & Milani, C. (2022). Ultrasonographic changes in fetal gastrointestinal motility during the last ten days before parturition in dogs. *Frontiers in Veterinary Science*, *9*, 1000975.

Silva, L. G. arcia, Portari, G. V. annucchi, Lúcio, C. F. átima, Rodrigues, J. A. guiar, Veiga, G. L. ima, & Vannucchi, C. I. nfantosi. (2015). The influence of the obstetrical condition on canine neonatal pulmonary functional competence. *Journal of Veterinary Emergency and Critical Care (San Antonio, Tex. : 2001)*, *25*(6), 725–730. https://doi.org/10.1111/vec.12368

Tal, S., Tikhonov, E., Aroch, I., Hefetz, L., Turjeman, S., Koren, O., & Kuzi, S. (2021). Developmental intestinal microbiome alterations in canine fading puppy syndrome: a prospective observational study. *Npj Biofilms and Microbiomes*, *7*(1). https://doi.org/10.1038/s41522-021-00222-7

Tesi, M., Miragliotta, V., Scala, L., Aronica, E., Lazzarini, G., Fanelli, D., Rota, A., & Abramo, F. (2021). Gross and histological findings in the canine placenta and amnion at term: What's normal, abnormal or pathological? *Reproduction in Domestic Animals*, *56*(5), 691–702. https://doi.org/10.1111/rda.13905

Uchańska, O., Ochota, M., Eberhardt, M., & Niżański, W. (2022). Dead or Alive? A Review of Perinatal Factors That Determine Canine Neonatal Viability. In *Animals* (Vol. 12, Issue 11). MDPI. https://doi.org/10.3390/ani12111402

van Ierland, Y., & de Beaufort, A. J. (2009). Why does meconium cause meconium aspiration syndrome? Current concepts of MAS pathophysiology. In *Early Human Development* (Vol. 85, Issue 10, pp. 617–620). https://doi.org/10.1016/j.earlhumdev.2009.09.009

Vassalo, F. G., Simões, C. R. B., Sudano, M. J., Prestes, N. C., Lopes, M. D., Chiacchio, S. B., & Lourenço, M. L. G. (2015). Topics in the Routine Assessment of Newborn Puppy Viability. *Topics in Companion Animal Medicine*, *30*(1), 16–21. https://doi.org/10.1053/j.tcam.2015.02.003

Veronesi, M. C. (2016). Assessment of canine neonatal viability—The Apgar score. *Reproduction in Domestic Animals*, *51*, 46-50. https://doi.org/10.1111/rda.12787

Veronesi, M. C., Bolis, B., Faustini, M., Rota, A., & Mollo, A. (2018). Biochemical composition of fetal fluids in at term, normal developed, healthy, viable dogs and preliminary data from pathologic littermates. *Theriogenology*, *108*, 277–283. https://doi.org/10.1016/j.theriogenology.2017.12.029

Veronesi, M. C., & Fusi, J. (2021). Neonatal severity indicators in dogs. *Revista Brasileira de Reprodução Animal*, *45*(4), 525–532. https://doi.org/10.21451/1809-3000.rbra2021.071

Veronesi, M. C., & Fusi, J. (2023). Biochemical factors affecting newborn survival in dogs and cats. *Theriogenology*, *197*, 150–158. https://doi.org/10.1016/j.theriogenology.2022.11.040

Vilar, J. M., Batista, M., Pérez, R., Zagorskaia, A., Jouanisson, E., Díaz-Bertrana, L., & Rosales, S. (2018). Comparison of 3 anesthetic protocols for the elective cesarean-section in the dog: Effects on the bitch and the newborn puppies. *Animal Reproduction Science*, *190*, 53–62. https://doi.org/10.1016/j.anireprosci.2018.01.007

Wiebe, V. J., & Howard, J. P. (2009). Pharmacologic advances in canine and feline reproduction. *Topics in companion animal medicine*, *24*(2), 71-99. https://doi.org/10.1053/j.tcam.2008.12.004

5. RESUMEN

CAPÍTULO 1. LA MADRE Y LA GESTACIÓN

La fisiología reproductiva de la perra presenta diferencias marcadas respecto a otros mamíferos domésticos. Las perras son monoestrales, no estacionales y poliovulatorias (Miranda et al., 2018). La gestación dura aproximadamente 65 ± 1 días desde el pico preovulatorio de la hormona luteinizante (LH), el cual se designa como el día 0 de gestación (Lopate, 2012).

Durante el período alrededor del parto ocurren importantes cambios fisiológicos y comportamentales como: fluctuaciones endocrinas, inicio de la lactación, involución uterina y aparición del comportamiento maternal (Arlt, 2020). Estos cambios son fundamentales, ya que los cachorros nacen con una inmadurez fisiológica importante y dependen durante las primeras semanas de los cuidados maternos. Por ello, el estado de salud y bienestar general de la madre son factores determinantes en la viabilidad neonatal y la supervivencia en las primeras semanas (Arlt, 2020).

Dado que muchos trastornos neonatales tienen su origen en alteraciones maternas, se debe realizar una evaluación clínica de la madre. Esta debe incluir el diagnóstico de alteraciones nutricionales, trastornos de lactación como agalactia o hipogalactia, mastitis, comportamiento maternal anormal o cambios patológicos en secreciones vaginales o uterinas (Pereira et al., 2024).

1.1 Fisiología de la gestación

La fase lútea en la perra, tanto en perras gestantes como no gestantes, dura aproximadamente dos meses, diferenciándose al final de esta etapa con el descenso en los niveles plasmáticos de progesterona, los que presentan un descenso más rápido y brusco en perras gestantes (Verstegen-Onclin et al., 2008). Aunque se desconoce mucho sobre los mecanismos que desencadenan el parto, se considera que la maduración del eje hipotálamo-hipófisis-adrenal fetal juega un papel central, con participación del cortisol fetal y materno como iniciadores clave (Arlt, 2020; Johnson, 2008).

Este eje endocrino activa una cascada hormonal que incluye estrógenos, oxitocina y relaxina, los cuales estimulan la producción de prostaglandinas, especialmente prostaglandina F2α, que induce la luteólisis y una caída brusca de la progesterona (Fusi et al., 2022). La relaxina, producida por la placenta, promueve la relajación de los ligamentos pélvicos y la maduración cervical, además de estimular de forma indirecta la secreción de prolactina (Verstegen-Onclin et al., 2008; Lopate, 2012).

La prolactina, hormona hipofisaria, se convierte en el principal factor luteotrópico a partir del día 25 de la gestación, junto con la LH (Fusi et al., 2022). Su concentración aumenta progresivamente, alcanzando su pico antes del parto. Además, se ha observado un incremento significativo de cortisol antes del parto, reflejo del estrés materno y fetal, más que un desencadenante directo (Fusi et al., 2022).

1.1.1 Progesterona

La progesterona es la hormona clave para el mantenimiento de la gestación en la perra (Lopate, 2012; Kowalewski, 2023). A diferencia de otras especies, la placenta canina no contribuye de forma significativa a su síntesis, siendo el cuerpo lúteo la única fuente encargada de su liberación durante toda la gestación (Hinderer, 2021).

La medición sérica de progesterona es una herramienta clínica valiosa para determinar la ovulación y predecir el parto. Niveles por debajo de 2 ng/mL se observan entre 24 y 48 horas antes del inicio del parto (Fusi et al., 2022; Siena y Chiara, 2021). Esta caída está inducida por el pico preparto de PGF2α que provoca luteólisis (Siena y Chiara, 2021). En gestaciones de un solo cachorro, esta señal puede ser insuficiente, provocando retraso en el parto (Cortes et al., 2025).

La progesterona también tiene un efecto termogénico, por lo que su descenso se asocia a una disminución de la temperatura rectal (hasta 0,8 °C), 12–24 horas antes del parto (Milani et al., 2020). Este parámetro, aunque útil, no debe utilizarse de forma aislada (Arlt, 2020).

1.2 Cambios fisiológicos durante la gestación

Durante la gestación se producen cambios hematológicos e inflamatorios. Es común una anemia normocítica y normocrómica desde la mitad de la gestación, a menudo con trombocitopenia leve (Arlt, 2020; Lopate, 2012). También se incrementan los niveles de fibrinógeno y proteína C reactiva entre los días 30 y 50, reflejando la invasión trofoblástica y el desarrollo placentario (Holst et al., 2018). Se ha descrito además una leucocitosis leve como parte de una respuesta inflamatoria fisiológica (Lopate, 2012). Este proceso parece estar modulado hormonalmente, en especial por la progesterona (Holst et al., 2018).

1.2 Placentación

La placenta canina es endoteliocorial, zonaria y alantoicocorial (Tesi et al., 2021). Presenta una banda central de intercambio feto-materno y zonas marginales con hematomas de coloración verdosa (Aralla et al., 2013). Histológicamente, se divide en zona laberíntica (intercambio), zona de unión y zona glandular (Premanandan & Runcan, 2019). La placenta también actúa como órgano endocrino, produciendo prostaglandinas luteolíticas como PGF2α en el momento del parto, y puede ser útil en el diagnóstico de infecciones uterinas como herpesvirus, brucelosis o leishmaniosis (Tesi et al., 2021).

1.3 Diagnóstico de gestación

El diagnóstico se puede realizar por palpación abdominal entre los días 25 y 35 post-pico de LH, aunque la obesidad y tensión muscular pueden dificultarla (Lopate, 2012). La detección de relaxina en suero a partir del día 19 post-LH es útil, aunque puede dar falsos positivos tras reabsorciones embrionarias (Evci et al., 2022).

La ecografía es la herramienta más fiable. Los sacos gestacionales se visualizan desde el día 19; aunque la actividad cardíaca fetal puede observarse a partir el día 24 (Lopate, 2012). Otros órganos, como riñones e intestinos, pueden observarse entre los días 35–63 (Milani et al., 2020). La motilidad intestinal es otro signo de madurez fetal, siendo visible en el último tercio de la gestación, especialmente en los últimos 10 días (Siena et al., 2022).

La monitorización de la frecuencia cardíaca fetal (FCF) es fundamental para evaluar la viabilidad fetal. FCF <180 lpm se considera preocupante y requiere de una intervención inmediata; <160 lpm indica hipoxia, lo que además indica que puede existir un compromiso fetal (Gil et al., 2014).

La radiografía es útil a partir del día 45, cuando ya hay mineralización esquelética. Una sola proyección lateral permite estimar el número de fetos (Lopate, 2008). No obstante, no es una herramienta útil al inicio de la gestación y no se utiliza para valorar la viabilidad neonatal.

1.5 Parto

La duración de la gestación canina varía entre 57 y 72 días tras la monta, dependiendo de la raza y la variabilidad individual (Uchanska et al., 2022). A medida que se acerca el parto, la perra presenta cambios fisiológicos y conductuales característicos. Entre los signos más fiables se incluyen el descenso de la progesterona sérica por debajo de 2 ng/mL dentro de las 24–48 horas previas al inicio del trabajo de parto, una disminución leve de la temperatura rectal y comportamientos como inquietud, jadeo, elaboración del nido, etc (Cortes et al., 2025).

Las herramientas clínicas más utilizadas para predecir el parto incluyen la monitorización de la temperatura corporal, mediciones de la progesterona y la evaluación ecográfica de la frecuencia cardíaca fetal (FCF) (Milani et al., 2020). Además, se puede determinar la existencia de peristaltismo intestinal y la maduración renal a través de ecografías (Siena et al., 2022).

El parto se divide en tres fases:

- Fase I: etapa preparatoria, donde se produce la dilatación cervical y contracciones uterinas iniciales. Su duración varía entre 6 y 12 horas, aunque puede prolongarse hasta 36 horas en perras primíparas o ansiosas (Lopate, 2012; Arlt, 2020).
- Fase II: fase activa de expulsión fetal, que suele durar entre 6 y 8 horas. Está mediada por contracciones uterinas y contracciones abdominales (Uchanska et al., 2022). Cuanto menor sea la duración de esta fase, mejor pronóstico de viabilidad neonatal (Fusi et al., 2021).
- Fase III: expulsión de las membranas fetales, que ocurre generalmente en los 15 minutos posteriores al nacimiento de cada cachorro. El proceso completo puede tardar hasta 2 horas tras el último nacimiento (Uchanska et al., 2022).

Durante el parto, se desencadenan adaptaciones cardiovasculares fetales críticas, mediadas por catecolaminas, que redistribuyen el flujo sanguíneo hacia órganos vitales (Kredatusova et al., 2011). En la madre, el dolor y el esfuerzo aumentan la ventilación, provocando alcalosis respiratoria seguida de acidosis metabólica compensatoria, que puede afectar el intercambio gaseoso feto-placentario (Kredatusova et al., 2011).

1.5.1 Distocia

La distocia, definida como dificultad durante el parto, afecta entre el 5% y 16% de los nacimientos caninos (Conze et al., 2022; Long et al., 2022), con una incidencia mucho mayor en razas miniatura o braquicéfalas, donde puede alcanzar hasta el 59.4% (Bolis et al., 2017). Las causas pueden ser maternas, fetales o combinadas (Lopate, 2012). Entre los factores de riesgo se encuentran: edad materna avanzada (>6 años), razas extremas (toy o gigantes), camadas de feto único o demasiado numerosas (>11), y razas con desproporción feto-pélvica como la mayoría de los braquicéfalos (Cornelius et al., 2019; Lopate, 2012).

La causa materna más común es la inercia uterina, la cual puede ser primaria (falta total de contracciones) o secundaria (cese de contracciones tras el nacimiento de uno o más cachorros (Lopate, 2012). La inercia primaria puede asociarse con camadas muy grandes o pequeñas, deficiencias nutricionales, enfermedades sistémicas, hipocalcemia, hipoglucemia o patologías uterinas como endometritis (de Araújo et al., 2025).

En cuanto a las causas fetales, estas incluyen presentación o posición anormal, anomalías estructurales (por ejemplo: anasarca, hidrocefalia, hernias) y fetos macroscópicos o muertos, que carecen de tono muscular para colocarse e el canal del parto provocando una distocia obstructiva (Lopate, 2012).

1.5.2 Cesárea

La cesárea electiva está indicada en perras con antecedentes de distocia, camadas únicas, razas predispuestas o condiciones clínicas como diabetes, toxemia o suplementación con progesterona (Lopate, 2012; Crummer et al., 2025). También se recomienda en razas braquicéfalas o en casos con FCF <180 lpm (Uchanska et al., 2022).

La cesárea puede clasificarse como:

- Electiva (antes del inicio del parto, con fetos maduros)
- Durante la Fase I (sin signos de sufrimiento fetal)
- De emergencia (por distocia o sufrimiento fetal) (Crummer et al., 2025)

Las cesáreas de emergencia tienen mayor riesgo de mortalidad neonatal (hasta 20%) y materna (1%) (Uchanska et al., 2022). La elección del momento quirúrgico debe basarse en la madurez fetal, la caída de progesterona (<2 ng/mL) y la FCF (Crummer et al., 2025).

Desde el punto de vista anestésico, se debe minimizar el intervalo de tiempo entre la inducción y la extracción de los neonatos, manteniendo la perfusión uterina y empleando fármacos con un bajo impacto fetal (Di Cesare et al., 2025; Navarro-Altuna et al., 2025).

1.5.3 Tratamiento no quirúrgico

En casos de distocia sin obstrucción ni sufrimiento fetal, puede intentarse tratamiento médico. El uso de oxitocina, gluconato de calcio y glucosa es común (Oliwia et al., 2022). Es imprescindible evaluar la FCF antes de iniciar tratamiento. Si es <180 lpm, está indicado realizar cesárea urgente (Runcan et al., 2018).

El protocolo incluye:

- Oxitocina: 0.25–1 UI IV, IM o SC, máximo 5 UI. Contraindicada si hay obstrucción (Runcan et al., 2018).
- Gluconato de calcio: 10–20 mg/kg IV lento, o SC diluido 1:1 con suero fisiológico (Runcan et al., 2018).
- Glucosa IV si hay hipoglucemia (Bergström et al., 2006).

La respuesta a la oxitocina puede verse afectada por el estrés materno o por desensibilización de los receptores (Münnich y Küchenmeister, 2009). En caso de utilizar el tratamiento médico, se recomienda comenzar con dosis bajas (0.25–0.5 UI). El uso excesivo puede causar hipoxia fetal o ruptura uterina (Karen et al., 2010).

1.6 Lactancia

La lactación está controlada por la prolactina (síntesis) y la oxitocina (eyección), sin participación directa de hormonas ováricas (Trass, 2008). Factores como hipotensión, dolor postoperatorio, malnutrición o deshidratación pueden reducir la producción láctea (Trass, 2008).

La transferencia de inmunidad pasiva a través del calostro es esencial. Una pérdida de peso mayor al 4% en las primeras 48 horas se asocia a mayor mortalidad (Chastant-Maillard et al., 2016). El calostro también constituye una fuente de energía, hormonas (cortisol, GH, insulina) y factores inmunitarios (IgG, IgA, células inmunes), promoviendo el desarrollo de órganos y microbiota intestinal (Pereira et al., 2023).

La producción láctea aumenta durante la lactancia máxima (2–4 semanas), variando según tamaño de camada (Chastant-Maillard, 2023). La administración de fármacos en la madre puede afectar a los neonatos a través de la leche, especialmente si tienen sistemas hepáticos y renales inmaduros (Ferrari et al., 2022). No obstante, se ha demostrado que muchos AINEs tienen una transferencia mínima y segura (Ferrari et al., 2022).

CAPÍTULO 2. NEONATOLOGÍA

La neonatología es una rama específica de la medicina veterinaria que se centra en la fisiología, patología y el manejo clínico del recién nacido (Pereira et al., 2024). En especies caninas y felinas, el período neonatal se extiende desde el nacimiento hasta el inicio del destete. Posteriormente, el animal se considera pediátrico hasta que alcanza la madurez sexual, que suele ocurrir alrededor de los seis meses de edad, dependiendo de la especie y la raza (Boller et al., 2025).

El nacimiento marca una transición brusca y crítica entre la vida intrauterina y extrauterina. Este proceso se conoce como adaptación neonatal e implica una compleja serie de ajustes fisiológicos que son necesarios para la supervivencia del neonato (Veronesi y Fusi, 2022; Plavec et al., 2022; Bolis et al., 2017). Su éxito dependerá de múltiples factores como la madurez fetal, la vitalidad neonatal y la eficacia de las respuestas fisiológicas frente al nuevo entorno (Brenda et al., 2022).

La mortalidad neonatal puede ocurrir en distintas fases: intrauterina, durante el parto, inmediatamente después del nacimiento o en las primeras horas de vida (Brenda et al., 2021). Sin embargo, la mayoría de las muertes se producen durante el parto o en los primeros siete días de vida, siendo la asfixia perinatal responsable de aproximadamente el 70% de estas pérdidas (Kusse et al., 2018; Reyes-Sotelo et al., 2021).

2.1 Anatomía fetal y neonatal

Los líquidos amniótico y alantoideo desempeñan roles fundamentales durante la gestación, proporcionando protección mecánica y un entorno estable para el desarrollo del feto (Tal et al., 2022). El líquido amniótico se compone de secreciones respiratorias, digestivas y urinarias fetales, además de exudados epidérmicos derivados de la cornificación cutánea. Por su parte, el líquido alantoideo tiene tanto un origen materno como fetal, y es crucial para la eliminación de desechos metabólicos y el mantenimiento de la hidratación (Tal et al., 2022).

La placenta canina se clasifica como zonal y endoteliocorial y constituye el principal órgano de intercambio entre la madre y el feto (Gloria et al., 2024). Presenta tres zonas funcionales: la banda placentaria (zona laberíntica), el hematoma marginal y la membrana corioalantoidea. La zona laberíntica es responsable del intercambio de gases y nutrientes; el hematoma marginal contiene productos de degradación hemática, y la membrana corioalantoidea sostiene la interfaz vascularizada entre la madre y el feto (Farias et al., 2023; Gloria et al., 2024).

Las alteraciones en la estructura o función placentaria pueden interferir con la gestación y aumentar el riesgo de pérdidas fetales, restricción del crecimiento intrauterino (RCIU) o enfermedades crónicas en la vida adulta (Sarli et al., 2021; Farias et al., 2023; Gloria et al., 2024).

2.2 Fisiología neonatal

2.2.1 Sistema cardiovascular

El sistema cardiovascular neonatal es funcionalmente inmaduro al momento del nacimiento, y requiere adaptaciones rápidas para garantizar la oxigenación y perfusión tisular (Veronesi y Fusi, 2022; Boller et al., 2025). La circulación fetal, de tipo paralelo, se transforma en una circulación en serie tras el cierre funcional del conducto arterioso y el foramen oval, lo que induce a un aumento del flujo pulmonar (Boller et al., 2025).

Los neonatos presentan una presión arterial baja (50–70 mmHg) y una resistencia vascular periférica disminuida, compensadas a su vez por frecuencias cardíacas elevadas (>200 lpm), ya que el volumen sistólico es limitado (Pereira et al., 2024; Da Cunha, 2024). La bradicardia neonatal se asocia más frecuentemente a hipoxemia que a estimulación vagal, por lo que el uso de atropina no está recomendado durante la reanimación(Da Cunha, 2015).

2.2.2 Sistema respiratorio

La adaptación respiratoria es el proceso más crítico para la supervivencia neonatal (Abreu et al., 2024). Los pulmones fetales están llenos de líquido, que debe ser eliminado durante el parto para permitir la ventilación alveolar (Azevedo et al., 2024). El surfactante pulmonar, se compone principalmente por fosfolípidos como lecitina y esfingomielina, siendo este esencial para reducir la tensión superficial alveolar (Silva et al., 2015).

La maduración pulmonar ocurre en varias fases: pseudoglandular, canalicular, sacular y alveolar (Benzato et al., 2017). La producción de surfactante comienza en la fase canalicular y es modulada por glucocorticoides fetales (Vannucchi et al., 2012).

Neonatos nacidos por cesárea presentan menor eliminación de líquido pulmonar y menor estimulación respiratoria, lo que puede traducirse en menor puntuación de vitalidad comparado con partos naturales (Silva et al., 2015; Reid y Donelly, 2024). La hipoxia prolongada durante el parto puede desencadenar acidosis metabólica, hipoglucemia y bradicardia (Veronesi y Fusi, 2022; Reyes-Sotelo et al., 2021).

2.2.3 Sistema hematopoyético

En neonatos, los valores hematológicos difieren notoriamente de los adultos. Se presenta una leucocitosis leve con valores inferiores a 30 g/l, y las enzimas hepáticas suelen estar elevadas, probablemente por la ingesta de calostro (Münnich et al., 2014).

2.2.4 Sistema nervioso

El sistema nervioso neonatal es inmaduro, aunque ya funcional en términos de reflejos básicos como el de succión (Fitzgerald y Newquist, 2010). La barrera hematoencefálica aún no está completamente desarrollada, lo que permite el paso de sustancias como ácido láctico o fármacos, aumentando el riesgo de toxicidad (Fitzgerald y Newquist, 2010).

2.2.5 Tracto gastrointestinal y hepático

El sistema digestivo neonatal inicia su actividad tras el nacimiento, con una rápida colonización bacteriana intestinal (Beretta et al., 2025). Aunque antes se consideraba estéril, estudios recientes sugieren colonización intrauterina (Beretta et al., 2025). La maduración hepática es incompleta, y el sistema enzimático P450 se desarrolla progresivamente (Da Cunha, 2015). El microbiota intestinal influye en el crecimiento y en la prevención de patologías neonatales (Nagendra Singh et al., 2025).

2.2.6 Sistema urinario

La nefrogénesis no se completa hasta la tercera semana de vida, presentando baja capacidad de concentración urinaria y reabsorción tubular (Fávera, 2015; Fitzgerald y Newquist, 2010). La orina presenta baja densidad (1.006–1.017) y puede contener proteínas, glucosa y aminoácidos (Molina et al., 2020). La inmadurez renal predispone a desequilibrios hídricos y electrolíticos (Kusse et al., 2018).

2.3 Índice Apgar Score

El Apgar Score permite evaluar la viabilidad neonatal en el momento del nacimiento. Para ello estudia cinco parámetros: color de mucosas, frecuencia cardíaca, esfuerzo respiratorio, tono muscular y respuesta refleja; asignando 0, 1 o 2 puntos por parámetro (Uchanska et al., 2022). Dependiendo de la puntuación, se clasifican en un grupo u otro. Puntuaciones de 7–10 indican buena vitalidad; 4–6 estrés moderado; y 0–3, depresión severa. En razas braquicéfalas se han propuesto adaptaciones específicas (Uchanska et al., 2022).

2.4 Reanimación neonatal

La reanimación neonatal incluye medidas inmediatas tras el parto, especialmente en cesáreas. Se recomienda un secado vigoroso, limpieza de las vías aéreas, estimulación táctil y evitar maniobras como el "swing" (Boller et al., 2025). En casos de depresión asociada a la anestesia, pueden utilizarse antagonistas como naloxona o atipamezol (Boller et al., 2025). El pinzamiento tardío del cordón umbilical puede favorecer la oxigenación y perfusión neonatal (Pereira et al., 2019).

2.5 Peso al nacimiento

El peso al nacimiento es un predictor clave de viabilidad. Neonatos con peso menor al 25% del promedio racial tienen una mortalidad 12 veces mayor (Mugnier et al., 2019; Schrank et al., 2020). Se recomienda control cada 12 horas, con un aumento diario del 10% (Boller et al., 2025). El bajo peso se asocia a hipoglucemia, hipoxia, dificultad para mamar y mayor riesgo de patologías neurológicas (Uchanska et al., 2022).

CAPÍTULO 3. PATOLOGÍAS NEONATALES: DIAGNÓSTICO Y TRATAMIENTO

La atención neonatal abarca múltiples aspectos, incluyendo la evaluación clínica del neonato, de la madre y de toda la camada. La neonatología es el área que se ocupa del cuidado, diagnóstico y tratamiento de los recién nacidos, comprendiendo tanto su fisiología como las patologías que pueden comprometer su viabilidad (Pereira et al., 2024; Beretta et al., 2025).

La etapa neonatal abarca las primeras 3 a 4 semanas de vida y se considera es una etapa crítica dado vulnerabilidad neonatal frente a factores ambientales, infecciosos y metabólicos. Durante este periodo, la tasa de mortalidad puede alcanzar hasta el 20%, siendo las causas más frecuentes: hipoxia, infecciones, malformaciones congénitas, hipotermia e hipoglucemia (Fuchs et al., 2023; Pereira, 2021).

3.1 Hipoglucemia

La hipoglucemia transitoria es una condición frecuente en neonatos, especialmente en aquellos con bajo peso al nacer, debido a la inmadurez hepática y a las reservas limitadas de glucógeno (Plavec et al., 2022; Fuchs et al., 2023). El hígado de los neonatos caninos no alcanza su madurez funcional hasta los 4–5 meses de edad (Fuchs et al., 2023), lo que condiciona la producción y almacenamiento de glucosa.

Durante las primeras 8–12 horas posparto, la ingesta de calostro es esencial para evitar el agotamiento de las reservas energéticas. La hipoglucemia se manifiesta clínicamente mediante vocalización, temblores, depresión mental, convulsiones y, en casos graves puede provocar estados comatosos o la muerte (Uchanska et al., 2022; Fuchs et al., 2023). En neonatos con glucosas inferiores a 40 mg/dL, se han relacionado con alteraciones significativas en los reflejos neurológicos y puntuaciones de Apgar (Fuchs et al., 2023).

El tratamiento incluye la administración de soluciones glucosadas al 10–12.5%, a dosis de 0.2–0.5 mL/100 g vía IV o intraósea. En hipoglucemias persistentes, se recomienda infusión continua de glucosa al 5% (6–18 mL/100 g/día) (Fuchs et al., 2023). La vía oral debe emplearse exclusivamente en neonatos normotérmicos y conscientes.

3.2 Hipotermia

Tras el nacimiento, los neonatos experimentan una caída brusca de la temperatura al exponerse al ambiente extrauterino. La inmadurez de los mecanismos termorreguladores durante las primeras semanas de vida favorecen la aparición de hipotermia, siendo esta más pronunciada en los neonatos de bajo peso (Uchanska et al., 2022; Veronesi et al., 2021).

Temperaturas rectales menores a 35.5 °C se consideran indicativas de hipotermia, y valores sobre los 33.9 ± 1.2 °C se han asociado con mayor riesgo de mortalidad (Vassalo et al., 2015). La hipotermia prolongada puede provocar depresión neurológica, pérdida del reflejo de succión, bradicardia, hipoglucemia, neumonía por aspiración y alteración inmunitaria (Veronesi et al., 2021; Boller et al., 2025).

3.3 Hipoxia

La hipoxia perinatal es responsable de hasta el 60% de las muertes neonatales en perros (Uchanska et al., 2022). Esta puede originarse durante el parto, especialmente en casos de distocia, parto prolongado o cesárea de urgencia. El compromiso respiratorio produce acidosis metabólica y bradicardia, provocando una isquemia multiorgánica si no se diagnostica e interviene rápidamente (Pereira et al., 2024).

3.4 Deshidratación

La inmadurez renal y la ingesta láctea insuficiente contribuyen a la deshidratación neonatal. La nefrogénesis se completa hacia las 2–3 semanas de vida, por lo que la capacidad de concentración urinaria es limitada (Uchanska et al., 2022). La reposición hídrica debe realizarse con soluciones isotónicas, a dosis de 80–120 mL/kg/día (Boller et al., 2025).

3.5 Neumonía por aspiración

La aspiración de meconio o leche puede desencadenar una respuesta inflamatoria pulmonar, hipoxia e hipertensión pulmonar persistente (Van Ierland & Beaufort, 2009; Mokra et al., 2013). Este riesgo aumenta en neonatos alimentados por sonda sin reflejo de deglución o en estado de hipotermia (Fitzgerald & Newquist, 2010).

3.6 Síndrome del cachorro desvanecido (Fading Puppy Syndrome)

Esta condición multifactorial afecta hasta el 30% de las camadas. Se caracteriza por la muerte súbita de neonatos aparentemente sanos en las primeras semanas de vida (Kusse et al., 2018; Nagendra Singh et al., 2025). Las causas incluyen hipoglucemia, hipotermia, infecciones, malformaciones y alteraciones inmunológicas. Histológicamente, se ha observado atrofia del tejido adiposo marrón y disminución del surfactante pulmonar (Blunden, 2012).

3.7 Malformaciones congénitas

Las anomalías congénitas afectan entre el 1% y 3% de los nacimientos caninos, siendo más frecuentes en razas braquicéfalas (Pereira et al., 2019; Uchanska et al., 2022). Entre las más comunes se encuentran: paladar hendido (2.8%), hidrocefalia (1.5%), anasarca (0.7%), y atresia anal (0.4%). Estas malformaciones pueden tener origen genético o ser provocadas por agentes teratogénicos durante la gestación (Pereira et al., 2019).

3.8 Septicemia neonatal

Las infecciones, especialmente bacterianas, constituyen la segunda causa de muerte neonatal (Pereira, 2021; Lima Gorza et al., 2020). Neonatos con inmunidad pasiva deficiente o expuestos a ambientes contaminados presentan mayor riesgo. Las vías de infección incluyen la ingestión de secreciones vaginales, vía umbilical, respiratoria o digestiva. El diagnóstico requiere cultivo microbiológico o PCR, ya que los hallazgos macroscópicos suelen ser inespecíficos (Lima Gorza et al., 2020).

CAPÍTULO 4. BIOMARCADORES NEONATALES

La identificación oportuna de neonatos en estado crítico es esencial para improvisar la supervivencia. Por ello, diversos biomarcadores han sido propuestos con fines diagnósticos o pronósticos. Entre ellos se encuentran: glucosa, lactato, cortisol, pH y parámetros del líquido amniótico (Valeria et al., 2018).

4.1 Glucosa

La hipoglucemia es común en neonatos y se asocia con la inmadurez hepática y deficiencia de reservas energéticas en el momento del nacimiento. Valores inferiores a 50 mg/dL son indicativos de riesgo, aunque aún no se ha establecido un umbral definitivo para predecir la mortalidad (Veronesi y Fusi, 2022). En estudios recientes, neonatos que no sobrevivieron presentaron niveles significativamente más bajos de glucosa en sangre y en líquido amniótico en relación con los que sobrevivieron (Plavec et al., 2022).

4.2 Lactato

El lactato sérico refleja la hipoxia tisular y el metabolismo anaeróbico. Se ha propuesto un valor de corte de 5 mmol/L como indicador de riesgo. No obstante, concentraciones mayores a 13 mmol/L se han asociado con muerte neonatal (Groppetti et al., 2010; Veronesi y Fusi, 2022). Una medición aislada no es suficiente para obtener información, por lo que se recomiendan mediciones seriadas para evaluar el estado del neonato (Castagnetti et al., 2017).

4.3 Cortisol

Concentraciones elevadas de cortisol en líquido amniótico o sangre neonatal se correlacionan con puntuaciones bajas del Apgar score y mayor mortalidad (Plavec et al., 2022; Groppetti et al., 2021). Aunque su utilidad como biomarcador único aún está en discusión, podría integrarse en protocolos multiparamétricos de evaluación de viabilidad neonatal.

4.4 pH y gases sanguíneos

El pH umbilical neonatal suele oscilar entre 7.1 y 7.2, lo que indica cierto grado de acidosis fisiológica (Veronesi y Fusi, 2022). La combinación de pH, lactato, PaO_2 y $PaCO_2$ en sangre umbilical permite una valoración más precisa del estado ácido-base y del sufrimiento perinatal (Plavec et al., 2022).

4.5 Biomarcadores en líquido amniótico

En medicina humana, el cociente lecitina/esfingomielina y la concentración de proteína surfactante A (SP-A) se utilizan como indicadores de madurez pulmonar. SP-A aumenta significativamente en el último tercio de la gestación y refleja la preparación para la vida extrauterina (Riva et al., 2023). Su aplicación en medicina veterinaria aún está en fase experimental.

6. OBJECTIVES

Artículo 1.
The present study was performed to assess the efficacy and reliability of alfaxalone in comparison with propofol to induce anesthesia in healthy bitches and bitches with pyometra undergoing an ovariohysterectomy. In addition, the use of propofol and alfaxalone as anesthetic induction agents for cesarean sections in bitches, and their influence on neonatal viability were also defined.

El presente estudio se realizó para evaluar la eficacia y la fiabilidad de la alfaxalona en comparación con el propofol para la inducción anestésica en perras sanas y perras con piómetra sometidas a ovariohisterectomía. Además, se definió el uso de propofol y alfaxalona como agentes de inducción anestésica para cesáreas en perras y su influencia en la viabilidad neonatal.

Artículo 2.
The objective of this study was to determine the distribution of the type of cesarean sections (scheduled cesarean sections, emergency cesarean sections) based on the breed type, as well as the influence of different maternal parameters. Likewise, neonatal mortality and the incidence of congenital malformations based on the type of cesarean section, breed, and different maternal parameters were recorded.

El objetivo de este estudio fue determinar la distribución del tipo de cesárea (cesárea programada, cesárea de emergencia) según el tipo de raza, así como la influencia de diferentes parámetros maternos. Asimismo, se registró la mortalidad neonatal y la incidencia de malformaciones congénitas según el tipo de cesárea, la raza y diferentes parámetros maternos.

Artículo 3.
Despite the growing use of ultrasonography in canine pregnancy monitoring, no published studies to date have examined the relationship between fetal kidney biometry and key gestational indicators such as biparietal diameter (BPD), maternal progesterone levels, and dam- specific factors. This study is the first to: (1) correlate fetal renal size with BPD; (2) explore its association with maternal hormonal profiles; (3) evaluate the influence of maternal characteristics on fetal kidney development; and (4) to evaluate the relationship between fetal renal biometry and biparietal diameter (BPD) as a potential indicator of fetal maturity.

A pesar del creciente uso de la ecografía en la monitorización de la gestación canina, hasta la fecha no se han publicado estudios que hayan examinado la relación entre la biometría renal fetal e indicadores gestacionales clave como el diámetro biparietal (DBP), los niveles de progesterona materna y factores específicos de la madre. Este estudio es el primero en: (1) correlacionar el tamaño renal fetal con el DBP; (2) explorar su asociación con los perfiles hormonales maternos; (3) evaluar la influencia de las características maternas en el desarrollo renal fetal; y (4) evaluar la relación entre la biometría renal fetal y el diámetro biparietal (DBP) como posible indicador de madurez fetal.

Artículo 4.
This study aimed to assess whether neonatal blood lactate can serve as an early biomarker of viability in puppies delivered via emergency cesarean section, but not to assess lactate as a long-term predictor of neonatal survival. Additionally, we assess the relationship between lactate levels with Apgar scores, glucose levels, and temperature to determine its clinical utility. Finally, this study aimed to value whether different maternal factors—including maternal weight, glucose levels, lactate levels, and age—could influence neonatal lactate concentration.

Este estudio tuvo como objetivo evaluar si el lactato sanguíneo neonatal puede servir como biomarcador temprano de viabilidad en cachorros nacidos por cesárea de emergencia, pero no evaluar el lactato como predictor a largo plazo de la supervivencia neonatal. Además, evaluamos la relación entre los niveles de lactato con las puntuaciones de Apgar, los niveles de glucosa y la temperatura para determinar su utilidad clínica. Finalmente, este estudio tuvo como objetivo evaluar si diferentes factores maternos, como el peso materno, los niveles de glucosa, los niveles de lactato y la edad, podrían influir en la concentración de lactato neonatal.

Artículo 5.
The objective of the present study was to analyze glucose levels and how they influence neonatal viability. Additionally, this study evaluated how maternal glucose and other factors could alter blood glucose concentrations. Currently, some authors have established cutoff values for neonatal glucose, showing differences among them; however, to the best of the authors' knowledge, no study has measured glucose levels immediately after birth without neonatal food intake and under conditions of dystocia or fetal stress. Furthermore, there are no authors who have assessed whether there are differences in the cutoff values between brachycephalic and non-brachycephalic neonates. Regarding the evaluation of maternal glucose concentrations, few authors have investigated how these levels change during gestation and how other factors, such as litter size, may influence them.

El objetivo del presente estudio fue analizar los niveles de glucosa y cómo influyen en la viabilidad neonatal. Además, este estudio evaluó cómo la glucosa materna y otros factores podrían alterar las concentraciones de glucosa en sangre. Actualmente, algunos autores han establecido valores de corte para la glucosa neonatal, mostrando diferencias entre ellos; sin embargo, hasta donde saben los autores, ningún estudio ha medido los niveles de glucosa inmediatamente después del nacimiento sin ingesta de alimentos neonatales y en condiciones de distocia o estrés fetal. Además, no hay autores que hayan evaluado si existen diferencias en los valores de corte entre neonatos braquicefálicos y no braquicefálicos. Con respecto a la evaluación de las concentraciones de glucosa materna, pocos autores han investigado cómo estos niveles cambian durante la gestación y cómo otros factores, como el tamaño de la camada, pueden influir en ellos.

7. ARTÍCULOS

animals

Article

Comparison of Propofol and Alfaxalone as Anesthesic Drugs in Bitches Undergoing Ovariohysterectomies (Healthy Bitches and with Pyometra) and Cesarean Sections

Raquel Rodríguez-Trujillo, Miguel Batista-Arteaga *, Kseniia Iusupova, Inmaculada Rosario-Medina and Desirée Alamo-Santana

Unit of Reproduction, Universitary Institute of Biomedical Research and Health, University of Las Palmas de Gran Canaria, Transmontaña s/n, 35413 Arucas, Las Palmas, Spain; raquel.rodriguez@ulpgc.es (R.R.-T.); ksfedorchak23@gmail.com (K.I.); inmaculada.rosario@ulpgc.es (I.R.-M.); dalamosantana@gmail.com (D.A.-S.)

* Correspondence: miguel.batista@ulpgc.es; Tel.: +34-928454356

Simple Summary: This study compared the effectiveness of two anesthesia drugs, alfaxalone and propofol, for female dogs undergoing an ovariohysterectomy (n = 69) or cesarean sections (n = 28). Maternal parameters were monitored during surgery, and neonatal viability was assessed post-delivery. The results showed no significant differences in maternal parameters recorded throughout surgery between the two drugs. Propofol required more additional doses for induction, the application of this agent was always the one recommended and did not exceed its maximum dose. Neonatal mortality rates were similar, but alfaxalone was associated with better neonatal viability and required less neonatal care compared to propofol. In summary, both drugs were equally effective for ovariohysterectomies, while alfaxalone showed potential benefits for neonatal health after cesarean sections.

Abstract: This study evaluated the efficacy and safety of two anesthetic agents, alfaxalone and propofol, on maternal physiological parameters (heart and respiratory rates, blood pressure, and temperature) on either ovariohysterectomies or cesarean sections in bitches. A total of 34 healthy and pyometra-affected females (classified as ASA II), were induced with IV propofol (4 mg/kg), while 35 females, both healthy and pyometra affected, were induced with IV alfaxalone (1 mg/kg). For cesarean sections, females (ASA II) were induced with propofol (n = 14) or alfaxalone (n = 14). Additionally, the neonatal viability and modified Apgar score were recorded at 5, 60, and 120 min post-delivery. There were no significant differences in the physiological parameters when comparing the use of propofol and alfaxalone in bitches undergoing ovariohysterectomies, regardless of their health status, nor when comparing cesarean sections. It was observed that bitches induced with propofol occasionally required an additional dose for maintenance of the anesthesia. Neonatal mortality rates were similar for both groups; however, alfaxalone was associated with higher neonatal viability as indicated by the Apgar scores. The findings suggest that both anesthetic protocols are effective and safe for use in canine reproductive surgeries, with no major differences in basic physiological parameters' alteration or neonatal outcomes between the two agents.

Keywords: propofol; alfaxalone; ovariohysterectomy; pyometra; cesarean section; Apgar test

check for updates

Citation: Rodríguez-Trujillo, R.; Batista-Arteaga, M.; Iusupova, K.; Rosario-Medina, I.; Alamo-Santana, D. Comparison of Propofol and Alfaxalone as Anesthesic Drugs in Bitches Undergoing Ovariohysterectomies (Healthy Bitches and with Pyometra) and Cesarean Sections. *Animals* **2024**, *14*, 1343. https://doi.org/10.3390/ani14091343

Academic Editors: Nicolo Columbano and Alberto Maria Crovace

Received: 18 April 2024
Revised: 22 April 2024
Accepted: 26 April 2024
Published: 29 April 2024

1. Introduction

In small animals, surgeries of the female reproductive tract are common procedures in veterinary practice. A routine ovariohysterectomy involves the removal of both ovaries and the uterine horns [1] and is usually performed via a ventral midline approach. Pyometra is a hormone-mediated disorder in which the uterine endometrium is altered due to a secondary bacterial infection; surgical treatment of pyometra includes an ovariohysterectomy similar to the one developed in healthy bitches, although it must be completed carefully due to the greater fragility of the uterine tissues [2]. During the cesarean section, a medial ventral incision is made to gain access to the abdominal cavity, then the uterine horns are carefully exteriorized, and a small incision is made at the uterine body to access the interior of the uterus; finally, a gentle massage is applied to the uterine horns to facilitate the delivery of the neonates [3]. For all procedures, preoperative and postoperative factors must be considered, and the anesthetic protocol should be optimized to minimize neurological and cardiorespiratory depression in females.

The goal of premedication is to prepare the animal for anesthesia, which may include sedation, analgesia, or both, depending on the individual's clinical needs. This approach can also have the benefit of sparing the amount of induction agents needed, which can be advantageous in managing the animal's physiological response to anesthesia. Propofol and alfaxalone are two drugs commonly used for anesthetic induction in veterinary medicine. Propofol is an alkylphenol-based intravenous anesthetic agent. It is known for its rapid anesthetic induction [4] and short recovery times, and it primarily acts on the gamma-aminobutyric acid (GABA), enhancing the inhibitory effect of the neurotransmitter GABA to produce anesthesia [5]. Alfaxalone, is a synthetic neuroactive steroid that interacts with the y-aminobutyric acid (GABA) receptors of the central nervous system, producing anesthesia and muscle relaxation [6]. While both agents act on the same receptor type, their effects on physiological parameters can differ; propofol is associated with decreases in heart rate and mean arterial pressure, and also promote respiratory depression [7]. Alfaxalone, on the other hand, is reported to maintain remarkably stable cardiovascular and respiratory parameters during general anesthesia [8]. Both propofol and alfaxalone exhibit rapid distribution, metabolism, and clearance, reducing the risk of accumulation and facilitating a quick recovery from anesthesia [7].

Different studies have reported the use alfaxalone for total intravenous anesthesia in healthy bitches undergoing an ovariohysterectomy [6,9,10]. In addition, the effectiveness of propofol or alfaxalone for anesthesic induction during cesarean sections has been also evaluated [11–14] and the influence on neonatal viability was determined, showing the best results when alfaxalone was used. Furthermore, only one study [15] has assessed the effectiveness of alfaxalone for total intravenous anesthesia in bitches undergoing elective cesarean sections. Other studies have described an anesthetic protocol consisting of premedication with an opioid, followed by induction and maintenance with propofol until delivery of neonates [3]. However, no studies have been performed on cesarean sections, comparing the efficacy of propofol or alfaxalone to perform the anesthesic induction and maintenance until the neonates' delivery. Finally, to our knowledge, no studies have been completed assessing the efficacy and safety of propofol versus alfaxalone in bitches with pyometra.

Several studies have considered the use of an Apgar score and a short-term survival prognosis in evaluating neonatal viability [16–20]. Neonatal parameters assessed in APGAR are heart rate, mucous membrane color, respiratory rate, stress-induced reflexes, and neonatal activity [3,17]. Temperature, glucose, and lactate plasma concentrations are also variables to consider in neonates [21,22]. The present study was performed to assess the efficacy and reliability of alfaxalone in comparison with propofol to induce anesthesia in healthy bitches and bitches with pyometra undergoing an ovariohysterectomy. In addition, the use of propofol and alfaxalone as anesthetic induction agents for cesarean sections in bitches, and their influence on neonatal viability were also defined.

2. Materials and Methods

2.1. Animals

This study enrolled ninety-nine privately owned female dogs, categorized by breed and age, and their 124 offspring. Before undergoing surgery, all bitches were subject to a thorough physical examination, which included heart rate, glucose levels, rectal temperature, breathing frequency, and mucus color evaluation, as well as a complete blood analysis. The females were divided into two groups based on the type of surgery they received: those undergoing ovariohysterectomy, which included both healthy females and those affected by pyometra, and those undergoing cesarean sections, which were exclusively brachycephalic breeds such as the American Bully, English Bulldog, and French Bulldog. The procedures took place at the Veterinary Hospital at the University of Las Palmas de Gran Canaria over a two-year period from 2020 to 2022. All protocols were carried out in accordance with the animal welfare committee's ethical guidelines ensuring the highest standard of care for all animals involved.

In this study, the bitches with pyometra as well as those who were pregnant and undergoing cesarean sections, were all classified as ASA II, indicating mild systemic disease with no significant functional limitations. This classification was based on the presence of mild systemic alterations, ensuring a homogeneous risk profile among the subjects for the purpose of the study. By categorizing both the pyometra bitches and the pregnant females as ASA II, we aimed to minimize the induction agent's variability due to differences in the sensorium's state. Therefore, it was anticipated that the amount of induction medication required would not vary widely among the subjects, reducing potential bias in the study's design and outcomes.

2.2. Experimental Design

The bitches were assigned to three experimental groups according to the type of surgery performed: ovariohysterectomy in healthy bitches (OVH), ovariohysterectomy in bitches with pyometra (OVHP), and cesarean section (CS). The selection of the anesthetic protocol was random for each bitch. The anesthetist applied the randomly selected anesthetic protocol, being the only one aware of it until the surgery was completed and all the results were obtained. Those responsible for collecting anesthetic data and determining the Apgar score were unaware of the induction agent used until the end to prevent any bias in the results of each measurement.

All females were premedicated with the IM administration of methadone (0.04 mg/kg; Semfortan 10 mg/mL. CIMAvet, Spain) and, after 10–15 min, anesthesia was induced. In the OVH group (n = 42), venous access was established in all females prior to induction; females showing agitation during the catheter placement were excluded from the study. After premedication with methadone, which did not result in heavy sedation, 20 females were induced with IV propofol (OVHP), and 22 females with IV alfaxalone (OVHA). Similarly, in the pyometra group (n = 27), after securing venous catheters without incident due to the animals remaining calm and not agitated, 14 females were induced with IV propofol (OVHPP) and 13 with IV alfaxalone (OVHPA). For both groups, the induction process was smooth, with no cases of agitation affecting catheter placement or the amount of induction agent administered. Subsequently, anesthesia was maintained with sevoflurane in all cases. Regarding cesarean sections, the venous catheter was placed in a similar way to previous groups, and then they were subdivided into two groups: in group CSP (n = 14) induction was performed with propofol and additional boluses of propofol were administered to maintain the anesthesia until the complete delivery of puppies, after which anesthesia was maintained with sevoflurane; in group CSA (n = 14), induction was completed with alfaxalone, additional boluses of alfaxalone were administered to maintain the anesthesia, and once the puppies were delivered, the anesthesia was maintained with sevoflurane. Physiological parameters (heart rate, breathing frequency, blood pressure, temperature, and oxygen saturation) were recorded during surgeries in the dams of the different groups; in addition, the viability and modified Apgar score were recorded in the neonates at 5, 60, and 120 min after birth. In all surgery groups, the bitches were organized uniformly (and randomly) according to age, weight, BCS, and breed.

2.3. Pre-Anesthetic Evaluation and Pre-Surgical Assessment

A clinical examination was conducted to measure the respiratory rate, heart rate, mucous membrane condition, and temperature before females were premedicated. A preanesthetic assessment was carried out, including blood tests (Procyte dx, IDEXX Laboratorios S.L, Barcelona, Spain), biochemistry analysis (Catalyst Dx, IDEXX Laboratorios, S.L, Barcelona, Spain), thorax radiography, and electrocardiogram. Regarding cesarean sections, plasmatic progesterone levels were determined before surgery and a transabdominal ultrasound (C4-1 Curved Array, ZONE Sonography®, Mindray Zonare Z.One PRO, Mountain View, CA, USA) was performed in the dams, and the neonatal heart rates were measured to check for fetal stress. All C-sections were scheduled between 59 and 61 days after ovulation.

Premedication included IM administration of methadone (0.04 mg/kg; Semfortan 10 mg/mL. Ecuphar, Barcelona, Spain). After the application, bitches due to have ovariohysterectomies (both healthy and those with pyometras) and cesarean sections were preoxygenated for 10 min and catheters were applied in the cephalic vein. Prior to induction, the operating room was prepared, particularly for cesarean sections.

2.4. Anesthetic Induction and Patient Preparation

Propofol was administered at a dose of 4 mg/kg (Propovet, 10 mg/mL, Esteve, Barcelona, Spain), while alfaxalone was used at 1 mg/kg dose (Alfaxan, 10 mg/mL, Jurox, Rutherford, Australia). During surgical procedures, including cesarean sections, after endotracheal intubation, bitches were connected to an automatic respirator. Anesthesia was initially maintained with sevoflurane post-induction for routine surgeries. However, for cesarean sections, sevoflurane was not used; instead, maintenance doses of propofol or alfaxalone were administered in boluses as needed. For all groups, the total safe dose of the induction agent was calculated based on the weight of the bitch and divided into four equal parts. If a bitch exhibited signs of pain, a positive palpebral reflex, or a superficial anesthetic plane, an additional boluse dose, equivalent to one-quarter of the total calculated dose, was given to maintain adequate anesthesia. Boluses were administered judiciously to avoid over-sedation and to ensure the safety of both the bitches and the puppies.

Continuous and automated monitoring of anesthetic parameters was conducted from induction to the end of surgery. This monitoring included heart rate, respiratory rate, blood pressure, temperature, oxygen saturation, and capnography, and was facilitated using a Ventilator GE Datex Ohmeda Anesthesia Machine (Vetmat, Bizkaia, Spain) equipped with a multi-parameter monitor that recorded these vital signs at 5-min intervals. Blood pressure was measured indirectly with an oscillometric cuff, and the cuff size was selected based on the patient's limb circumference to ensure accuracy. To guarantee dependable readings, the equipment was calibrated prior to each surgery.

During surgery, if a female dog exhibited physiological responses indicative of nociception, such as an increase in heart rate, blood pressure, or respiratory rate, fentanyl (Fentadon 50 µg/mL, Northwich, United Kingdom) was administered intravenously. The dose of Fentanyl for rescue analgesia was 1 µg/kg. The administration was performed slowly to prevent potential respiratory depression and other opioid-related side effects. Crystalloid fluids (Ringer Lactate® 3 mL/kg/h, Braun, Barcelona, Spain) were given to support blood pressure before, during, and after the procedure.

In the case of cesarean sections, once the neonates were safely removed, sevoflurane was then used for the remainder of the anesthetic maintenance.

2.5. Surgical Procedure

Ovariohysterectomies (in both healthy bitches and bitches with pyometra) were performed using a conventional approach. An incision was made in the skin and subcutaneous tissues to expose the linea alba, and once the abdominal cavity was opened, the uterine horns and ovaries were located. Ovarian and uterine vessels were ligated, and the reproductive tract was removed. Finally, the closure was made in layers; the abdominal wall and the subcutaneous tissue were sutured in a continuous pattern; finally, the skin was closed with an intradermal suture.

A standard surgical technique was used for the cesarean section. The uterine body and horns were carefully exposed, and a 4–10 cm incision was made in the uterine body. The neonates were swiftly extracted and transferred to the neonatal resuscitation area. After the removal of the puppies, the uterine mucous membrane and submucosa were closed using a monofilament absorbable suture (Monosyn® 3/0, HR22, B. Braun Surgical SA, Rubí, Spain) in a single continuous pattern and then an inverting pattern (Cushing) was performed. Afterwards, intravenous oxytocin (1-4 IU Oxiton®, Ovejero, León, Spain) was administered to facilitate the expulsion of the placental membrane. Finally, the closure was performed in layers, in the same manner as the ovariohysterectomies.

2.6. Neonatal Resuscitation

The veterinarians responsible of the resuscitation procedure always used the same reanimation protocol without knowing the anesthetic protocol employed. Electric blankets were used to regulate their temperature. There were enough personnel in the resuscitation area to care for up to two puppies each.

The ABC protocol (airway/breathing/cardiac) was used to properly manage newborns. The airways were cleared by suctioning the mouths and noses with a bulb syringe, removing any fluids or meconium, and ensuring that the mouth and nose were devoid of fetal membrane. The newborns were rubbed vigorously with a towel, and the animal's head was tipped downward to allow fluids to drain from the airway. The pups were not swung, thus avoiding causing excessive intracranial pressure, subdural hemorrhage, or the pass of stomach content into the airway.

Oxygenation and a heat source were available during the process. The respiratory rate was closely monitored and if the neonates were not breathing adequately, they were placed on an oxygen mask. If newborns were auscultated and found to have no heartbeat, emergency neonatal resuscitation began with heart compressions (2–3 compressions per second). If the newborn's vitality did not improve after several minutes of resuscitation, a catheter was inserted into the jugular vein for intravenous administration of drugs. Medicaments used were as follows: naloxone (0.05–0.1 mg/kg) to reverse the effects of opioids given to the mother during premedication; heptaminol (0.1 mg/kg) to support systole and blood pressure; and epinephrine (0.2 mg/kg) to increase heart rate. ABC was stopped if neonates did not show a favorable evolution after 45 min of resuscitation.

After the puppies were stabilized, the placenta was removed, and blood was drawn to measure biochemical parameters. Also, the temperature was measured, and the puppies were weighed on the scale. Afterward, the newborns were placed in an incubator (Vetario© S40, Vetario, Weston-super-Mare, UK) at a temperature of 33–34 °C and fed before 60 min after their birth. Puppies with physical deformities such as anasarca, pronounced cleft palate, or wide fontanelle were registered. If the newborns' welfare was incompatible due to congenital malformations, they were euthanized.

2.7. Apgar Test Evaluation

The Apgar test was performed to assess neonatal viability [3]. The Apgar test included five parameters: heart rate, respiratory rate, mucosal appearance, neonatal mobility, and reflex/irritability status. The heart rate was measured using a stethoscope and an ultrasound scan; the respiratory rate was determined by counting the number of spontaneous breaths per minute; the color of the mucous membrane was by direct examination; mobility was based on spontaneous movement; and reflex irritability was determined by the response to external stimuli, such as compression of a paw tip. Each parameter received a score of 0, 1, or 2 with a final score ranging from 0 to 10, as described Table 1. Puppies were classified into three categories based on their final Apgar score: normal neonates (score > 7), moderate viability neonates (score: 3–7), and critical neonates (score < 3). The Apgar test was performed at 0 (Apgar 0), 60 (Apgar 60), and 120 (Apgar 120) minutes after birth.

Table 1. Apgar score protocol used in the present study.

Parameter	Score		
	0	1	2
Mucus color	Cyanotic	Pale	Pink
Heart rate (bpm)	<180 bpm	180–220 bpm	>220 bpm
Reflex irritability	Absent	Grimace	Vigorous
Mobility	Flaccid	Some flexions	Active motion
Respiratory rate (rpm)	<6 rpm	6–15 rpm	>15 rpm

2.8. Statistical Analysis

The SPSS 10.0 software package was used to analyze the data (SPSS Inc., Chicago, IL, USA). Normality of continuous data was assessed by visual inspection of the histogram and normal quantile plot. Continuous data that are normally distributed are presented as mean ± SD; categorical variables are reported as a fraction (%). The Shapiro–Wilk test was performed to evaluate normality in data intra-operative parameters (propofol/alfaxalone doses, time intervals, secondary effects, fentanyl intraoperative, and maternal physiological parameters). Normal data (parametric) were examined with repeated measures analysis of variance (ANOVA), and a Bonferroni post-hoc test for multiple comparisons was conducted when a statistical significance was found. All data regarding Apgar evaluation and puppies' viability (puppies born alive, neonates stillborn, and total neonatal mortality) were analyzed using the general linear models (GLMs). The lineal model included the effects of the anesthetic protocol (2 protocols), the Apgar scores (critical neonates, moderate viability neonates, and normal viability neonates), and the time of Apgar evaluation (Apgar0, Apgar60, and Apgar 120) as well as the interactions between them. Values were considered to be statistically significant when $p < 0.05$.

3. Results

3.1. Ovariohysterectomies in Healthy Bitches and Pyometra-Affected Females

The mean weight and age of healthy females ($n = 42$) were 14.34 kg and the age ranged between 8 months and 4 years. In contrast, bitches with pyometra ($n = 27$) had a mean weight of 17.12 kg and their age varied between 7 and 11 years. There were no significant differences in weight or age between the propofol and alfaxalone groups. Table 2 presents the percentage of females requiring additional doses of propofol/alfaxalone to complete the induction, observed secondary effects, and the administration of fentanyl during surgery. A higher percentage of females required additional doses to complete induction when propofol was administered (55.9%, 19/34) compared to alfaxalone (28.6%, 10/35); with no significant differences between the OVH and OVHP groups. Notably, transient tachycardia was observed as a secondary effect exclusively in the alfaxalone groups (11.4% vs. 0.0%, $p < 0.05$), which normalized after 5 min. Regarding the administration of intraoperative fentanyl, no significant differences were observed between the propofol and alfaxalone groups.

Table 2. Percentage of additional doses of propofol/alfaxalone required to complete induction, side effects after induction, and fentanyl administration during surgery in the OVHP, OVHA, OVHPP, and OVHPA groups.

Groups	Extra Doses	Secondary Effects	Intraoperative Fentanyl
OVH_P	55.0% ^{a} (11/20)	0.0% (0/20)	40.0% (8/20)
OVH_A	31.8% ^{b} (7/22)	4.5% (1/22)	27.3% (6/22)
$OVHP_P$	57.1% ^{a} (8/14)	0.0% (0/14)	42.8% (6/14)
$OVHP_A$	23.1% ^{b} (3/13)	23.1% (3/13)	46.1% (6/13)
$OVH_P + OVHP_P$	55.9% ^{a} (19/34)	0.0% ^{a} (0/34)	41.1% (14/34)
$OVH_A + OVHP_A$	28.6% ^{b} (10/35)	11.4% ^{b} (4/35)	34.3% (12/35)

^{ab}: Different letters in the same row and category denote significant differences ($p < 0.05$).

Table 3 illustrates the mean blood pressure and heart rate in the OVH and OVHP groups. Blood pressure did not show significant differences between the groups. In females induced with alfaxalone (OVHA and OVHPA), the heart rate was significantly higher ($p < 0.05$) during the first 10 min of surgery compared to those induced with propofol (OVHP and OVHPP). However, in the second half of surgery, both groups exhibited similar values. Additionally, temperature changes throughout the surgery were minimal (range: 37.1–37.7 °C), with no significant differences observed between the groups. Heart rate and temperature were measured at 0, 30, and 60 min after surgery, and did not show significant differences between the protocols. However, a decrease in body temperature one hour after surgery in all groups was noted, but was not statistically significant (36.8, 36.9, 36.7, and 37.0 °C, mean values for OVH, OVHP, OVHA, and OVHPA, respectively).

Table 3. Mean (±sd) blood pressure and heart rate during ovariohysterectomies in healthy bitches (OVH) and bitches with pyometra (OVHP).

	Blood Pressure (mm Hg)				Heart Rate (beats/min)			
Minutes	**OVH_P**	**OVH_A**	**$OVHP_P$**	**$OVHP_A$**	**OVH_P**	**OVH_A**	**$OVHP_P$**	**$OVHP_A$**
0	82.9 ± 2.1	89.0 ± 1.2	83.6 ± 3.2	85.6 ± 3.0	88.9 ± 5.1 [a]	117.5 ± 6.2 [b]	99.5 ± 4.1 [a]	122.1 ± 2.9 [b]
5	85.2 ± 2.3	80.5 ± 2.7	81.3 ± 4.2	82.2 ± 3.1	79.4 ± 4.4 [a]	110.7 ± 4.7 [b]	96.3 ± 3.2 [a]	111.5 ± 4.0 [b]
10	81.5 ± 3.1	72.5 ± 3.2	74.6 ± 2.2	75.4 ± 2.8	78.5 ± 3.0	92.6 ± 5.2	92.7 ± 6.0	102.5 ± 4.6
15	81.6 ± 2.5	75.6 ± 2.3	73.1 ± 3.4	73.2 ± 2.5	79.0 ± 3.9	85.7 ± 3.3	93.8 ± 3.5	97.5 ± 3.1
20	82.9 ± 2.1	89.0 ± 1.2	83.6 ± 3.2	85.6 ± 3.0	88.3 ± 4.2	96.6 ± 6.2	93.5 ± 3.4	90.0 ± 3.9
25	79.0 ± 3.3	86.2 ± 1.9	75.2 ± 2.9	79.6 ± 5.1	83.1 ± 4.3	81.5 ± 2.9	93.1 ± 2.1	94.1 ± 4.7
30	--	--	73.2 ± 2.3	70.6 ± 4.1	--	--	104.5 ± 5.3	106.3 ± 3.7

[ab]: Different letters in the same file and parameter (blood pressure/heart rate) denote significant differences ($p < 0.05$).

3.2. Cesarean Sections

Bitches that underwent cesarean sections had a mean weight and age of 23.5 kg and 3.4 years, respectively, with no significant differences between the propofol and alfaxalone groups. A higher percentage of females required additional doses of propofol for maintaining the anesthetic plane until the delivery of all neonates (Table 4), compared to alfaxalone (64.3% vs. 28.6%, CSP and CSA, respectively; $p < 0.05$). No differences were observed between the groups regarding the administration of fentanyl during surgery, with values ranging between 43% and 50%. Finally, secondary effects (transient tachycardia) immediately after induction did not show differences between the groups.

Table 4. Percentage of additional doses of propofol/alfaxalone, side effects after induction, and fentanyl administration during cesarean sections in experimental groups.

	Additional Doses	**Secondary Effects**	**Intraoperative Fentanyl**
CS_P	64.3% [a] (9/14)	7.1% (1/14)	50.0% (7/14)
CS_A	28.6% [b] (4/14)	14.2% (2/14)	42.8% (6/14)

[ab]: Different letters in the same row denote significant differences ($p < 0.05$).

Table 5 presents the mean blood pressure and heart rate recorded in bitches throughout the cesarean sections. Blood pressure did not exhibit significant differences between the groups, with only a slight decrease observed in the mean values at the end of the cesarean sections. Similar to ovariohysterectomies, the heart rate showed higher values ($p < 0.05$) in the first 10 min of surgery when alfaxalone was administered, but thereafter the heart rate was similar between both protocols. Regarding temperature, mean values ranged between 37.5 and 38 °C throughout surgery. All females survived the cesarean section and experienced an appropriate postoperative recovery; bitches were consciousness within 30 min after surgery, being monitored for the next 3 h.

Table 5. Mean (±sd) blood pressure and heart rate during cesarean section in bitches induced with propofol (CS_P) or with alfaxalone (CS_A).

Minutes	Blood Pressure (mm Hg) CS_P	Blood Pressure (mm Hg) CS_A	Heart Rate (beats/min) CS_P	Heart Rate (beats/min) CS_A
0	82.5 ± 3.1	88.4 ± 1.9	92.1 ± 4.1 [a]	118.4 ± 3.9 [b]
5	82.9 ± 4.1	80.1 ± 2.3	88.5 ± 4.5 [a]	102.8 ± 5.1 [b]
10	79.1 ± 5.3	81.5 ± 1.5	87.2 ± 4.9	94.6 ± 5.5
15	78.2 ± 2.5	80.8 ± 1.4	85.3 ± 3.8	91.4 ± 3.0
20	79.4 ± 2.8	81.2 ± 1.8	83.7 ± 4.0	88.3 ± 3.4
25	70.4 ± 3.7	72.5 ± 3.5	80.5 ± 3..4	84.7 ± 3.7
30	69.5 ± 5.2	70.1 ± 3.3	81.0 ± 3.7	84.5 ± 5.2
35	74.5 ± 3.3	72.7 ± 2.8	86.2 ± 4.0	89.1 ± 4.2

[ab]: Different letters in the same file and parameter (blood pressure/heart rate) denote significant differences ($p < 0.05$).

The incidence of neonatal mortality is detailed in Table 6. The total neonatal mortality was 8.1% (10/124), but when stillborn puppies (6/10) were excluded, the neonatal mortality after cesarean section decreased to 3.2% (4/124). No significant differences were observed between CSP and CSA, with comparable percentages of stillborn puppies and puppies that were born alive but subsequently died between 15 and 30 min after being delivered. The percentage of bitches exhibiting neonatal mortality was quite similar (35.7% vs. 28.6%, CSP, and CSA, respectively), and once the surgeries with stillborn puppies were excluded, neonatal mortality was recorded in only 10.71% (3/28) of the cesarean sections.

Table 6. Neonatal viability after cesarean sections in bitches induced with propofol (CS_P) or with alfaxalone (CS_A).

	CS_P	CS_A	Total
Neonates stillborn	4.7% (3/64)	5.0% (3/60)	4.8% (6/124)
Neonates born alive that died	3.1% (2/64)	3.3% (2/60)	3.2% (4/124)
Total neonatal mortality	7.8% (5/64)	6.7% (4/60)	8.1% (10/124)

The mean average of the Apgar test increased slightly at each measurement in both protocols, and the difference between the Apgar 0 evaluation (7.0 ± 0.2 and 8.1 ± 0.2, CSP and CSA, respectively) and the Apgar 120 score (9.1 ± 0.2 and 9.7 ± 0.1, CSP and CSA, respectively) was markedly significant ($p < 0.01$). When both protocols were compared, it was observed that the alfaxalone protocol had higher Apgar test values ($p < 0.05$) in all three periods studied (Apgar 0: 7.0 ± 0.2 vs. 8.1 ± 0.2; Apgar 60: 8.2 ± 0.2 vs. 9.1 ± 0.1, Apgar 120: 9.1 ± 0.2 vs. 9.7± 0.1, CSP and CSA, respectively). Finally, initial viability scores (critical, moderate, and normal) for each period in both protocols are shown in Table 7. In the CSP group, immediately after birth, about 60% of the puppies were classified as normal neonates (score > 7), significantly higher ($p < 0.01$) than that recorded in moderate and critical neonates. In addition, neonates in critical condition decreased markedly at 60 and 120 min after birth, while the percentage of neonates in moderate or normal condition remained consistent. Regarding the CSA group, the percentage of neonates with normal viability was notably higher ($p < 0.005$) than the number of critical puppies in the Apgar 0 evaluation, and all neonates reached the best score at 120 min after birth. When both protocols were compared, it was observed that the percentage of normal neonates was higher in group CSA in Apgar 0, and this difference became more evident ($p < 0.01$) at 60 and 120 min after birth, with values between 95 and 100% of normal neonates in the CSA group, while in CSP group they were between 75 and 80%. Finally, in both protocols, no neonate decreased their score over time.

Table 7. Neonatal initial viability based on the Apgar score in bitches induced with propofol (CS_P) or with alfaxalone (CS_A).

	Groups	Neonatal Score		
		Critical (Score < 3)	Moderate (Score: 3–7)	Normal (>7)
Apgar 0	CS_P	9.3% [a,1] (5/54)	33.3% [b] (18/54)	57.4% [c] (31/54)
	CS_A	1.7% [a,2] (1/58)	25.9% [b] (15/58)	65.5% [c] (38/58)
Apgar 60	CS_P	1.8% [a] (1/54)	22.2% [b,1] (12/54)	75.9% [c,1] (41/54)
	CS_A	0% [a] (0/58)	3.4% [b,2] (2/58)	96.6% [c,2] (56/58)
Apgar 120	CS_P	0% [a] (0/54)	20.4% [b,1] (11/54)	79.6% [c,1] (43/54)
	CS_A	0% [a] (0/58)	0% [b,2] (0/58)	100% [c,2] (58/58)

[abc] Different letters in the same file and Apgar score denote significant differences ($p < 0.01$); [1,2] Different numbers in the same row and Apgar score denote significant differences ($p < 0.01$).

4. Discussion

Different studies have assessed the use of alfaxalone for the induction and maintenance of anesthesia in bitches. However, to our knowledge, this study describes for the first time the comparison between propofol and alfaxalone for inducing and maintaining anesthesia in all the basic reproductive surgical procedures (ovariohysterectomies, pyometras, and cesarean sections) usually performed on the bitch.

When comparing the use of propofol and alfaxalone in ovariohysterectomy procedures on healthy bitches (OVH) and those with pyometra (OVHP), no significant differences were observed in the maternal parameters assessed. During cesarean sections, propofol or alfaxalone was administered in intermittent boluses to maintain anesthesia, without the use of sevoflurane; once all neonates were delivered, sevoflurane was applied to maintain anesthesia, and no further boluses of the induction agents were given. It is important to note that this approach constituted a partial intravenous anesthesia (PIVA) rather than a total intravenous anesthesia (TIVA), due to the combination of intravenous agents with inhalant anesthesia (sevoflurane) used in different stages of the surgical procedures. Different studies have pointed out that the drug dosages may be fine-tuned based on the type of premedication used [23], and that a slower infusion rate could lead to reduced induction doses for both propofol and alfaxalone. It has been reported that alfaxalone administration can lead to an increase in heart rate as well as hypotension, especially at higher doses; whereas propofol is primarily associated with hypotension [24,25]. In our study, a transient tachycardia appeared in 9.09% of the female dogs treated with alfaxalone, but the heart rate was similar between protocols less than 10 min after anesthetic induction. During surgery, the mean arterial pressure (MAP) showed the same behavior in both protocols. A mild to moderate decrease in MAP has been described in dogs after anesthetic induction using therapeutic doses [24,25], and another study [9] reported significant changes in MAP when the suspensory ligament was pulled in female dogs if the induction and anesthetic maintenance was performed with propofol. In our study, no significant differences were observed between the protocols, probably because sevoflurane was used to maintain the anesthesia. Intraoperative pain was primarily monitored controlling the heart rate and MAP; when the heart rate increased, a microdose of fentanyl (1 microgram/kg) was administered to the bitches, with slightly higher values in the propofol group. This finding is consistent with reported results when alfaxalone is used instead of propofol for induction and/or maintenance [9].

No studies have directly compared anesthetic parameters in female dogs with pyometras using propofol or alfaxalone. In our study, we documented hemodynamic parameters in bitches with pyometra, which were classified as ASA II due to moderate analytical alterations in the red and white blood cells series. These dogs were deemed fit for surgery but, due to their condition (either being pregnant or suffering from pyometra), they were categorized as hemodynamically "unstable" in the context of the ASA classification system. Despite these moderate analytical changes, the animals were compensated and could undergo anesthesia and surgery without being considered critical. Our study demonstrated that alfaxalone and propofol can be used effectively as induction agents in these patients. In the present study, different parameters such as temperature, heart rate, and mean arterial pressure (MAP) were monitored and recorded. In both groups (alfaxalone and propofol), female dogs experienced transient tachycardia after induction, but no instances of apnea were observed. The temperature decreased in both groups during surgery, which was associated with the influence of the anesthetic agents [24]. When values were compared with those during ovariohysterectomy performed in healthy bitches, no significant differences in heart rate, temperature, or MAP were found. Some studies have reported a harder recovery when alfaxalone was used for induction and maintenance, but this may be related to the level of sedation prior to surgery [25–27]. However, in our study, the recovery profiles were similar across all protocols, both in healthy bitches and in bitches with pyometra.

Several studies have found that neonates delivered by cesarean sections showed a mortality rate ranging from 4 to 15% [3,13,17,28–31]. In the present study, neonatal mortality was 8.1%, and after removing the stillborn neonates, only 3.1% (4/124) of the newborns were born alive but died within 2 h; one neonate had a severe congenital pathology (anasarca), and the other three came from cesarean sections with fetal stress. Some studies [3] reported lower neonatal mortality in cesarean sections with smaller litters (<4 puppies), but other studies have reported greater neonatal viability in caesarean sections of large litters [29]. Our study showed that neonatal mortality was more frequent in bitches with large litters and those belonging to brachycephalic breeds. This finding has been reported in other studies that show a high neonatal mortality rate (immediately after birth) associated with brachycephalic breeds [30,31].

In our study, when propofol was used during cesarean sections, additional doses were frequently required to maintain adequate anesthesia, with the requirement for these top-up doses being up to four times higher than that registered for bitches induced with alfaxalone. These extra doses were not part of the initial induction phase but were administered to sustain the depth of anesthesia throughout the surgical procedure. Our findings agree with different studies [12] that confirm a greater efficacy of alfaxalone in inducing a correct anesthetic plan to subsequently carry out cesarean sections, using practically similar induction doses, either propofol (4 mg/kg) or alfaxalone (2–3 mg/kg). Similarly, the physiological parameters of the mother during surgery were similar in both surgical protocols, indicating that anesthetic maintenance (until puppy delivery) can be carried out interchangeably with propofol or alfaxalone, without evident changes in temperature, heart rate, or MAP of the dam. In addition, both protocols presented the same degree of neonatal viability, similar to different studies that did not detect differences in the rate of neonatal mortality when comparing both anesthetic protocols [12,29].

The Apgar test is a useful tool to assess the neonatal viability immediately after birth or a cesarean section [18]. In our study, a lower score was measured immediately after birth (Apgar 0) and increased 120 min following delivery (Apgar 120), similar to the results described in different studies [3,31] reporting mean values between 7.5 and 8.8. The anesthetic protocol showed influence over the Apgar test, with a higher proportion of neonates in optimal condition (score 7–10) immediately after delivery when alfaxalone was used, and these results are in agreement with previous studies [11]. It has been reported that alfaxalone reduced the duration of apnea in the mother, and as a result, the neonatal score at birth was higher when compared to propofol [12], and a better viability was observed in neonates born of mothers where alfaxalone was used [11].

Based on the results of this study, the influence of the anesthetic protocol can be linked to neonatal viability. Mortality was observed in puppies showing a lower Apgar score at birth, but 100% survival was found when neonates had an Apgar score >5, independently of the anesthetic protocol. However, in the alfaxalone group, neonates presented a better Apgar test immediately after birth, confirming this trend in the following measurements. Some studies have reported that the neonates awaken from anesthesia faster and with greater vitality [11] when alfaxalone is used. This could explain that neonates born via caesarean section with propofol had a lower mean score in the first Apgar measurement when compared to the alfaxalone group. All anesthetic drugs can cross the placenta, so propofol or alfaxalone used to perform induction and anesthetic maintenance in dams may affect the fetuses. As a side note, the placental transfer of propofol is quick [32] and intravenous propofol applied until the removal of all neonates could produce the same effect (respiratory depression) on the neonates as it did on the mother. Furthermore, propofol promotes the appearance of hypotension and decreases the resistance in the circulatory system and blood flow from the heart; these effects can also be observed in neonates. However, when alfaxalone was used, maternal cardiovascular depression was minimal, resulting in a compensatory increase in heart rate. If these same effects appear in neonates, it may explain why puppies born via caesarean section with propofol had a lower Apgar score and a lower vitality immediately after birth. Regardless of the anesthetic protocol, viability improved in neonates 120 min after birth, most likely because puppies had metabolized the anesthetic drugs received through the placenta, normalizing their vital functions. Some authors have suggested that once hypoxia is removed, the respiratory and cardiovascular functions quickly return to normal, improving neonatal viability [33,34].

5. Conclusions

Based on the findings of this study, propofol and alfaxalone showed similar efficacy for healthy bitches and bitches with pyometra undergoing an ovariohysterectomy, with no significant differences in hemodynamic stability during surgery. Both anesthetic protocols proved to be effective for cesarean sections. Alfaxalone was associated with a higher neonatal vitality as indicated by immediate post-birth Apgar scores. However, it is important to note that this did not translate into a statistically significant difference in neonatal survival rates between the two protocols.

Author Contributions: Conceptualization, M.B.-A.; methodology, M.B.-A., R.R.-T., D.A.-S. and K.I.; formal analysis, M.B.-A., R.R.-T. and K.I.; writing—original draft preparation, M.B.-A., I.R.-M. and R.R.-T.; writing—review and editing, M.B.-A., R.R.-T., D.A.-S. and I.R.-M.; visualization, M.B.-A. and K.I.; supervision, M.B.-A. and D.A.-S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding. The authors acknowledge support from the Veterinary Hospital of the University of Las Palmas de Gran Canaria.

Institutional Review Board Statement: The present study did not require ethical approval. All the veterinary activities were carried out according to the Spanish legislation about animal care (L7/2023, 28 March 2023) and the European Guidelines on Animal Welfare (Directive 2010/63/EU).

Informed Consent Statement: A written informed consent was signed by each owner to submit the bitches to ovariohysterectomy and cesarean section, to allow all the needed clinical procedures on females and newborns, and allow the use of the clinical records for research purposes.

Data Availability Statement: Data will be available for all readers upon request.

Acknowledgments: Miguel Batista acknowledge the excellent technical work carried out by the veterinarian assistant at the Veterinary Hospital of the University of Las Palmas de Gran Canaria.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Van Goethem, B.; Schaefers-Okkens, A.; Kirpensteijn, J. Making a rational choice between ovariectomy and ovariohysterectomy in the dog: A discussion of the benefits of either technique. *Vet. Surg.* **2006**, *35*, 136–143. [CrossRef]
2. Hagman, R. Pyometra in small animals. *Vet. Clin. N. Am. Small Anim. Pract.* **2018**, *48*, 639–661. [CrossRef]
3. Batista, M.; Moreno, C.; Vilar, J.; Golding, M.; Brito, C.; Santana, M.; Alamo, D. Neonatal viability evaluation by Apgar score in puppies delivered by cesarean section in two brachycephalic breeds (English and French bulldog). *Anim. Reprod. Sci.* **2014**, *46*, 218–226. [CrossRef]
4. Campagna, I.; Schwarz, A.; Keller, S.; Bettschart-Wolfensberger, R.; Mosing, M. Comparison of the effects of propofol or alfaxalone for anaesthesia induction and maintenance on respiration in cats. *Vet. Anaesth. Analg.* **2015**, *42*, 484–492. [CrossRef]

5. Bornkamp, J.L.; Robertson, S.; Isaza, N.M.; Harrison, K.; Digangi, B.A.; Pablo, L. Effects of anesthetic induction with a benzodiazepine plus ketamine hydrochloride or propofol on hypothermia in dogs undergoing ovariohysterectomy. *Am. J. Vet. Res.* **2016**, *77*, 351–357. [CrossRef]
6. Dehuisser, V.; Bosmans, T.; Kitshoff, A.; Duchateau, L.; Rooster, H.; Polis, I. Cardiovascular effects, induction and recovery characteristics and alfaxalone dose assessment in alfaxalone versus alfaxalone-fentanyl total intravenous anaesthesia in dogs. *Vet. Anaesth. Analg.* **2017**, *44*, 1276–1286. [CrossRef]
7. Pasloske, K.; Ranasinghe, M.G.; Sauer, S.; Hare, J. The bioequivalence of a single intravenous administration of the anesthetic alfaxalone in cyclodextrin versus alfaxalone in cyclodextrin plus preservatives in cats. *J. Vet. Pharmacol. Ther.* **2018**, *41*, 437–444. [CrossRef]
8. Whittem, T.; Pasloske, K.S.; Heit, M.C.; Ranasinghe, M.G. The pharmacokinetics and pharmacodynamics of alfaxalone in cats after single and multiple intravenous administration of Alfaxan® at clinical and supraclinical doses. *J. Vet. Pharmacol. Ther.* **2008**, *31*, 571–579. [CrossRef]
9. Suarez, M.A.; Dzikiti, B.T.; Stegmann, F.G.; Hartman, M. Comparison of alfaxalone and propofol administered as total intravenous anaesthesia for ovariohysterectomy in dogs. *Vet. Anaesth. Analg.* **2012**, *39*, 236–244. [CrossRef]
10. Herbert, G.L.; Bowlt, K.L.; Ford-Fennah, V.; Covey-Crump, G.L.; Murrell, J.C. Alfaxalone for total intravenous anaesthesia in dogs undergoing ovariohysterectomy: A comparison of premedication with acepromazine or dexmedetomidine. *Vet. Anaesth. Analg.* **2013**, *40*, 124–133. [CrossRef]
11. Doebeli, A.; Michel, E.; Bettschart, R.; Hartnack, S.; Reichler, I.M. Apgar score after induction of anesthesia for canine cesarean section with alfaxalone versus propofol. *Theriogenology* **2013**, *80*, 850–854. [CrossRef]
12. Metcalfe, S.; Hulands-Nave, A.; Bell, M.; Kidd, C.; Pasloske, K.; O'Hagan, B.; Perkins, N.; Whittem, T. Multicentre, randomised clinical trial evaluating the efficacy and safety of alfaxalone administered to bitches for induction of anaesthesia prior to caesarean section. *Aust. Vet. J.* **2014**, *92*, 333–338. [CrossRef]
13. Melandri, M.; Alonge, S.; Peric, T.; Bolis, B.; Veronesi, M.C. Effects of Alfaxalone or Propofol on Giant-Breed Dog Neonates Viability During Elective Caesarean Sections. *Animals* **2019**, *9*, 962. [CrossRef]
14. Groppetti, D.; Di Cesare, F.; Pecile, A.; Cagnardi, P.; Merlanti, R.; D'Urso, E.S.; Gioeni, D.; Boracchi, P.; Ravasio, G. Maternal and neonatal wellbeing during elective C-section induced with a combination of propofol and dexmedetomidine: How effective is the placental barrier in dogs? *Theriogenology* **2019**, *129*, 90–98. [CrossRef]
15. Conde-Ruiz, C.; Del Carro, A.P.; Rosset, E.; Guyot, E.; Maroiller, L.; Buff, S.; Portier, K. Alfaxalone for total intravenous anaesthesia in bitches undergoing elective caesarean section and its effects on puppies: A randomized clinical trial. *Vet. Anaesth. Analg.* **2016**, *43*, 281–290. [CrossRef]
16. Lúcio, C.F.; Silva, L.C.G.; Rodrigues, J.A.; Veiga, G.A.; Vannucchi, C.I. Acid-base changes in canine neonates following normal birth or dystocia. *Reprod. Domest. Anim.* **2009**, *44*, 208–210. [CrossRef]
17. Groppetti, D.; Pecile, A.; Del Carro, A.P.; Copley, K.; Minero, M.; Cremonesi, F. Evaluation of newborn canine viability by means of umbilical vein lactate measurement, Apgar score and uterine tocodynamometry. *Theriogenology* **2010**, *74*, 1187–1196. [CrossRef]
18. Veronesi, M.; Panzani, S.; Faustini, M.; Rota, A. An Apgar scoring system for routine assessment of newborn puppy viability and short-term survival prognosis. *Theriogenology* **2009**, *72*, 401–407. [CrossRef]
19. Veronesi, M.C. Assessment of canine neonatal viability—The Apgar score. *Reprod. Domest. Anim.* **2016**, *51*, 46–50. [CrossRef]
20. Veronesi, M.C.; Faustini, M.; Probo, M.; Rota, A.; Fusi, J. Refining the APGAR Score Cutoff Values and Viability Classes According to Breed Body Size in Newborn Dogs. *Animals* **2022**, *12*, 1664. [CrossRef]
21. Castagnetti, C.; Cunto, M.; Bini, C.; Mariella, J.; Capolongo, S.; Zambelli, D. Time-dependent changes and prognostic value of lactatemia during the first 24 h of life in brachycephalic newborn dogs. *Theriogenology* **2017**, *94*, 100–104. [CrossRef] [PubMed]
22. Bolis, B.; Prandi, A.; Rota, A.; Faustini, M.; Veronesi, M.C. Cortisol fetal fluid concentrations in term pregnancy of small-sized purebred dogs and its preliminary relation to first 24 hours survival of newborns. *Theriogenology* **2018**, *88*, 264–269. [CrossRef]
23. Antończyk, A.; Ochota, M. Is an epidural component during general anaesthesia for caesarean section beneficial for neonatal puppies' health and vitality? *Theriogenology* **2022**, *187*, 1–8. [CrossRef] [PubMed]
24. Grint, N.J.; Alderson, B.; Dugdale, A.H.A. A comparison of acepromazine-buprenorphine and medetomidine-buprenorphine for preanesthetic medication of dogs. *J. Am. Vet. Med. Assoc.* **2010**, *237*, 1431–1437. [CrossRef] [PubMed]
25. Muir, W.; Lerche, P.; Wiese, A.; Nelson, L.; Pasloske, K.; Whittem, T. Cardiorespiratory and anesthetic effects of clinical and supraclinical doses of alfaxalone in dogs. *Vet. Anaesth. Analg.* **2008**, *35*, 451–462. [CrossRef]
26. Muir, W.; Lerche, P.; Wiese, A.; Nelson, L.; Pasloske, K.; Whittem, T. The cardiorespiratory and anesthetic effects of clinical and supraclinical doses of alfaxalone in cats. *Vet. Anaesth. Analg.* **2009**, *36*, 42–54. [CrossRef]
27. Jiménez, C.P.; Mathis, A.; Mora, S.S.; Brodbelt, D.; Alibhai, H. Evaluation of the quality of the recovery after administration of propofol or alfaxalone for induction of anaesthesia in dogs anaesthetized for magnetic resonance imaging. *Vet. Anaesth. Analg.* **2012**, *39*, 151–159. [CrossRef] [PubMed]
28. Wydooghe, E.; Berghmans, E.; Rijsselaere, T.; Van Soom, A. International breeder inquiry into the reproduction of the English bulldog. *Vlaams Diergenskun. Tijds.* **2013**, *82*, 38–43.
29. De Cramer, K.G.; Joubert, K.E.; Nöthling, J.O. Puppy survival and vigor associated with the use of low dose medetomidine premedication, propofol induction and maintenance of anesthesia using sevoflurane gas-inhalation for cesarean section in the bitch. *Theriogenology* **2017**, *96*, 10–15. [CrossRef]

30. Adams, D.J.; Ellerbrock, R.E.; Wallace, M.L.; Schmidt, C.W.; Sutherland, B.J.; Grimes, J.A. Risk factors for neonatal mortality prior to hospital discharge in brachycephalic and nonbrachycephalic dogs undergoing cesarean section. *Vet. Surg.* **2022**, *51*, 1052–1060. [CrossRef]
31. Vilar, J.M.; Batista, M.; Pérez, R.; Zagorskaia, A.; Jouanisson, E.; Díaz-Bertrana, L.; Rosales, S. Comparison of 3 anesthetic protocols for the elective cesarean-section in the dog: Effects on the bitch and the newborn puppies. *Anim. Reprod. Sci.* **2018**, *190*, 53–62. [CrossRef] [PubMed]
32. Sánchez-Alcaraz, A.; Quintana, M.B.; Laguarda, M. Placental transfer and neonatal effects of propofol in caesarean section. *J. Clin. Pharm. Ther.* **1998**, *23*, 19–23. [CrossRef] [PubMed]
33. Silva, L.C.G.; Lúcio, C.F.; Veiga, G.A.L.; Rodrigues, J.A.; Vannucchi, C.I. Neonatal clinical evaluation, blood gas and radiographic assessment after normal birth, vaginal dystocia or caesarean section in dogs. *Reprod. Domest. Anim.* **2009**, *44*, 160–163. [CrossRef]
34. Traas, A.M. Resuscitation of canine and feline neonates. *Theriogenology* **2008**, *70*, 343–348. [CrossRef] [PubMed]

Theriogenology 227 (2024) 144–150

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Original Research Article

Maternal and fetal factors for determining the cesarean section type (scheduled/emergency) in bitches

Raquel Rodríguez, Dácil Alemán, Miguel Batista *, Carla Moreno, Melania Santana, Kseniia Iusupova, Desirée Alamo

Department of Animal Reproduction, Veterinary Faculty (IUBS), University of Las Palmas de Gran Canaria, Transmontaña S/n 35416, Arucas, Las Palmas, Spain

A R T I C L E I N F O

Keywords:
Cesarean section
Bitch
Maternal features
Neonatal mortality
Congenital malformations

A B S T R A C T

One hundred and forty bitches and their offspring (689 puppies) were involved in this study. The influence of different maternal features such as age, breed (brachycephalic/non-brachycephalic), previous births (primiparous/multiparous), health status (complete/incomplete) and litter size over the type of cesarean sections (scheduled/emergency), the neonatal survival, and the incidence of congenital malformations were also examined. Scheduled cesareans were predominant (104/140), of which 90 % were brachycephalic breeds and females were mostly between 2 and 4 years old (54.8 %), multiparous (88.4 %) and with a correct health status (67.3 %). Emergency cesarean sections mainly involved non-brachycephalic breeds (80 %) and were carried out mostly in females under 4 years of age (72.2 %), primiparous (77.7 %), with incomplete health status and a large litter size (47.2 %). Perinatal mortality was notably higher in emergency C-sections (3.25 % and 13.3 %, scheduled and emergency C-sections, respectively); the highest incidence of neonatal mortality was recorded in young females (<2, 2–4 years old), primiparous and with incomplete health status. Congenital anomalies were observed in 4.50 % (31/689) of the puppies, with anasarca (38.71 %) and cleft palate (29.03 %) being the most frequently observed malformations. A higher incidence of congenital malformations was detected in puppies from dams with incomplete sanitary health and from inbreeding cross. Overall, the study provides valuable insights into the complex interplay between maternal characteristics and cesarean outcomes. Appropriate genetic selection, good sanitary health conditions, and the age of the reproducers, are pivotal factors in planning for gestation and improving the survival of neonates.

1. Introduction

Parturition represents the end of pregnancy, and it is essential to identify situations where veterinary intervention is necessary. When parturition cannot proceed in a physiological way, a dystocic birth occurs, which may be due to maternal factors such as uterine inertia, fetal factors such as malpresentation, malformations, or fetal oversize, or a combination of both [1]. The number of previous births, the age of the mother [2] and the nature of the crossbreeding (inbreeding/outbreeding) have also been cited as causes that may predispose to dystocia in the canine species [3]. Once dystocia is detected, medical or surgical treatment can be performed based on the type of dystocia. Cesarean section is a common procedure performed in bitches and is often recommended in brachycephalic breeds due to the high risk of complications during birth [4]. Approximately 16 % of bitches experience dystocia, and 60 % of those with dystocia require a cesarean section [5].

Neonatal viability is a critical aspect in the study of the health and survival of puppies during their initial weeks of life. Neonatal mortality varies significantly depending on whether the delivery is natural or via cesarean section. In natural deliveries, the transition of neonates from the intrauterine to the extrauterine environment is particularly delicate [6]. Overall, neonatal mortality in puppies following a natural delivery range between 20 and 40 %, including an 11 % stillbirth rate and neonatal death2s comprising 8–31 % [7]. Cesarean sections are often associated with different rates of neonatal mortality, influenced by factors such as the urgency of the surgery [3] and the condition of the bitch and puppies at the time of delivery [4]. Neonatal mortality rates across all breeds from cesarean sections range from 2.3 % to 8 % [7]. However, in brachycephalic breeds such as English Bulldogs or French Bulldogs, the neonatal mortality rate is usually higher, approximately 11.6 %–14.9 % [4]. Understanding these differences is crucial for improving care strategies and outcomes for both mothers and their

* Corresponding author.
E-mail address: miguel.batista@ulpgc.es (M. Batista).

https://doi.org/10.1016/j.theriogenology.2024.07.020
Received 11 June 2024; Received in revised form 23 July 2024; Accepted 24 July 2024
Available online 25 July 2024

Table 1
Distribution of programmed C-sections and emergency C-sections in brachycephalic and non-brachycephalic bitches.

	Brachycephalic	Non-brachycephalic	Total
Programmed C-sections	64.42%[a] (67/104)	35.58%[b] (37/104)	74.28 %[1] (104/140)
Emergecy C-sections	19.44%[a] (7/36)	80.56%[b] (29/36)	25.71 %[2] (36/140)

[ab] Different letters in the same file denote significant differences (P < 0.01).
[12]Different numbers in the same row and category denote significant differences (P < 0.05).

Table 2
The distribution of programmed and emergency C-sections based on different maternal parameters.

Maternal parameters		Programmed C sections	Emergency C- sections
Age	<2 years	2.8 % (3/104)[1]	22.2 % (8/36)[1]
	2–4 years	54.8 % (57/104)[2]	50.0 % (18/36)[2]
	4–6 years	28.8 % (30/104)[3]	22.2 % (8/36)[1]
	>6 years	12.5 % (13/104)[4]	9.3 % (3/36)[3]
Number of births	No previous births	11.5 % (12/104)[1]	77.7 % (28/36)[1]
	≥1 birth	88.4 % (92/104)[2]	22.2 % (8/36)[2]
Sanitary status	Complete	67.3 % (70/104)[1]	5.5 % (2/36)[1]
	Incomplete	28.8 % (30/104)[2]	38.9 % (14/36)[2]
	Unknow	3.84 % (4/104)[3]	55.5 % (20/36)[3]
Litter size	<3	25.0 % (26/104)[1]	30.5 % (11/36)[1]
	3–6	42.3 % (44/104)[2]	22.2 % (8/36)[1]
	>6	32.7 % (34/104)[1]	47.2 % (17/36)[2]

[123]: Different numbers in the same row and category (age, number of births, sanitary status, litter size) denote significant differences (P < 0.05).

Table 3
Neonatal mortality in emergency and scheduled cesarean sections in the brachycephalic and non-brachycephalic breeds.

	C-sections	Brachycephalic	Non-brachycephalic	Total
After delivery	Programmed	2.97 %[1](9/303)	2.91 %[1] (7/240)	2.94 %[1] (16/543)
	Emergency	8.97 %[2,a] (7/78)	19.11 %[2,b] (13/68)	13.70 %[2] (20/146)
First h. after birth	Programmed	1.98 %[1] (6/303)	2.8 %[1] (5/240)	2.02 %[1] (11/543)
	Emergency	11.53 %[2] (9/78)	13.53 %[2] (9/68)	12.32 %[2] (18/146)
Fisrt 48 h after birth	Programmed	2.97 % (9/303)	2.50 % (6/240)	2.76 % (15/543)
	Emergency	2.56 % (2/78)	2.94 % (2/68)	2.73 % (4/146)
Total		11.02 % (42/381)	13.63 % (42/308)	12.19 % (84/689)

[12]:Different numbers in the same row and category (immediately after delivery, first h after birth, first 48 h after birth) denote significant differences (P < 0.05).
[ab] Different letters in the same file denote significant differences (P < 0.01).

offspring, especially in breeds that are predisposed to higher risks.

Among the causes of neonatal mortality, congenital defects and malformations are significant factors. These defects cause structural and functional abnormalities across various organs and can result in the death of neonates [8]. The prevalence of these malformations generally ranges from 1 to 3 % [1], although some authors have reported rates as high as 6.7–6.9 % [9]. The development of these anomalies can be attributed to genetic factors or teratogenic influence, such as nutritional deficiencies, exposure to irradiation, toxins, or infectious agents during pregnancy. Consanguinity is another contributing factor [10], and while direct associations have not been extensively documented, an increased incidence of malformations has been reported in cases of close kinship [11]. Additionally, maternal age plays a crucial role, with older dogs over 7 years more likely to produce offspring with malformations compared to younger mothers [2]. Cleft palate, abdominal wall defects, and anasarca are more common in brachycephalic breeds, indicating a strong genetic influence [2]. Specific malformations like hydrocephalus are responsible for 11.3 % of neonatal deaths within the first 4 weeks of life, while cleft palate accounts for 50 % of such mortality during the same period [12].

The Apgar score is a crucial tool used to assess neonatal viability immediately after birth, evaluating five key parameters: mucous membrane color, heart rate, reaction to irritability, neonatal motion, and respiratory rate [12]. Scores range from 0 to 10, with neonates scoring 0–3 classified as critical, 4–6 as moderate viability, and 7–10 as normal viability [7,12,13]. The Apgar score has been specially adapted for brachycephalic breeds like English Bulldogs and French Bulldogs due to their unique physiological characteristics [14]. Studies indicate that neonates born via cesarean section generally score lower on the Apgar scale compared to those born natural [1], highlighting the importance of immediate and effective resuscitation to reduce neonatal mortality [12].

The objective of this study was to determine the distribution of the type of cesarean sections (scheduled cesarean sections, emergency cesarean sections) based on the breed type, as well as the influence of different maternal parameters. Likewise, neonatal mortality and the incidence of congenital malformations based on the type of cesarean section, breed, and different maternal parameters were recorded.

2. Material and methods

2.1. Animals

One hundred and forty (140) privately owned bitches and 689 puppies were enrolled in this study. The bitches were classified as either brachycephalic (n = 74) or non-brachycephalic (n = 66). Data on their age, reproductive history, potential for inbreeding (endogamy) or outbreeding, health status, and dietary habits were recorded prior to surgery. Furthermore, the bitches were categorized into two groups: scheduled cesarean section and emergency cesarean section. All cesarean sections were performed by a board-certified surgeon at a private clinic from 2016 to 2021. Neonatal assessments were conducted by a Spanish neonatology specialist. This study adhered to the European

Table 4
Influence of maternal parameters over the percentage of neonatal mortality in programmed and emergency C-sections.

Maternal parameters		Scheduled C-sections	Emergency C- sections
Age	<2 years	6.8%[a,1] (3/44)	50.0%[b,1] (6/12)
	2–4 years	9.8%[a,1] (32/327)	39.65%[b,1] (23/58)
	4–6 years	4.47%[a,2] (6/134)	21.73%[b,2] (10/46)
	>6 years	2.63%[b,3] (1/38)	10.00%[b,2] (3/30)
Number of births	No previous births	12.79%[a,1] (11/86)	31.13%[b,1] (33/106)
	>1 birth	6.78%[a,2] (31/457)	22.25%[b,2] (9/40)
Sanitary status	Complete	3.25%[a,3] (11/338)	13.3%[b,3] (10/75)
	Incomplete	14.89%[a,1] (21/141)	51.28%[b,1] (20/39)
	Unknow	3.12%[a,2] (2/64)	28.57%[b,2] (12/42)
Litter size	<3	6.97%[a] (3/43)	28.57%[b] (4/14)
	3–6	8.09%[a] (17/210)	28.00%[b] (7/25)
	>6	7.58%[a] (22/290)	29.90%[b] (32/107)

[ab] Different letters in the same file denote significant differences ($P < 0.01$).
[123]Different numbers in the same row and category (age, number of births, sanitary status, litter size) denote significant differences ($P < 0.05$).

Table 5
Percentage of females showing neonatal mortality based on the type of C-section and breed.

	Breed			Type of C-section		
	Brachycephalic	Non-brachycephalic	Total	Scheduled	Emergency	Total
Immediately after C-section	12.16%[a] (9/74)	22.72%[b] (15/66)	17.14 %[1] (24/140)	5.09%[a] (8/104)	44.44%[b] (16/36)	17.14 %[1] (24/140)
First hour after birth	8.11 % (6/74)	7.57 % (5/66)	7.86 %[2] (11/140)	7.69 % (8/104)	8.33 % (3/36)	7.85 %[2] (11/140)
First 24 h after birth	12.16%[a] (9/74)	7.57%[b] (5/66)	10.0 %[2] (14/140)	9.61 % (10/104)	11.11 % (4/36)	10.00 %[2] (14/140)
Total	33.78 % (25/74)	36.36 % (24/66)	35.0 % (49/140)	24.04%[a] (25/104)	66.66%[b] (24/36)	35.0 % (49/140)

[ab] Different letters in the same file and category (breed/type of C-section) denote significant differences ($P < 0.01$).
[12] Different numbers in the same row denote significant differences ($P < 0.05$).

animal welfare laws and was conducted following the guidelines of the Bioethics Committee at the University Veterinary Hospital of Las Palmas.

2.2. Experimental design

In the experimental design of our study, we evaluated multiple maternal characteristics in dogs and their potential influence on birth outcomes. The bitches included were classified as either brachycephalic or non-brachycephalic breeds. Regarding the delivery procedure, we distinguished between scheduled and emergency cesarean sections. A total of 104 bitches underwent scheduled cesareans, with 67 being brachycephalic and 37 non-brachycephalic. In contrast, 36 bitches underwent emergency cesareans, with 7 brachycephalic and 29 non-brachycephalic. Furthermore, the bitches were categorized by age into four groups: <2, 2–4, 4–6 and >6 years old. We also considered the number of previous births, differentiating between primiparous (first birth) and multiparous (more than one birth) bitches. The health plan for each animal was assessed and classified into three categories: complete, incomplete, or unknown, which included aspects such as vaccinations and deworming. The size of the litters was another factor, classified into three categories: fewer than 3 puppies, between 3 and 6 puppies, and more than 6 puppies. Lastly, consanguinity in the breeding was evaluated to determine the presence of any consanguinity between the parents. This comprehensive experimental design was aimed at thoroughly addressing the factors that could influence the health and well-being of both the mothers and the puppies.

2.3. Cesarean sections classification

Cesarean sections were classified as either programmed or emergency. Programmed C-sections were scheduled for 59–61 days after ovulation, defined when plasma progesterone levels reached 6–8 ng/ml. C-sections were scheduled for bitches from breeds predisposed to dystocia, such as brachycephalic breeds, owners who preferred cesarean delivery over natural birth, or females that had previously undergone cesarean sections. During gestation, three ultrasound evaluations were conducted: the first between 25 and 30 days after insemination/mating, assessing the total number of embryos and their viability (heartbeat); the second between 43 and 46 days, where fetal heartbeat, size, congenital anomalies, and placental status were evaluated; the final ultrasound, conducted between 57 and 59 days of gestation, determined the biparietal diameter and heartbeat in approximately 50 % of the fetuses. All ultrasound evaluations were performed by the same veterinarian using a GE Medical Systems Ultrasound scanner (GE Medical Systems Ultrasound scanner LOGIQ™, Model MWA150019A, GE HealthCare, United Kingdom).

Emergency C-sections were indicated when parturition commenced (first stage of labor) and complications arose that impeded normal birth (dystocia) or when fetal distress was diagnosed. Prior to the cesarean sections, a comprehensive clinical evaluation of the bitches was conducted, including heart rate, blood pressure, temperature, mucosa examination, plasma progesterone levels, and hematological (Procyte dx, IDEXX Laboratorios S.L., Spain) and biochemical (Catalyst Dx, IDEXX Laboratorios S.L., Spain) analyses. Additionally, thorax radiography and electrocardiograms were performed.

2.4. Premedication, anesthetic and surgical procedures

Initially, premedication was administered to the dogs. Premedication protocols included administering a microdose of IV dexmedetomidine (1 μg/kg, Dexdomitor® 0.5 mg, Zoetis Spain S.L.U., Madrid, Spain) and metoclopramide (0.1 mg/kg, Primepran® 1 mg/ml, Zoetis Spain S.L.U., Madrid, Spain). Bitches were then oxygenated using a face mask, and an intravenous catheter was inserted into the cephalic vein. Induction of anesthesia was completed with fentanyl (2 μg/kg, Fentanest® 0.05 mg/ml, Kern Pharma, Barcelona, Spain) and propofol (2 mg/kg, Propovet® 10 mg/ml, Esteve, Barcelona, Spain), followed by endotracheal intubation. The females were then connected to an automatic respirator delivering 3–4 % oxygen, with 2–3 % isoflurane administered for

Table 6
Congenital anomalies recorded in brachycephalic and non-brachycephalic females and malformations distribution.

Congenital anomalies	Breed		Total
	Brachycephalic	Non-brachycephalic	
Anasarca	1.83 % (7/381)	1.62 % (5/308)	1.74 %[1] (12/689)
Cleft palate	1.31 % (5/381)	1.30 % (4/308)	1.31 %[2] (9/689)
Cleft lip	0.78 % (3/381)	0.32 % (1/308)	0.58 %[3] (4/689)
Hernia	0.26 % (1/381)	0.32 % (1/308)	0.29 %[4] (2/689)
Missing limbs	0.26 % (1/381)	0.32 % (1/308)	0.29 %[4] (2/689)
Monster	0.0 % (0/381)	0.32 % (1/308)	0.14 %[4] (1/689)
Eviscerated	0.26 % (1/381)	0.00 % (0/308)	0.14 %[4] (1/689)
Total	4.72 % (18/381)	4.22 % (13/308)	4.50 % (31/689)

[1234]:Different numbers in the same row denote significant differences (P < 0.05).

Table 7
Percentage of congenital malformations and bitches showing puppies with anomalies based on different maternal parameters.

Maternal parameters		Litters showing malformations	Percentage of neonates with malformations
Age	<2 years	18.18 %[12] (2/11)	6.81 % (3/44)
	2–4 years	12.00 %[1] (9/75)	4.58 % (15/327)
	4–6 years	21.05 %[2] (8/38)	7.46 % (10/134)
	>6 years	18.75 %[12] (3/16)	7.89 % (3/38)
Number of births	No previous births	17.50 % (7/40)	4.68 % (9/192)
	>1 birth	14.00 % (14/100)	4.42 % (22/497)
Sanitary status	Complete	12.50 %[1] (9/72)	2.90 %[1] (12/413)
	Incomplete	25.00 %[2] (11/44)	9.44 %[2] (17/180)
	Unknow	8.33 %[3] (2/24)	3.77 %[1] (4/106)
Litter size	<3	10.81 %[1] (4/37)	7.01 %[1] (4/57)
	3–6	13.46 %[1] (7/52)	3.83 %[2] (9/235)
	>6	21.57 %[2] (11/51)	4.53 %[2] (18/397)
Mating	Inbreeding	21.74 %[1] (5/33)	11.72 %[1] (17/145)
	Outbreeding	8.24 %[2] (8/97)	2.40 %[2] (12/499)
	Unknow	20.0 %[1] (2/10)	4.44 %[1] (2/45)

[123]Different numbers in the same row and category (age, number of births, sanitary status, litter size, mating) denote significant differences (P < 0.01).

maintenance [5,13].

To stabilize blood pressure before and after the procedure, crystalloid fluids (3–4 ml/kg/h Ringer Lactate®, Braun, Rubí, Spain) were administered. Continuous monitoring during the surgery was ensured using a Ventilator GE Datex Ohmeda Anesthesia Machine (Ventilator GE Datex Ohmeda Anesthesia Machine, Vetmat, Bizkaia). Throughout the surgical procedure, vital signs such as heart rate, respiratory rate, oxygen saturation, carbon dioxide levels, and pressures were monitored using a cuff system. Additional analgesia was provided with intravenous fentanyl (2 μg/kg, Fentanest® 0.05 mg/ml, Kern Pharma, Barcelona, Spain) if any signs of pain were observed during the procedure [5,13].

Local anesthesia at the surgical site was achieved with lidocaine (Lidocaína B. Braun 20 mg/ml, Braun, Rubí, Spain), followed by a 6–12 cm incision through the skin and subcutaneous tissue to access the linea alba [14]. Upon entering the abdominal cavity, the uterine horns were exposed, and a 6–10 cm incision was made in the uterine body to extract the neonates, who were then immediately transferred to the neonatal resuscitation area. The duration from propofol induction to neonate extraction ranged between 10 and 15 min.

If an ovariohysterectomy was indicated, it was performed following standard procedures. Alternatively, if the uterus was preserved, closure was achieved using a double inverting pattern (Cushing) with a monofilament absorbable suture (Monosyn® 3/0, B. Braun Surgical, Rubí, Spain). The abdominal wall and subcutaneous tissues were also closed using Monosyn® in a discontinuous pattern, and the skin was closed with an intradermal suture (Monosyn® 3/0, B. Braun Surgical, Rubí, Spain) to facilitate postoperative care [1].

After surgery, the bitches were monitored continuously in the post-surgery area and meloxicam (0.2 mg/kg SC, Inflacam® 5 mg/ml, Virbac, Barcelona, Spain), amoxicillin (20 mg/kg IM, Clamoxyl® 150 mg/ml, Zoetis, Madrid, Spain) and oxytocin (0.1 IU/kg IV, Oxiton® 10 UI/ml, Laboratorios Ovejero, León, Spain) were administered.

2.5. Neonatal care

Prior to the commencement of cesarean sections, a dedicated reanimation area was prepared, equipped with oxygenation facilities and electric blankets to maintain optimal temperature. The resuscitation team, comprising one person for every two puppies and a technician coordinating between the surgery and reanimation areas, was on standby.

The established protocol for managing newborns was the ABC (Airway, Breathing, Cardiac) sequence. Initially, the airways were cleared using a nasal aspirator to remove fluids and any remnants of the fetal membrane from the mouth and nose. Newborns were then vigorously dried with a towel, and their heads were positioned downward to facilitate further drainage of fluids from the airways. Respiratory rates were continuously monitored. Newborns exhibiting inadequate breathing were assisted with an oxygen mask and administered atipamezole (0.01 ml/100 g SC, Revazol® 5 mg/ml, Eurovet Animal Health BV, Handelsweg, Netherlands) and naloxone (0.05–0.1 mg/kg, SC, Naloxona Kern Pharma® 0.4 mg/ml, Kern Pharma, Barcelona, Spain). During this process, neonates were also auscultated to assess heart rate; if it fell below 100 beats per minute, emergency neonatal resuscitation procedures were initiated. This included the insertion of a jugular vein catheter for administering heptaminol (Vetecardiol® 100 mg/ml, Merck Sharp, Salamanca, Spain) to support blood pressure and heart rate, along with adrenaline to enhance cardiac output. If no heartbeat was detected, cardiac compressions at a rate of 2–3 per second were started, accompanied by subsequent doses of adrenaline administered intravenously. If neonatal viability did not improve, the steps of nasal aspiration, oxygenation, and drug administration were repeated. The ABC protocol was discontinued if there was no favorable response after 45 min [1].

Once stabilized, the puppies had the placenta removed, and the umbilical cord clamped. Neonatal temperatures were recorded and the puppies were weighed before placement in an incubator (Vetario© S40 Intensive Care Unit, Buckingham Road, Weston Industrial Estate, Weston-super-Mare) set at 33–34 °C under continuous supervision. Between 60 and 90 min post-delivery, puppies received 1–3 ml of reconstituted milk (Lacta diet®, Pharmadiet, Barcelona, Spain); those lacking a sucking reflex were fed via a nasogastric tube.

Puppies identified with congenital anomalies were assessed, and those with malformations deemed incompatible with life were humanely euthanized. For survival data analysis, newborns were categorized as follows: stillborn; born alive with severe birth defects; born alive but deceased within 4 h; or viable and alive after 4 h. Neonatal viability was further evaluated at 24 and 48 h post-birth.

2.6. Apgar test assessment

Neonatal viability was assessed using the Apgar test, applicable to all puppies whether they required neonatal reanimation [1]. The evaluation involved five parameters: heart rate, respiratory rate, mucous membrane color, neonatal mobility, and reflex/irritability status. The heart rate was determined using a stethoscope, while the respiratory rate was measured by recording the number of breaths per minute. Mucous membrane color was evaluated through direct visual inspection. Neonatal mobility was assessed based on the observation of spontaneous movement, and reflex/irritability was gauged by the response to external stimuli, such as compression of the tip of the paw.

Each parameter received a score of 0, 1, or 2, with the total possible score ranging from 0 to 10. Based on their final Apgar scores, puppies were classified into three categories: critical (score <3), moderate viability (score 3–7), and normal viability (score >7). The Apgar test was administered immediately after birth (Apgar 1) and again 60 min postpartum (Apgar 60) [1,12,15].

2.7. Statistical analysis

Frequency and proportion of categorical variables and mean and standard deviation of scale were calculated. Data of bitches and puppies represented as continuous variables were evaluated for normality using the Shapiro–Wilk test. To explore the homogeneity of baseline data, bitch data in continuous variables (age, health status, number of births) were compared between the type of cesarean section (programmed and emergency cesarean sections) using one-way analysis of variance with Bonferroni adjustment. Comparisons of discrete data (litter size, and number of puppies born following cesarean section) between groups were calculated using the Kruskal–Wallis test with Dunn's test for multiple comparisons. The number of bitches in each type of surgery (programmed and emergency) was analyzed using the Chi-square test with Pearson's correlation coefficient. Puppies' viability (puppies born alive, birth defects and neonatal mortality) were analyzed using the general linear models (GLM). The linear model included the effects of type of cesarean section (programmed and emergency), age (<2, 2–4, 4–6 and >6 years old), number of births, sanitary status (complete, incomplete, unknonw) and litter size (<3, 3–6, >6 puppies) as well as the interactions between them. Pairwise comparisons were adjusted for multiplicity using the Bonferroni test. All analyses were performed using the STATA software (Stata 18, StataCorp LLC, USA). Analysis with $p < 0.05$ was interpreted as statistically significant.

3. Results

A total of 140 cesarean sections were carried out and almost 75 % of which were previously scheduled (Table 1), with a higher frequency in brachycephalic breeds (90 %). Regarding emergency cesarean sections, a higher incidence ($p < 0.01$) was observed in non-brachycephalic breeds. Table 2 describes the distribution C-sections based on different maternal parameters; in scheduled cesarean section, females were mostly in 2–4 years old bitches (54.8 %), with more than one birth (88.4 %) and with a correct health status (67.3 %). Regarding emergency cesarean sections, they were carried out mainly in females under 4 years of age (72.2 %), without previous births (77.7 %), showing an incomplete or unknown health status (94.5 %) and with a large litter size (47.2 %).

Neonatal mortality immediately after cesarean section (Table 3) had a mean value of 5.22 % (36/689), being significantly higher in emergency cesarean sections, both in the number of newborns dead immediately after the cesarean section (13.70 % vs. 2.94 %, emergency vs. programmed C-sections; $p < 0.01$) and in the first hour after birth (12.32 % *vs* 2.94 %, emergency *vs* programmed C-sections; $p < 0.01$). When breeds were compared, the differences were only detected in the percentage of puppies dead before cesarean section, which was higher in non-brachycephalic breeds. Finally, neonatal mortality was similar in the first 48 h after birth, regardless of the breed and the type of cesarean section.

Table 4 shows the percentage of neonatal mortality based on maternal parameters in programmed and emergency C-sections. The highest incidence of neonatal mortality was recorded in young females (<2, 2–4 years) and when both types of cesarean sections were compared, a higher incidence of mortality was observed in emergency cesarean sections, regardless of the female's age. In addition, a higher incidence of neonatal mortality was found in primiparous females, exceeding 30 % of neonatal mortality in emergency cesarean sections and showing the lowest incidence in multiparous bitches with scheduled cesarean section. Bitches with a complete health status showed the lowest incidence of perinatal mortality and the highest values were recorded in females with an incomplete health status and more frequently (about 50 %) in emergency cesarean sections. Litter size did not show the influence on neonatal mortality, with very uniform values within each group of cesarean sections; however, in emergency cesarean sections, mortality was 4–5 times higher when compared to scheduled cesarean sections, within the same age range. The percentage of bitches that showed neonatal mortality is described in Table 5. Neonatal mortality was notably higher in emergency cesarean sections (66.6 % *vs* 24.04 %, emergency *vs.* scheduled C-sections, $p < 0.01$). A lower incidence of neonatal mortality was recorded in brachycephalic bitches just before cesarean sections (12.16 % *vs* 22.72 %, $p < 0.01$; brachycephalic *vs* non-brachycephalic, respectively), while in the first 24 h after birth, the mortality of puppies was slightly higher ($p < 0.05$) in brachycephalic bitches.

The total number of congenital anomalies showed a value of 4.5 % (31/689), with no significant differences between brachycephalic and non-brachycephalic breeds (Table 6). The most frequently malformations were anasarca and cleft palate, which represented 66.67 % (12/18) and 69.23 % (9/13) of the total congenital malformations, in brachycephalic and non-brachycephalic females, respectively. The percentage of litters with congenital anomalies and its distribution based on maternal parameters are described in Table 7. Bitches between 2 and 4 years old had the lowest incidence of litters (12 %) with congenital malformations, but the mother's age did not show influence over the percentage of congenital anomalies of puppies. In addition, Bitches with an incomplete health status had the highest number of litters with congenital anomalies (25 %) and about 21.5 % (11/51) of congenital anomalies were observed in bitches with large litters. Finally, pregnant bitches resulted from inbreeding showed an incidence of congenital malformations almost 3 times higher ($p < 0.01$) than bitches whose pregnancy was the result of outcrossing.

4. Discussion

This retrospective study has defined the percentage of scheduled and emergency cesarean sections in a private clinic, considering different maternal features such as breed, age, previous births, sanitary status, and litter size. In addition, neonatal mortality and congenital malformations were also registered, assessing the same factors previously described, as well as the inbreeding/outbreeding of the mating.

Cesarean sections are a common procedure in veterinary clinics, especially in breeds with a higher predisposition to dystocia, as is the case with brachycephalic breeds [4]. The risk of dystocia is related to abnormal pelvic conformation, a narrow birth canal, and the long biparietal diameter, which predisposes to the inability of natural birthing to occur [4]. In the current study, a total of 75 % scheduled cesareans were performed, of which 90 % were brachycephalic breeds, while 80 % of the emergency cesareans involved non-brachycephalic breeds. This can be explained by scheduling cesareans for breeds known to be at risk of dystocia, thereby reducing the probability of them suffering from dystocia and needing emergency care. In contrast, owners of bitches with a lower predisposition to suffer from dystocia may be more likely to have been unaware of the pregnancy [16] and may not know how a birth occurs normally or when dystocia begins. About 53 % of the cesarean sections (scheduled and emergency C-sections) were performed in brachycephalic breeds, consistent with routine clinical practices and findings from other studies [4,16,15].

Different retrospective studies [4,16] reported an incidence between 34.4 and 50 % of primiparous females that underwent cesarean sections, with no differences between emergency and scheduled cesarean sections. In our study, about 71.4 % of females that underwent cesarean section were multiparous, although it was detected that in emergency cesarean sections, there were more primiparous females; likewise, bitches between 2 and 4 years old represented the highest percentage of females undergoing cesarean section, both in scheduled or emergency cesarean sections. Previous studies did not find a strong relationship between the age of the bitch and the incidence of cesarean sections, but Schrank et al. [17] reported a higher incidence of cesarean sections with advanced maternal age. On the other hand, the lowest incidence of emergency C-section was observed in bitches with a complete sanitary status and a litter size between 3 and 6 puppies, while in scheduled C-sections, bitches with a complete sanitary status and with a litter size between 1 and 6 puppies represented more than 66 % of the total females. No studies have assessed the influence of the sanitary status on the type of cesarean sections, and there were different results regarding the incidence of litter size over the presentation of cesarean sections [18–20].

Neonatal mortality, once excluded puppies with life-incompatible congenital malformations, was around 7.5 % immediately after birth, being notably higher in emergency C-section (15.7 %) compared with scheduled C-sections (1.84 %). Our results agree with those reported by Adams et al. [4] with values of neonatal mortality of 1.2 % and 19.6 % in scheduled C-sections and emergency C-sections, respectively, highlighting the critical impact of the type of cesarean on neonatal survival. Veronesi and Fusi [7] reported a neonatal mortality range of 20 %–40 % during the first three weeks of life, which includes 11 % of neonates born dead and 8–31 % dying within this period. Batista et al. [1] found a lower mortality rate (below 5 %) immediately after birth, although the range across studies varied from 8 to 14 % [21,22]. In our research, the overall neonatal mortality was 12.19 % (84/689), with higher rates observed in emergency cesareans (13.7 %) compared to scheduled ones (2.94 %). This trend was particularly evident in brachycephalic breeds [4], where the survival rate post-scheduled cesarean was 98.8 % (166/168 neonates), significantly better than the 80.4 % survival rate (37 out of 46 neonates) seen after emergency cesareans. Furthermore, our data suggest that most neonatal deaths occur in the first days of life, which can account for up to 67 % of all neonatal fatalities, often due to complications such as hypoxia, hypoglycemia, and hypothermia, which may subsequently lead to infectious diseases [6].

Our study assessed also the influence of maternal age, litter size and health status of the dam on neonatal mortality. Bitches <2 years old showed a particularly high mortality rate, especially during emergency cesareans (50 %) compared to scheduled cesareans (6.8 %). Some studies reported a mortality rate of 38.9 % in bitches over 7 years old [3] tend to have low birth weight and immature neonates, which favors higher mortality [3]. The size of the litter also plays a crucial role in neonatal outcomes; several studies have found a negative correlation between neonatal survival and larger litter size, with litters of more than 12 puppies experiencing a significant impact on survival [4,23]. Uteri from large litters are less able to contract, often leading to dystocia [23], and the increased number of fetuses reduces uterine space, potentially resulting in low-weight neonates. Low birth weight is a known risk factor for increased neonatal mortality due to associated lower glucose concentrations and temperatures [6]. However, in our study, we did not find significant differences in neonatal mortality based on litter size, with values very similar inside each type of cesarean sections (scheduled/emergency). The health status of the mother, encompassing vaccination, deworming, and nutrition, plays a vital role in breeding success. According to our findings, bitches that were well-vaccinated and dewormed exhibit significantly lower neonatal mortality rates. In contrast, bitches with incomplete health plans experienced much higher mortality rates, up to 48 %. This result highlights the critical need for thorough vaccination, deworming, and balanced nutrition to ensure fewer pregnancy complications and improved outcomes for neonates. It is acknowledged that certain pathogens are more likely to cause abortions and the development of congenital malformations [9,24], with these outcomes being considerably more prevalent in bitches with poor or nonexistent health care.

In our study, the 4.5 % of the total neonates exhibited congenital malformations, with a significant prevalence in brachycephalic breeds, which accounted for over 60 % of these abnormalities. The incidence of congenital anomalies has been reported in different studies, with values ranging between 2.8 and 14 % [1,2,4,9,21,25,26] but the influence of maternal factors over the presentation of congenital malformations has not been described deeply. Congenital malformations were slightly less common in bitches aged 2–4 years compared to those under 2 years and over 6 years, with rates ranging from 18 to 21 %, though the differences were not statistically significant Furthermore, neonates born from mothers lacking adequate health care displayed a 9.34 % incidence of malformations. This higher incidence of malformations may be connected to deficiencies in prenatal care, such as insufficient folic acid supplementation, which is crucial for reducing malformation risks and managing conditions that can complicate pregnancies, like hypothyroidism and diabetes mellitus [21]. Overall, our research underscores the importance of comprehensive health management for breeding bitches. This highlights the critical need for thorough vaccination, deworming, and balanced nutrition to ensure fewer pregnancy complications and improved outcomes for neonates [27]. Proper maternal healthcare not only decreases the risk of congenital malformations but also enhances neonatal survival rates. Moreover, congenital malformations can arise from genetic factors or from inbreeding, with inbreeding seen more commonly in large breeds than in small breeds [10,21]. This could explain the higher incidence observed in brachycephalic breeds which tend to involve large-breed dogs [1]. Breeders often select individuals for breeding based on phenotypic characteristics and behavior, which can create genetic bottlenecks. This selection, particularly prevalent in brachycephalic dogs, tends to reduce fertility, increase reproductive issues, and contributes to the occurrence of dystocia due to anatomical disproportion in some breeds [21]. This observation is consistent with the patterns noted in our study, highlighting a critical area for intervention to improve canine breeding outcomes. Finally, litters with 6 or more puppies showed a higher incidence of congenital malformations in agreement with Batista et al. [1] that proposed a predisposition to the development of congenital malformations when the uterus is subjected to an overuse of its functional capacity.

The most common malformations identified were anasarca and cleft palate, representing more than 60 % of the total congenital anomalies, as reported in previous studies [1,2,26,28]. Some studies have reported a higher incidence of anasarca and clef palate in brachycephalic breeds, suggesting the influence of genetic factors in the development of anasarca in the English bulldog [9,29] and a 30 % risk factor for the development of cleft palate in puppies of brachycephalic breeds [30,31]. However, in our study, non-brachycephalic and brachycephalic bitches showed a similar incidence of puppies with anasarca and clef palate and these findings may be due to the low number of inbreeding mating recorded in our study, since inbreeding practices tend to increase the occurrence of congenital malformations [3,29,32]. In addition, hydrocephalus is other congenital malformations usually described in puppies, but this alteration was not observed in our study; this may be related to the breeds involved in each study, with some malformations being more common in large breeds, whereas in small breeds, the appearance of hydrocephalus is more common [9].

5. Conclusion

The present study confirmed the influence of maternal factors over the type of cesarean sections and the incidence of neonatal mortality. Scheduled cesareans were more frequent in bitches with a complete health status, multiparous and a litter size between 4 and 6 puppies, while the higher incidence in emergency C-sections was recorded in bitches without previous birth, with incomplete health status and a big litter size (>6 puppies). Neonatal mortality was notable higher in emergency C-sections, in bitches without previous births and with a not proper sanitary management. Finally, inbreeding and incomplete sanitary health conditions were factors that could favor a higher incidence of congenital malformations. The comprehensive analysis of the study indicates that proper reproductive management is crucial for gestation and the safe delivery of neonates. Appropriate genetic selection of breeders, maintaining good sanitary health conditions, and considering the age of the reproducers, are pivotal factors in planning for gestation. Moreover, owners should be counseled to implement strict control and monitoring during gestation to prevent conditions that could lead to dystocia, which would necessitate emergency cesarean sections and potentially result in higher neonatal mortality rates.

CRediT authorship contribution statement

Raquel Rodríguez: Writing – original draft, Formal analysis, Data curation, Conceptualization. **Dácil Alemán:** Methodology, Investigation, Formal analysis. **Miguel Batista:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Carla Moreno:** Methodology, Investigation, Formal analysis. **Melania Santana:** Methodology, Formal analysis. **Kseniia Iusupova:** Writing – review & editing, Formal analysis. **Desirée Alamo:** Writing – review & editing, Validation.

References

[1] Batista M, Moreno C, Vilar J, Golding M, Brito C, Santana M, Alamo D. Neonatal Viability evaluation by Apgar score in puppies delivered by cesarean section in two brachycephalic breeds (English and French bulldog). Anim Reprod Sci 2014;146:218–26. https://doi.org/10.1016/j.anireprosci.2014.03.003.

[2] Estevam MV, Beretta S, Smargiassi NF, Apparício M, Toniollo GH, Pereira GT. Congenital malformations in brachycephalic dogs: a retrospective study. Front Vet Sci 2022;9:981923. https://doi.org/10.3389/fvets.2022.981923.

[3] Pereira KHN, Fuchs K da M, Corrêa JV, Chiacchio SB, Lourenço ML. Neonatology: topics on puppies and kittens neonatal management to improve neonatal outcome. Animals 2022;12:e3426. https://doi.org/10.3390/ani12233426.

[4] Adams DJ, Ellerbrock RE, Wallace ML, Schmiedt CW, Sutherland BJ, Grimes JA. Risk factors for neonatal mortality prior to hospital discharge in brachycephalic and non-brachycephalic dogs undergoing cesarean section Vet. Surgery (St Louis) 2022;51:1052–60. https://doi.org/10.1111/vsu.13868.

[5] Doebeli A, Michel E, Bettschart R, Hartnack S, Reichler IM. Apgar score after induction of anesthesia for canine cesarean section with alfaxalone versus propofol. Theriogenology 2013;80:850–4. https://doi.org/10.1016/j.theriogenology.2013.07.006.

[6] Mila H, Grellet A, Delebarre M, Mariani C, Feugier A, Chastant-Maillard S. Monitoring of the newborn dog and prediction of neonatal mortality. Prev Vet Med 2017;143:11–20. https://doi.org/10.1016/j.prevetmed.2017.05.005.

[7] Veronesi MC, Fusi J. Neonatal severity indicators in dogs. Rev Bras Reprod Anim 2021;45:525–32. https://doi.org/10.21451/1809-3000.rbra2021.071.

[8] Tønnessen R, Sverdrup Borge K, Nødtvedt A, Indrebø A. Canine perinatal mortality: a cohort study of 224 breeds. Theriogenology 2012;77:1788. https://doi.org/10.1016/j.theriogenology.2011.12.023. 01.

[9] Estevam MV, Toniollo GH, Apparício M. The most common congenital malformations in dogs: literature review and practical guide. Res Vet Sci 2024;171:105230. https://doi.org/10.1016/j.rvsc.2024.105230.

[10] Pereira KHN, Burini CHP, Oliveira ELR, Canuto LEF, dos Santos Correia LEC, Lopes MD, Lourenço MLG. Hypoplasia of ribs associated with cleft palate, cleft lip, and unilateral renal agenesis in a neonate dog of undefined breed. Semina Ciências Agrárias 2019;40:497. https://doi.org/10.5433/1679-0359.2019v40n1p497. 02.

[11] Yordy J, Kraus C, Hayward JJ, White ME, Shannon LM, Creevy KE, Promislow DEL, Boyko AR. Body size, inbreeding, and lifespan in domestic dogs. Conserv Genet 2020;21:137–48. https://doi.org/10.1007/s10592-019-01240-x.

[12] Veronesi MC. Assessment of canine neonatal viability—the Apgar score. Reprod Domest Anim 2016;51:46–50. https://doi.org/10.1111/rda.12787.

[13] Veronesi MC, Bucci R, Probo M, Faustini M, Fusi J. Apgar score for newborn dog viability assessment: differences between English and French bulldogs born via cesarean section. Animals 2023;13:e3318. https://doi.org/10.3390/ani13213318.

[14] Fusi J, Faustini M, Bolis B, Veronesi MC. Apgar score or birthweight in Chihuahua dogs born by elective Caesarean section: which is the best predictor of the survival at 24 h after birth? Acta Vet Scand 2000;62:1–8. 10.1186%2Fs13028-020-00538-y.

[15] Hyndman TH, Fretwell S, Bowden RS, Coaicetto F, Irons PC, Aleri JW, Metcalfe SS. The effect of doxapram on survival and APGAR score in newborn puppies delivered by elective caesarean: a randomized controlled trial. J Vet Pharmacol Therapeut 2023;46:353–64. https://doi.org/10.1111/jvp.13388.

[16] Guest KE, Ellerbrock RE, Adams DJ, Reed RA, Grimes JA. Performing an ovariohysterectomy at the time of c-section does not pose an increase in risk of mortality, intra-or postoperative complications, or decreased mothering ability of the bitch. J Am Vet Med Assoc 2023;261:837–43. https://doi.org/10.2460/javma.23.01.0012.

[17] Schrank M, Sozzi M, Mollo A. Prevalence of cesarean sections in swiss Bernese Mountain Dogs (2001–2020) and identification of risk factors. Acta Vet Scand 2022;64:42. https://doi.org/10.1186/s13028-022-00664-9.

[18] Borge KS, Tønnessen R, Nødtvedt A, Indrebø A. Litter size at birth in purebred dogs—a retrospective study of 224 breeds. Theriogenology 2011;75:911–9. https://doi.org/10.1016/j.theriogenology.2010.10.034.

[19] Dolf G, Gaillard C, Russenberger J, Moseley L, Schelling C. Factors contributing to the decision to perform a cesarean section in Labrador retrievers. BMC Vet Res 2018;14:57. https://doi.org/10.1186/s12917-018-1381-8.

[20] Proctor-Brown LA, Cheong SH, Diel de Amorim M. Impact of decision to delivery time on fetal mortality in canine caesarean section in a referral population. J Vet Med Sci 2019;5:336–44. https://doi.org/10.1002/vms3.163.

[21] Uchańska O, Ochota M, Eberhardt M, Niżański W. Dead or alive? A review of perinatal factors that determine canine neonatal viability. Animals 2022;12:e1402. https://doi.org/10.3390/ani12111402.

[22] Mugnier A, Gaillard V, Chastant S. Relative impact of birth weight and early growth on neonatal mortality in puppies. Animals 2023;13:e1928. https://doi.org/10.3390/ani13121928.

[23] Cornelius AJ, Moxon R, Russenberger J, Havlena B, Cheong SH. Identifying risk factors for canine dystocia and stillbirths. Theriogenology 2019;128:201–6. https://doi.org/10.1016/j.theriogenology.2019.02.009.

[24] Hopper BJ, Richardson JL, Lester NV. Spontaneous antenatal resolution of canine hydrops fetalis diagnosed by ultrasound. J Small Anim Pract 2024;45:2–8. https://doi.org/10.1111/j.1748-5827.2004.tb00191.x.

[25] Moon-Massat PF, Erb HN. Perioperative factors associated with puppy vigor after delivery by cesarean section. J Am Anim Hosp Assoc 2002;38:90–6. https://doi.org/10.5326/0380090.

[26] Vilar JM, Batista M, Pérez R, Zagorskaia A, Jouanisson E, Díaz-Bertrana L, Rosales S. Comparison of 3 anesthetic protocols for the elective cesarean-section in the dog: effects on the bitch and the newborn puppies. Anim Reprod Sci 2018;190:53–62. https://doi.org/10.1016/j.anireprosci.2018.01.007.

[27] Schreiber P, Sanquer A, Martin V, Fontaine C, Gueguen S. Vaccines for pregnant bitches and their offspring. J Vet Sci Med 2015;3:6. https://doi.org/10.13188/2325-4645.1000015.

[28] Wydooghe E, Berghmans E, Rijsselaere T, Van Soom A. International breeder inquiry into the reproduction of the English bulldog. Vlaams Diergen. Tijds 2013;82:38–43. https://doi.org/10.21825/vdt.v82i1.16726.

[29] Cunto M, Zambelli D, Castagnetti C, Linta N, Bini C. Diagnosis and treatment of foetal anasarca in two English bulldog puppies. Pak Vet J 2014;35:251–3. http://www.pvj.com.pk.

[30] Peralta S, Fiani N, Kan-Rohrer KH, Verstraete FJ. Morphological evaluation of clefts of the lip, palate, or both in dogs. Am J Vet Res 2017;78:926–33. https://doi.org/10.2460/ajvr.78.8.926.

[31] Roman N, Carney PC, Fiani N, Peralta S. Incidence patterns of orofacial clefts in purebred dogs. PLoS One 2019;14:e0224574. https://doi.org/10.1371/journal.pone.0224574.

[32] Sousa CGA, Gonçalves IFL, Souza ALM, Fernandes DA, Hyppolito RRC, Rodrigues ABF. Morphological analysis of the cleft palate in dogs (Canis lupus familiaris). J Health Sci 2018;6:427–31. https://doi.org/10.17265/2328-7136/2018.06.005.

Theriogenology 248 (2025) 117612

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Original Research Article

Fetal renal ultrasonography in canine pregnancy: relationship with maternal and fetal metrics for assessing fetal maturity

Rodríguez Raquel, Miguel Batista *, Alonso Sara, Iusupova Kseniia

Unit of Reproduction, University Institute of Biomedical Research and Health, University of Las Palmas de Gran Canaria, Transmontaña s/n 35413, Arucas, Las Palmas, Spain

ARTICLE INFO

Keywords:
Gestacional age
Kidney measurement
Neonate
Canine
Pregnancy

ABSTRACT

Accurate estimation of gestational age is essential in canine obstetrics to optimize the timing of parturition and reduce neonatal risk. This study investigated the correlation between fetal kidney biometry and established gestational indicators in pregnant bitches. Fifty clinically healthy bitches underwent serial ultrasonographic evaluations between Days 40 and 63 of gestation. A total of 147 fetal kidney measurements were recorded, including longitudinal length, vertical width, and cortex-to-medulla (C/M) ratio. Biparietal diameter (BPD), maternal serum progesterone concentration, body weight, and litter size were also assessed. Fetal kidney area showed a strong positive correlation with gestational age ($r = 0.64$, $p < 0.001$), BPD ($r = 0.80$, $p < 0.001$), and maternal body weight ($r = 0.43$, $p < 0.001$), and a moderate negative correlation with maternal progesterone levels ($r = -0.47$, $p < 0.001$). A receiver operating characteristic (ROC) curve was constructed to evaluate the diagnostic performance of fetal kidney area in discriminating between fetal categories defined by biparietal diameter (BPD). The analysis identified an optimal cut-off value of 6.78 cm^2, achieving a sensitivity of 100 % and a specificity of 65.3 %. Linear regression revealed a predictive model between renal area and BPD ($R^2 = 0.623$; $p < 0.001$). The C/M ratio did not significantly correlate with gestational age or maternal factors. These findings support fetal kidney area as a reliable ultrasonographic parameter for estimating gestational age in bitches, particularly in late pregnancy. Its application may complement traditional biometric markers and improve clinical decision-making regarding optimal timing of parturition.

1. Introduction

The accurate prediction of parturition in canine pregnancies is a key clinical challenge, critical for the safe scheduling of cesarean sections and ensuring optimal conditions for natural whelping [1]. Incorrectly timed elective cesareans may lead to pulmonary immaturity, hypoxia, and increased neonatal mortality [2,3]. Timely intervention has been associated with a significant reduction in neonatal mortality, currently reported to range between 20 % and 30 % [4,5], and contributes to improved perinatal outcomes [6]. In dogs, unlike other domestic species, the date of mating is not a reliable predictor of parturition due to wide variability in gestation length (57–72 days post-coitus). Therefore, additional parameters such as hormonal profiling, vaginal cytology, and ultrasonographic biometry are commonly employed to improve predictive accuracy [1,6–11].

Among hormonal tools, serum progesterone remains one of the most accurate indicators. Concentrations of 2–3 ng/mL typically reflect the LH surge, while levels of 4–10 ng/mL correspond to ovulation [12,13]. Progesterone levels usually drop below 2 ng/mL approximately 24 h before parturition [6,7]. When levels remain above this threshold, daily monitoring becomes necessary [12,13].

Ultrasonography is pivotal for gestational assessment. In early pregnancy, inner chorionic cavity diameter is useful around Days 20–25 post-ovulation. Later, biparietal diameter (BPD) is the most widely used parameter, especially with breed-specific formulas [1,10]. However, its accuracy decreases during the final week of gestation [12], limiting its utility for precise scheduling of delivery. As a result, several novel ultrasonographic biomarkers have been investigated to enhance the prediction of parturition during the final stages of gestation. One such parameter is fetal intestinal development, which typically becomes detectable by ultrasonography from Day 57 post-LH surge. This phase is characterized by marked peristaltic activity, indicative of advanced intestinal maturation [14]. However, functional assessment of fetal intestines remains challenging using ultrasonography alone, limiting its

* Corresponding author.
E-mail address: Miguel.batista@ulpgc.es (M. Batista).

https://doi.org/10.1016/j.theriogenology.2025.117612
Received 17 June 2025; Received in revised form 28 July 2025; Accepted 28 July 2025
Available online 30 July 2025

clinical utility [15]. Additionally, recent studies have explored the association between fetal heart rate variability (HRV) and the umbilical artery resistive index (RI). A HRV greater than 27.92 % combined with an RI below 0.7 in all fetuses has been proposed as a potential indicator of imminent parturition, occurring within 12 h [16].

More recently, fetal renal development has gained attention as a promising ultrasonographic marker of gestational age. Fetal kidneys can be visualized between Days 37 and 45 post-ovulation, with progressive cortico-medullary differentiation becoming increasingly pronounced as term approaches [3,17]. The most consistent and reliable measurements appear to be obtained between Days 48 and 52 of gestation [6]. However, renal pelvic dilation has not demonstrated consistent correlation with fetal maturity, and the absence of comparative analyses with well-established parameters—such as biparietal diameter (BPD), maternal serum progesterone levels, or fetal heart rate—limits the current clinical applicability of renal biometry [12,18,19].

Despite the growing use of ultrasonography in canine pregnancy monitoring, no published studies to date have examined the relationship between fetal kidney biometry and key gestational indicators such as biparietal diameter (BPD), maternal progesterone levels, and dam-specific factors. This study is the first to: (1) correlate fetal renal size with BPD; (2) explore its association with maternal hormonal profiles; (3) evaluate the influence of maternal characteristics on fetal kidney development; and (4) to evaluate the relationship between fetal renal biometry and biparietal diameter (BPD) as a potential indicator of fetal maturity. By addressing these gaps, this work offers a clinically relevant, non-invasive, and easily implementable tool for enhancing the precision of gestational age assessment in bitches, particularly during the critical final phase of pregnancy. While direct correlation with gestational age remains the most robust approach, exploring these relationships may help identify alternative or complementary indicators of fetal maturity, especially in cases where ovulation timing is unknown or gestational age estimation is uncertain.

2. Materials and methods

2.1. Animals

This prospective observational study was conducted on 60 clinically healthy pregnant bitches (*Canis lupus familiaris*) that underwent routine prenatal ultrasonographic monitoring at the Veterinary Hospital of the University of Las Palmas de Gran Canaria between 2024 and 2025.

Inclusion criteria encompassed pregnant bitches of any breed, age, body weight, or litter size, provided that serial ultrasonographic examinations could be performed throughout gestation. Bitches that experienced embryonic resorption or were unavailable for follow-up assessments were excluded from the final analysis. Singleton pregnancies were not included in the present study, as their unique growth dynamics could significantly influence fetal biometry and potentially bias the interpretation of renal measurements. Parturition occurred either via natural delivery ($n = 14$) or elective cesarean section ($n = 46$).

The enrolled animals ranged in age from 1.2 to 6.0 years and in body weight from 2.5 to 45.5 kg. Litter size varied between 1 and 11 puppies. A total of 146 complete fetal renal measurements were obtained and included in the final dataset. Detailed information regarding breed distribution and litter size is provided in Table 1. All procedures performed in this study were non-invasive and formed part of routine clinical monitoring aimed exclusively at ensuring maternal and fetal well-being throughout gestation. In accordance with institutional and national ethical regulations, formal approval by an animal ethics committee was not required. Nevertheless, informed consent was obtained from all dog owners prior to inclusion in the study.

2.2. Experimental design

To evaluate the potential influence of maternal body size on fetal renal biometry, bitches were categorized into three groups based on body weight: <5 kg ($n = 10$ mothers), 5–15 kg ($n = 31$), and >15–45 kg ($n = 19$). These intervals were selected based on the distribution of the sample to ensure balanced group sizes and adequate statistical power. The categorization was not based on breed size, but rather on the observed spread of data within the study population. Maternal body weight was measured at each clinical visit, on the same day as the ultrasound and blood sampling. The corresponding body weight value was used for each individual data point in the analysis. Although weight gain occurred during gestation, no bitch experienced a shift in body weight category. Therefore, the classification remained unchanged throughout the study.

Table 1
Number of bitches and neonates based on breed.

Breed	Bitches (n = 60)	Neonates (n = 146)
Labrador Retriever	1	3
Dachshund	17	40
Staffordshire Bull-Terrier	9	6
Chihuahua	10	18
American Bully	2	3
Spanish Water Dog	1	1
Shih Tzu	7	21
Bloodhound	1	2
Meegrel	4	16
Pastor alemán	1	3
Podenco	2	6
Presa canario	5	15

Similarly, gestational age at the time of ultrasonographic examination was stratified into three intervals: 40–50 days ($n = 14$ mothers; $n = 32$ neonates), 50–55 days ($n = 12$ mothers; $n = 37$ neonates), and 55–63 days ($n = 34$ mothers; $n = 77$ neonates). Gestational age was determined using two approaches, depending on the clinical context. In bitches prospectively monitored for breeding management, ovulation was estimated by serial serum progesterone measurements, and gestational age was calculated from the presumed day of ovulation (Day 0). In emergency cases without prior reproductive monitoring, gestational age was estimated retrospectively based on the reported date of mating and corrected using the actual date of parturition. These cases were primarily included for cross-sectional measurements, and their gestational age was interpreted with appropriate caution.

In each ultrasound evaluation, biparietal diameter (BPD), heart rate (HR) and kidney measurements (longitudinal length, vertical width, cortical and medullary thickness) were assessed. In addition, blood samples were taken at the same time, and progesterone plasmatic levels were determined. Finally, after natural parturition or cesarean section, the neonatal survival was defined.

2.3. Ultrasound imaging and techniques

At each visit, a thorough anamnesis was performed. In addition to recording the bitch's age, date of mating or artificial insemination, and prior assessment of serum progesterone concentrations, further information was collected regarding the animal's reproductive history (e.g., number of previous pregnancies or litters), presence of any chronic diseases, current health status, and whether routine preventive care (vaccination and deworming) had been maintained. Two-dimensional ultrasonographic evaluations were performed using a MINDRAY VETUS 7 ultrasound system equipped with a linear-array transducer (7.5–10 MHz). All ultrasound examinations were conducted by the same experienced clinician to minimize interobserver variability.

Each bitch underwent at least three ultrasonographic examinations during gestation: the first between Days 25–27 (pregnancy confirmation), the second between Days 40–45 (fetal and maternal assessment), and the third between Days 53–55 (evaluation of fetal maturity and peripartum planning). In selected cases, particularly when cesarean section was being considered, a fourth ultrasound was performed between Days 57–59 to refine the estimation of parturition date. Although pregnancy diagnosis and general fetal monitoring began earlier (around Days 25–28 post-ovulation), renal ultrasonography was initiated from Day 41 onward. This decision was based on the fact that fetal kidneys are not consistently visible until mid-gestation [17]. According to previous ultrasonographic studies in dogs, renal structures typically become detectable between Days 40 and 45. In the final prenatal evaluation, an abdominal radiograph was performed as part of the service protocol to estimate litter size.

During each session, fetal biparietal diameter (BPD), fetal heart rate (FHR), and organ development were evaluated. BPD was measured when the fetal head was visualized in a transverse plane (Fig. 1), recording the maximum distance between the outer margins of the parietal bones. Measurements followed the technique described by Batista et al. (2021) [20] to ensure consistency with previously validated protocols. Fetal kidneys were identified in both longitudinal and transverse planes (Figs. 2–4). For each bitch and ultrasound session, renal measurements were obtained from at least two fetuses per dam. In most cases, approximately 50 % of the fetuses were assessed per litter, based on the total number of fetuses previously confirmed via radiographic examination performed in late gestation. To avoid duplicating measurements from the same fetus, a standardized scanning protocol was applied. Each examination began at the pelvic inlet, followed by a counterclockwise sweep of the uterus: the left uterine horn was scanned from cranial to caudal, then the right horn, and finally a return to the pelvic region. This approach allowed systematic identification of fetuses and minimized the likelihood of repeated measurements.

The following parameters were recorded for each kidney: longitudinal length (measured from the cranial to the caudal pole), vertical width (measured at the widest perpendicular point in a transverse plane), cortical thickness (measured from the renal capsule to the corticomedullary junction) and medullary thickness-renal pelvis (measured from the corticomedullary junction to the central echoic region corresponding to the renal pelvis. All measurements were performed on frozen images, using electronic calipers, and recorded to the nearest 0.1 mm.

During ultrasonographic examination, differentiation between the right and left fetal kidney was made when possible. To standardize the protocol, the right kidney was prioritized for measurement. However, in cases where the right kidney could not be adequately visualized, the left kidney was assessed instead. In a subset of fetuses, both kidneys were measured and showed no significant differences in size. Therefore, for the purposes of this study, kidney side was not differentiated in the statistical analysis.

Fetal renal area was estimated from longitudinal ultrasonographic images using the formula for the area of an ellipse: $A = \pi \times a \times b$, where "a" corresponds to the semi-major axis (i.e., half the renal length) and "b" to the semi-minor axis (i.e., half the renal width). Renal length and width were measured manually using the ultrasound system's caliper tool, and all values were recorded in centimeters (cm). The final renal area was expressed in square centimeters (cm^2). All renal area measurements were calculated using the same standardized formula, based on fetal renal length and width. This uniform approach allowed us to evaluate whether maternal size, breed, or age had a significant impact on fetal kidney development. Rather than using breed-specific correction formulas, our objective was to detect whether such adjustments would be necessary by analyzing renal area trends across the full data set. This method ensured measurement consistency and facilitated statistical analysis of potential influencing factors.

Fig. 1. Ultrasonographic image showing the measurement of fetal biparietal diameter (BPD) at its maximal width in a transverse plane in a canine fetus.

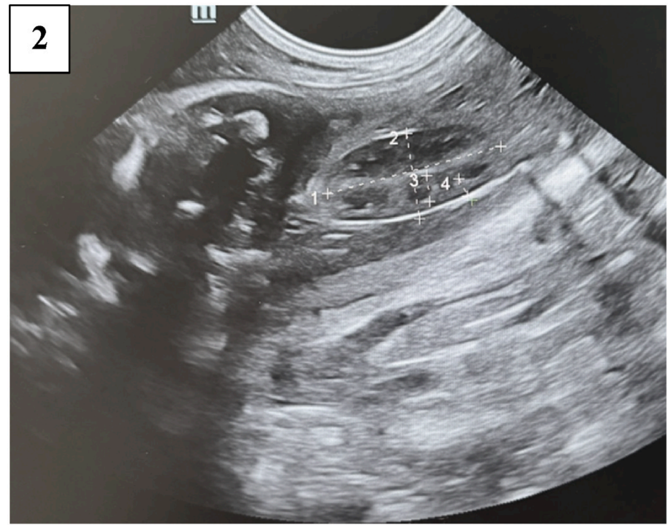


Fig. 2. Ultrasonographic images of fetal kidneys illustrating biometric measurements: (1) longitudinal length (cranial to caudal pole), (2) vertical height (maximum perpendicular width), (3) medullary thickness, and (4) cortical thickness.

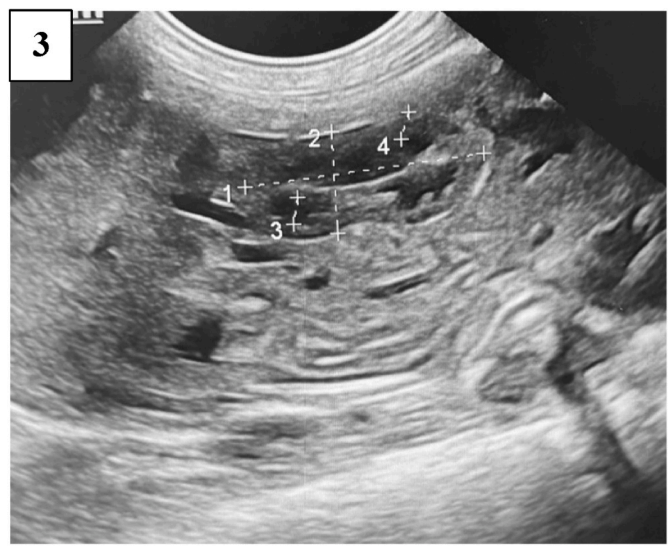


Fig. 3. Ultrasonographic images of fetal kidney illustrating: (1) longitudinal length, (2) vertical height, (3) medullary thickness, and (4) cortical thickness.

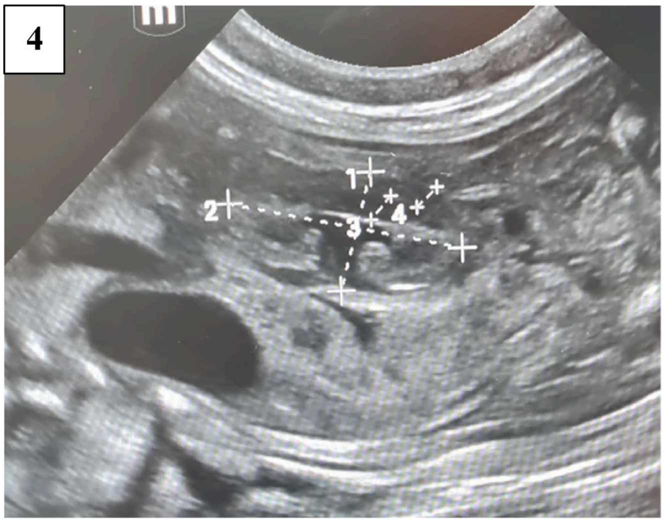


Fig. 4. Additional ultrasonographic view of a fetal kidney: (1) vertical height, (2) longitudinal length, (3) medullary thickness, and (4) cortical thickness.

2.4. Determination of progesterone concentration

Blood samples for serum progesterone determination were collected from each bitch using a sterile 5 mL syringe and a 23-gauge needle, via either the cephalic or jugular vein. Samples were immediately transferred into serum separator tubes (SST II Advance, BD Vacutainer®) and subsequently, the samples were centrifuged at 2000 revolutions per minute (rpm) for 10 min. Progesterone concentrations were measured using an automated chemiluminescent immunoassay analyzer (VIRBAC, Barcelona, Spain), with results typically available within 15 min. Hormone levels were expressed in nanograms per milliliter (ng/mL). According to the manufacturer's validation data, the test shows a 93.5 % agreement with the gold-standard radioimmunoassay (RIA), supporting its clinical reliability in canine reproductive monitoring.

2.5. Cesarean section

All cesarean sections included in this study were performed at the Veterinary Clinical Hospital (HCV) of Las Palmas de Gran Canaria. Both elective and emergency cesarean sections were represented in the study population. In the case of elective cesarean sections, typically indicated in brachycephalic or high-risk breeds, the decision to proceed was based on serial monitoring of maternal serum progesterone and fetal biometry. Progesterone levels were assessed every 48 h once concentrations dropped below 6 ng/mL, and cesarean section was scheduled when values fell below 2 ng/mL in combination with biparietal diameter (BPD) measurements compatible with full-term gestation. This approach aimed to minimize the risk of dystocia and ensure neonatal viability. In urgent cases (e.g., prolonged labor, fetal distress, or uterine inertia), cesarean section was performed based on clinical and ultrasonographic findings.

Emergency cesarean sections were performed when clinical signs of dystocia, fetal distress, or failure of progression during stage II labor were observed, regardless of progesterone concentration. The decision for surgical intervention was based on clinical indicators suggestive of term gestation and fetal compromise, including: (1) onset of labor with no progression after more than 4 h of active contractions; (2) partial delivery followed by secondary uterine inertia, characterized by a complete absence of contractions for 60 min; and (3) ultrasonographic evidence of fetal distress, defined as a fetal heart rate below 180 bpm, and considered critical when <160 bpm. These criteria were used to confirm that these cases were consistent with term gestation despite their emergency nature.

2.6. Statistical analysis

Statistical analyses were performed using Microsoft Excel for Microsoft 365 MSO (Version 16.0.18623.20116) with the Real Statistics Resource Pack add-in. The normality of continuous variables was assessed using the Shapiro–Wilk test. As the data followed a normal distribution, descriptive statistics are presented as mean ± standard error of the mean (SEM), and a p-value <0.05 was considered statistically significant. Linear associations between fetal renal biometric variables (length and area) and continuous parameters such as maternal serum progesterone concentration, biparietal diameter (BPD), gestational age, litter size, and maternal body weight were assessed using Pearson correlation coefficients. To evaluate the effect of categorical variables on renal morphology, one-way analysis of variance (ANOVA) was conducted. The categorical variables included gestational age (40–50 days, 50–55 days, 55–61 days), maternal weight (<5 kg, 5–15 kg, >15–45 kg), serum progesterone level (<2 ng/mL, 2–8 ng/mL, >8 ng/mL), and BPD categories (<2.3 cm, 2.3–2.6 cm, >2.6 cm). However, cortex-to-medulla ratio and renal area were compared only across gestational age and BPD categories, as these two variables are more directly and physiologically linked to fetal development and maturation, and were the primary focus of our hypothesis. For this reason, they were not evaluated against other maternal or litter-related factors. Additionally, to explore the potential of fetal renal size as a maturity marker, receiver operating characteristic (ROC) curve analysis was performed using MedCalc® Statistical Software version 20.218 (MedCalc Software Ltd, Ostend, Belgium). The area under the curve (AUC) was calculated to assess diagnostic performance. The optimal cut-off point was determined using the Youden Index (sensitivity + specificity – 1), and the corresponding sensitivity, specificity, and 95 % confidence intervals were reported.

3. Results

Maternal survival was 100 %, with no intraoperative or postpartum complications recorded in any of the bitches included in the study. Of the 146 ultrasound measurements analyzed, 102 corresponded to pregnancies that concluded via elective or emergency cesarean section. Regarding neonatal outcome, a total of 102 puppies were delivered via cesarean section, of which 86 were born through emergency procedures. Among these, 11 were stillborn and 1 was born alive but died within the first days of life due to a congenital malformation. Thus, 90 puppies survived the neonatal period (0–7 days postpartum). Of the 12 perinatal deaths, three neonates presented visible congenital anomalies: one case of anasarca, one of cheiloschisis (cleft lip), and one with abdominal evisceration. Unfortunately, detailed neonatal data were not consistently available for litters born via vaginal delivery; therefore, neonatal outcomes for this group were not included in the present analysis.

A total of 146 fetal kidney measurements were obtained and analyzed. Renal area and biparietal diameter (BPD) increased significantly with advancing gestational age ($p < 0.001$) and were also positively associated with maternal body weight ($p < 0.001$). The cortex-to-medulla (C/M) ratio showed significant differences among gestational age groups ($p = 0.04$), but not among maternal weight groups ($p = 0.09$). These findings are summarized in Table 2.

Fetal kidney area (Table 3) varied significantly according to maternal serum progesterone levels and fetal biparietal diameter (BPD). Specifically, lower progesterone concentrations were associated with larger renal areas ($p < 0.05$), suggesting that fetal kidney growth may parallel hormonal changes in late gestation. In contrast, the cortex-to-medulla (C/M) ratio remained constant across progesterone groups, indicating limited sensitivity of this parameter to endocrine status. Regarding BPD, both renal area and C/M ratio demonstrated significant differences between groups ($p < 0.001$ and $p = 0.01$, respectively), reinforcing their potential as indirect indicators of fetal maturation.

Table 4 presents the Pearson correlation coefficients (r) and

Table 2
Mean, standard error (SE) and p-value calculated for renal area, cortex/medulla ratio (C/M-r) and Biparietal Diameter (BPD) between groups of gestational age and bitch weight.

Groups	*Classification*	*Parameters*	*Mean*		*SE*	*p-value*
GESTATIONAL AGE	40–50 days	Renal Area (cm)	3.1[a]		0.07	<0.001
	50–55 days		5.07[b]		0.06	
	55–61 days		7.31[c]		0.03	
	40–50 days	C/M-r (cm)	0.91[a]		0.01	0.04
	50–55 days		0.63[b]		0.01	
	55–61 days		0.78[c]		0.01	
	40–50 days	BPD (cm)	1.94[a]		0.02	<0.001
	50–55 days		2.31[b]		0.01	
	55–61 days		2.68[c]		0.01	
BITCH WEGHT (kg)	<5 kg	Renal Area (cm)	4.57[a]	0.13	<0.001	
	5–15 kg		5.08[b]	0.03		
	>15–45 kg		7.65[c]	0.07		
	<5 kg	C/M-r (cm)	0.64	0.02	0.09	
	5–15 kg		0.78	0.01		
	>15–45 kg		0.90	0.01		
	<5 kg	BPD (cm)	2.28[a]	0.06		
	5–15 kg		2.34[b]	0.04	0.03	
	>15–45 kg		2.62[c]	0.07		

[abc]: Different letters in the same row and category denote significant differences ($p < 0.05$).

Table 3
Fetal kidney measurements (means and std Err) by progesterone levels and Biparietal Diameter (BPD).

Groups	Classification	Parameters	Mean	SE	p-value
PROGESTERONE LEVELS (ng/ml)	<2 ng/ml	Renal Area (cm)	7.03[a]	0.07	<0.001
	2–8 ng/ml		5.79[b]	0.05	
	>8 ng/ml		4.52[c]	0.05	
	<2 ng/ml	C/M-r (cm)	0.83	0.01	1
	2–8 ng/ml		0.80	0.01	
	>8 ng/ml		0,80	0.01	
BIPARIETAL DIAMETER (cm)	<2,3 cm	Renal Area (cm)	3.28[a]	0.04	<0.001
	2.3–2.6 cm		5.82[b]	0.04	
	>2.6 cm		7.75[c]	0.03	
	<2,3 cm	C/M-r (cm)	0.92[a]	0.01	
	2.3–2.6 cm		0.66[b]	0.01	0.01
	>2.6 cm		0.80[c]	0.01	

[abc]: Different letters in the same row and category denote significant differences ($p < 0.05$).

Table 4
Pearson correlation coefficients (r) and p-values between fetal kidney size (length and area) and maternal (gestational age, bitch weight, litter size, progesterone levels) and fetal variables (biparietal diameter).

Paramethers	Fetal kidney parameters			
	Length		Area	
	Correlation (*r*)	p-value	Correlation	p-value
Progesterone levels	−0.39[a]	<0.001	−0.47[a]	<0.001
Biparietal Diameter	0.73[a]	<0.001	0.80[a]	0
Gestational age	0.57[a]	<0.001	0.64[a]	<0.001
Litter size	0.08[ns]	0.44	0.14 [ns]	0.22
Bitch weight	0.33[a]	0.02	0.43[a]	<0.001

[a] Correlation was significant at 0,5 % ($p < 0.001$); **Correlation was significant at 0,5 % ($p < 0.005$), * Correlation is significant at 1 % level ($p < 0.05$); ns Correlation was not significant.

corresponding p-values between fetal kidney length and area and the following continuous variables: maternal serum progesterone concentration (P4), fetal biparietal diameter (BPD), gestational age, litter size, and maternal body weight. A strong and statistically significant positive correlation was observed between BPD and both fetal kidney length and area ($r = 0.73$; $p < 0.001$). Gestational age also showed a significant positive correlation with kidney length and area ($r = 0.57$; $p < 0.001$). Maternal body weight was moderately and positively correlated with kidney length and area, with both associations reaching statistical significance ($r = 0.33$ and $r = 0.43$ respectively; $p < 0.05$). In contrast, serum progesterone concentration was negatively correlated with both kidney parameters, and these correlations were statistically significant ($r = -0.39$ and $r = -0.47$ respectively; $p < 0.05$). No significant correlations were found between litter size and either kidney length or area ($r = 0.08$ and $r = 0.14$; $p > 0.05$).

A receiver operating characteristic (ROC) curve was generated to assess the diagnostic performance of fetal kidney area in discriminating between biparietal diameter (BPD) thresholds. The ROC analysis demonstrated excellent discriminatory ability, with an area under the curve (AUC) of 1.00 (Fig. 5), indicating perfect classification within the current sample.

The analysis identified an optimal cut-off value for fetal kidney area at 6.78 cm^2, yielding a sensitivity of 100 % and a specificity of 65.3 %, with a corresponding Youden index of 0.347. The most effective cut-off values ranged from 6.5 to 7.3 cm^2, with progressive increases in the Youden index. The maximum index was reached at a kidney area of 13.52 cm^2, at which both sensitivity and specificity were 100 %.

A linear regression analysis was performed to evaluate the predictive relationship between fetal biparietal diameter (BPD) and renal area. The resulting model demonstrated a statistically significant association, with a multiple correlation coefficient (R) of 0.79 and an adjusted coefficient of determination (R^2) of 0.623, indicating that approximately 62.3 % of the variability in renal area can be explained by BPD measurements. The regression equation derived from the model was: Renal area (cm^2) $= -6.93 + 5.16 \times$ BPD (cm). The model exhibited a mean squared error (MSE) of 0.422 and a standard error of 0.234. The analysis of variance (ANOVA) confirmed the significance of the model ($F(1,138) = 230.74$, $p < 0.001$). The scatter plot with the fitted regression line is shown in Fig. 6, illustrating the positive linear relationship between BPD and fetal kidney area.

A receiver operating characteristic (ROC) curve was constructed to evaluate the diagnostic performance of fetal kidney area in predicting maternal serum progesterone concentrations. The area under the curve (AUC) was 0.70, indicating moderate discriminatory capacity (Fig. 7). The optimal cut-off point, based on the highest Youden index (0.333), was identified at a fetal kidney area of 4.22 mm^2, yielding a sensitivity of 100 % and a specificity of 33.3 %. However, specificity values remained

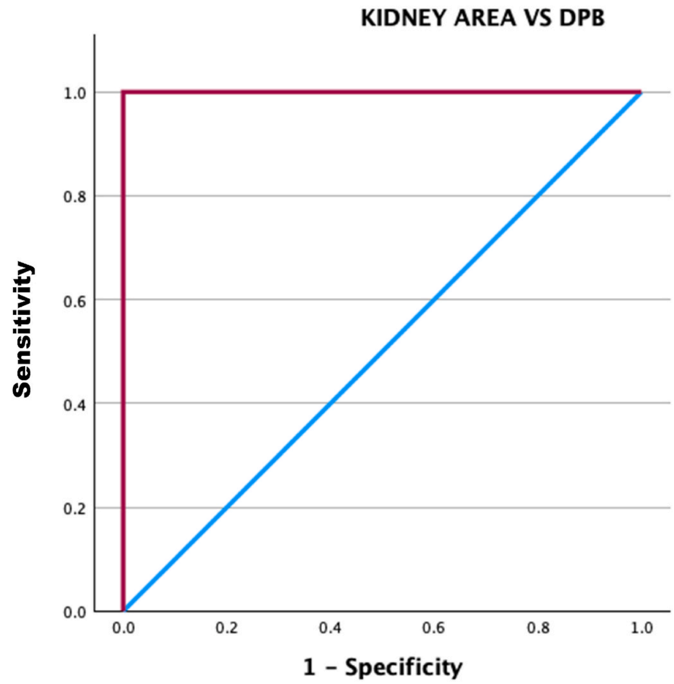


Fig. 5. Receiver operating characteristic (ROC) curve illustrating the diagnostic performance of fetal kidney area in predicting fetal biparietal diameter (BPD) thresholds.

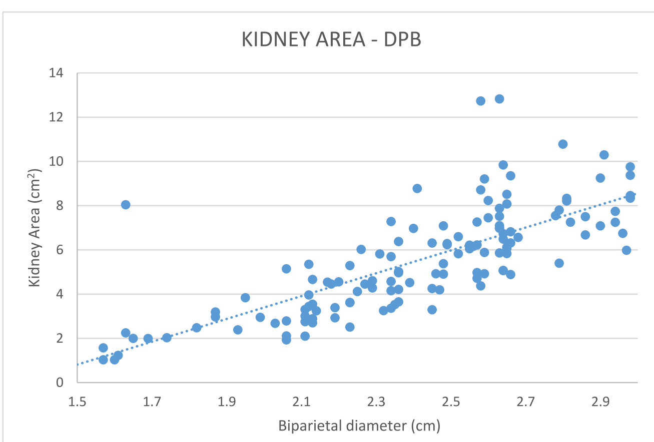


Fig. 6. Scatter plot showing the linear relationship between fetal biparietal diameter (BPD) and fetal kidney area. The dotted line represents the fitted linear regression model.

low across most cut-off values, limiting the overall diagnostic utility of this parameter when used independently.

4. Discussion

Accurate prediction of the parturition date is critical in canine obstetrics to prevent dystocia, reduce perinatal mortality, and optimize the management of high-risk pregnancies and neonatal care. Current estimation methods [1,6,12] primarily rely on maternal indicators, such as serum progesterone concentrations, and fetal measurements, particularly biparietal diameter (BPD) [21]. However, BPD alone may have limitations in certain clinical scenarios [22], including brachycephalic breeds [23] or late gestation. In this context, the present study investigated fetal renal biometry—specifically kidney area and cortex-to-medulla (C/M) ratio—as potential complementary

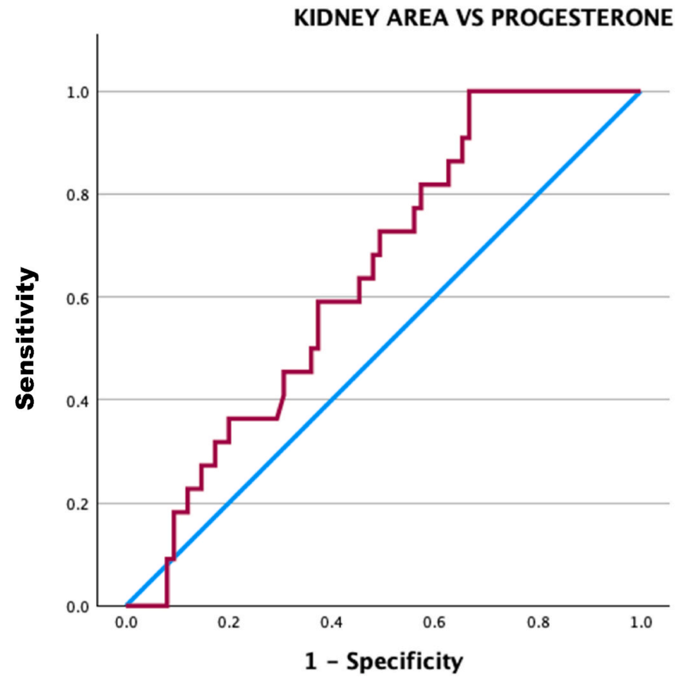


Fig. 7. Receiver operating characteristic (ROC) curve showing the diagnostic performance of fetal kidney area in predicting maternal serum progesterone concentration.

ultrasonographic markers to assess fetal development and estimate gestational age more accurately, thereby improving the prediction of the expected date of parturition. Among the variables analyzed, fetal kidney area showed the strongest correlation with biparietal diameter (BPD), suggesting that renal area may serve as a reliable marker of fetal development, particularly in cases where visualization of the fetal head is suboptimal or not feasible. To the authors' knowledge, this is the first study in pregnant bitches to report a direct association between fetal renal area and BPD, underscoring its potential clinical relevance.

It is well documented that the predictive accuracy of BPD decreases during the final stages of gestation, with reported accuracies ranging from 50.9 % (±1 day) to 69.8 % (±2 days) during the ninth week [12,24]. Furthermore, several authors have reported significant variability in biparietal diameter (BPD) measurements among different breeds [25,26] and body sizes of dogs and cats [27]. These findings suggest that breed-specific or size-adjusted predictive formulas may be more appropriate to improve the accuracy of gestational age estimation and parturition prediction [3,6]. In this context, renal biometry—especially kidney length—has been proposed as an alternative parameter, and a recent study developed a regression-based formula using renal length to estimate gestational age [28]. In addition to predicting parturition timing, fetal renal biometry may offer indirect information about the progression of renal development as part of overall fetal maturation. Although renal ontogenesis in the canine species is considered to occur relatively early in gestation, deviations from expected renal size relative to fetal head dimensions might reflect broader developmental delays. Thus, establishing reference associations between renal area and biparietal diameter could aid in assessing whether organ growth is appropriate for a given fetal size, potentially contributing to the evaluation of fetal well-being and postnatal viability. This approach may offer a non-invasive tool to support clinical decision-making in high-risk pregnancies, contributing to improved neonatal outcomes. Furthermore, although renal area may be less affected by skull morphology than BPD — particularly in brachycephalic or dolichocephalic breeds — it is nonetheless significantly influenced by maternal body weight. This suggests that renal area, while potentially more consistent across cranial conformations, is not entirely breed-independent and should be interpreted within the context of maternal size [23,29]. In contrast, renal development appears to follow a more conserved ontogenic trajectory, potentially minimizing interindividual variation and enhancing the applicability of predictive models based on renal biometry across different canine morphotypes. Neither renal area nor kidney length showed a statistically significant correlation with litter size, consistent with previous findings [12]. Although the present study included 147 fetal kidney measurements, further research incorporating breed-specific subgroups and stratification by maternal body weight could yield more refined conclusions.

This finding aligns with the general concept that larger bitches may provide a more favorable intrauterine environment for fetal growth, possibly due to enhanced uteroplacental blood flow, greater uterine surface area, or overall improved nutrient transfer capacity. Although no previous studies have specifically linked maternal weight to fetal renal growth, Luvoni and Beccaglia (2006) reported that fetal biometric parameters such as biparietal diameter (BPD) vary according to maternal size and breed, indirectly supporting the hypothesis that maternal phenotype can influence fetal organogenesis [3]. Although maternal body weight was categorized into three groups to account for size variability, further stratification by breed or body conformation was not performed due to sample size limitations. Future studies with larger and more balanced populations may allow for more detailed assessments of the potential effects of maternal size on fetal renal biometry.

In contrast, the cortex-to-medulla (C/M) ratio showed no significant correlation with maternal weight, serum progesterone levels, or gestational age. Milani et al. (2021) emphasized that although renal length and width are consistent and practical indicators of gestational progression, echotextural features like the C/M ratio may be influenced by multiple factors, including inter-fetal variability, ultrasound resolution, and operator subjectivity [6]. Interestingly, the lack of a significant correlation between the C/M ratio and gestational age observed in this study limits its applicability as a predictor of parturition timing or fetal maturity. One possible explanation is that corticomedullary differentiation in the canine fetus may follow a non-linear progression, with most morphological changes occurring within a relatively narrow window near term [18]. In such a scenario, cross-sectional assessments may fail to capture meaningful variation unless data collection is specifically focused on the final stages of gestation. Alternatively, the C/M ratio may not serve as a direct indicator of renal structural maturation, but rather be modulated by transient physiological factors such as fetal hydration, renal perfusion, or functional status. These findings underscore the need for future longitudinal studies involving serial ultrasonographic evaluations of individual fetuses across multiple time points, ideally integrating morphometric and functional parameters to better characterize renal development and its clinical implications. The integration of advanced imaging modalities, such as contrast-enhanced ultrasonography or elastography, may provide deeper insights into the structural and functional maturation of fetal kidneys in the canine species.

Both fetal kidney length and area were significantly associated with gestational age, exhibiting a progressive increase as parturition approached. This trend parallels the growth pattern observed for biparietal diameter (BPD), further supporting the potential utility of renal area as an indirect estimator of fetal maturity. These findings suggest that kidney biometry may not only complement BPD in clinical estimations but could also be incorporated into novel predictive models to improve the accuracy of parturition date estimation. To date, only one published formula includes fetal kidney length as a predictor, and its applicability is limited to the final 10 days of gestation [19]. However, its predictive precision remains suboptimal, particularly in estimating the exact interval until delivery. The present results indicate that renal area may provide a broader window of applicability throughout the second half of gestation and could enhance the performance of existing models when used in combination with established parameters. The observed correlation between renal area and gestational age likely reflects not only morphometric growth but also the underlying functional maturation of the fetal kidney. Although ultrasonographic biometry does not allow direct assessment of renal function, the consistent increase in kidney area throughout gestation may reflect ongoing nephrogenesis and structural complexity. Although our findings suggest that fetal kidney area increases progressively throughout gestation and correlates with parameters such as biparietal diameter (BPD) and gestational age, we do not propose that renal biometry alone should be used to determine the timing of elective cesarean section. Instead, we suggest that it may serve as an additional non-invasive marker of fetal maturation, particularly in breeds or cases where gestational timing is uncertain. Further studies with larger sample sizes and breed-specific reference values are needed to establish clinical cut-offs and validate this approach.

Renal biometry has been evaluated in other species such as humans, cattle, sheep, and horses [29–31]. In human medicine, several studies have demonstrated a positive correlation between fetal kidney size and gestational age, supporting its potential as a reliable indicator of fetal development [32,33]. Notably, renal measurements are reported to be less affected by fetal positioning compared to biparietal diameter (BPD), which may enhance their reliability in routine clinical practice [30,31]. In equine fetuses, although limited in number, studies have shown a positive association between renal area and gestational progression, suggesting that renal biometry could serve as a complementary parameter for fetal age estimation [32]. Despite the scarcity of equivalent studies in canine reproduction, these findings support the hypothesis that renal area may indirectly reflect functional maturation. Future research integrating renal biometry with functional tools such as Doppler velocimetry or postnatal renal performance indices could provide a more comprehensive understanding of fetal renal development.

Although a significant correlation was identified between renal area and maternal serum progesterone concentrations, the clinical utility of kidney biometry for predicting hormonal changes appears limited. A decrease in serum progesterone to below 2 ng/mL is generally considered a reliable indicator of imminent parturition within 24 h [1,7]. However, hormonal monitoring requires serial blood sampling and is subject to individual variability, particularly in singleton pregnancies, where incomplete luteolysis may delay the expected drop in progesterone levels [34,35]. Several authors have described significant variability in prepartum progesterone concentrations under specific clinical conditions, such as singleton pregnancies, in which levels may not decline below 2 ng/mL near term [28]. This observation raises concerns regarding the predictive reliability of serum progesterone when used as a sole indicator of impending parturition, particularly in atypical gestational scenarios. In this context, renal biometry may offer a non-invasive adjunct in reproductive monitoring protocols, although further validation is required. The use of progesterone monitoring in this study served as an auxiliary tool to assess the proximity of parturition, as progesterone levels are known to decline in the final days of gestation. While progesterone levels values were not used to diagnose pregnancy, they provided context for interpreting fetal development and timing labor. The observed correlation between progesterone levels and kidney area was not intended to imply causality, but to explore whether hormonal and anatomic indicators of maturity might progress in parallel. This perspective may prove useful in future studies investigating the integration of hormonal and morphometric data to improve obstetric decision-making in the bitch.

5. Conclusion

Fetal kidney area, in particular, may serve as an indirect marker of fetal maturity and could be clinically relevant in the context of elective cesarean section planning. Assessing whether fetal organ development is sufficient prior to delivery is essential to prevent iatrogenic prematurity, especially in high-risk pregnancies and brachycephalic breeds predisposed to dystocia. If further validated, fetal renal biometry could complement current protocols by providing additional criteria for the optimal timing of surgical parturition. While ultrasonography is widely established in veterinary obstetrics, additional research focusing on fetal organ development—particularly renal growth patterns—in pregnant bitches is warranted. The development of robust, breed-adjusted predictive models based on kidney biometry holds promise for improving clinical decision-making, minimizing perinatal morbidity, and enhancing the safety of canine deliveries.

CRediT authorship contribution statement

Rodríguez Raquel: Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Miguel Batista:** Writing – review & editing, Supervision, Conceptualization. **Alonso Sara:** Methodology, Investigation, Formal analysis. **Iusupova Kseniia:** Writing – review & editing, Formal analysis.

Declaration of competing interest

None.

References

[1] Beccaglia M, Alonge S, Trovo' C, Luvoni GC. Determination of gestational time and prediction of parturition in dogs and cats: an update. Reprod Domest Anim 2016; 51:12–7. https://doi.org/10.1111/rda.12782. 2016.

[2] Groppetti D, Pecile A, Del Carro AP, Copley K, Minero M, Cremonesi F. Evaluation of newborn canine viability by means of umbilical vein lactate measurement, apgar score and uterine tocodynamometry. Theriogenology 2010;74(7):1187–96. https://doi.org/10.1016/j.theriogenology.2010.05.020.

[3] Luvoni GC, Grioni A. Determination of gestational age in medium and small size bitches using ultrasonographic fetal measurements. J Small Anim Pract 2000;41 (7):292–4. https://doi.org/10.1111/j.1748-5827.2000.tb03204.x.

[4] Xavier GM, Bittencourt RF, Planzo Fernandes M, Biscarde CEDA, Carneiro IDMB, Costa EO. & loiola, M. V. G. Evaluation of embryo–foetal biometry and its correlation with parturition date in toy poodle bitches. Reprod Domest Anim 2024; 59(6):e14621. https://doi.org/10.1111/rda.14621.

[5] Bolis B, Prandi A, Rota A, Faustini M, Veronesi MC. Cortisol fetal fluid concentrations in term pregnancy of small-sized purebred dogs and its preliminary relation to first 24 hours survival of newborns. Theriogenology 2017;88:264–9. https://doi.org/10.1016/j.theriogenology.2016.09.037.

[6] Milani C, Rota A, Olsson U, Paganotto A, Holst BS. Serum concentration of mineralocorticoids, glucocorticoids, and sex steroids in peripartum bitches. Domest Anim Endocrinol 2021;74:106558. https://doi.org/10.1016/j.domaniend.2020.106558.

[7] De Cramer KGM, Nöthling JO. The precision of predicting the time of onset of parturition in the bitch using the level of progesterone in plasma during the preparturient period. Theriogenology 2018;107:211–8. https://doi.org/10.1016/j.theriogenology.2017.11.018.

[8] Kutzler MA, Mohammed HO, Lamb SV, Meyers-Wallen VN. Accuracy of canine parturition date prediction from the initial rise in preovulatory progesterone concentration. Theriogenology 2003;60(6):1187–96. https://doi.org/10.1016/s0093-691x(03)00109-2.

[9] Kutzler MA, Yeager AE, Mohammed HO, Meyers-Wallen VN. Accuracy of canine parturition date prediction using fetal measurements obtained by ultrasonography. Theriogenology 2003;60(7):1309–17. https://doi.org/10.1016/S0093-691X(03)00146-8.

[10] Pedersen MD, Klesiewicz AB, Marqvorsen HM, Pedersen HG, Secher JBM. Ultrasonographic determination of day of parturition based on fetal biparietal diameter in pregnant bitches: comparison of simple linear regression and mixed linear regression in breed-specific models and maternal weight grouped models. Theriogenology 2022;184:124–31. https://doi.org/10.1016/j.theriogenology.2022.03.001.

[11] Arlt S. Canine ovulation timing: a survey on methodology and an assessment on reliability of vaginal cytology. Reprod Domest Anim 2018;53:53–62.

[12] Siena G, Milani C. Usefulness of maternal and fetal parameters for the prediction of parturition date in dogs. Animals 2021;11(3):878. https://doi.org/10.3390/ani11030878.

[13] Conley AJ, Gonzales KL, Erb HN, Christensen BW. Progesterone analysis in canine breeding management. Vet Clin: Small Animal Practice 2023;53(5):931–49.

[14] Siena G, Romagnoli S, Drigo M, Contiero B, Di Nardo F, Milani C. Ultrasonographic changes in fetal gastrointestinal motility during the last ten days before parturition in dogs. Front Vet Sci 2022;9:1000975.

[15] Gil EM, Garcia DA, Froes TR. In utero development of the fetal intestine: sonographic evaluation and correlation with gestational age and fetal maturity in dogs. Theriogenology 2015;84(5):681–6. https://doi.org/10.1016/j.theriogenology.2015.04.030.

[16] Giannico AT, Garcia DAA, Gil EMU, Sousa MG, Froes TR. Assessment of umbilical artery flow and fetal heart rate to predict delivery time in bitches. Theriogenology 2016;86(7):1654–61. https://doi.org/10.1016/j.theriogenology.2016.03.042.

[17] Siena G, di Nardo F, Contiero B, Banzato T, Milani C. Preliminary evaluation of cortical and medullary echogenicity in normal canine fetal kidneys during the last 10 days of pregnancy. Vet Sci 2023;10(11):639.

[18] Gil EMU, Garcia DAA, Giannico AT, Froes TR. Early results on canine fetal kidney development: ultrasonographic evaluation and value in prediction of delivery time. Theriogenology 2018;107:180–7. https://doi.org/10.1016/j.theriogenology.2017.11.015.

[19] Siena G, di Nardo F, Contiero B, Milani C. Clinical use of the canine foetal kidney formula in dogs of different maternal sizes during the last ten days before parturition. Vet Res Commun 2023;47(3):1653–63. https://doi.org/10.1007/s11259-023-10120-2.

[20] Batista M, Moreno C, Vilar J, Golding M, Brito C, Santana M, Alamo D. Neonatal viability evaluation by apgar score in puppies delivered by cesarean section in two brachycephalic breeds (english and French bulldog). Anim Reprod Sci 2014;146 (3–4):218–26.

[21] Lopate C. Estimation of gestational age and assessment of canine fetal maturation using radiology and ultrasonography: a review. Theriogenology 2008;70(3): 397–402. https://doi.org/10.1016/j.theriogenology.2008.05.034.

[22] Arlt SP. The bitch around parturition. Theriogenology 2020;150:452–7. https://doi.org/10.1016/j.theriogenology.2020.02.046.

[23] Maronezi MC, Simões APR, Uscategui RA, Pavan L, Rodrigues MG, Mariano RS, Feliciano MA. Gestational echobiometry in brachycephalic bitches using high-definition ultrasonography. Pesqui Vet Bras 2021;41:e06650. https://doi.org/10.1590/1678-5150-PVB-6650.

[24] De Cramer KGM, Nöthling JO. Is the biparietal diameter of fetuses in late gestation too variable to predict readiness for cesarean section in dogs? Theriogenology 2018;113:50–5. https://doi.org/10.1016/j.theriogenology.2018.02.005.

[25] Vieira CDA, Bittencourt RF, Biscarde CEA, Fernandes MP, Nascimento AB, Romão EA, Loiola MVG. Estimated date of delivery in Chihuahua breed bitches, based on embryo-fetal biometry. Anim Reprod 2020;17:e20200037. https://doi.org/10.1590/1984-3143-AR2020-0037. assessed by ultrasonography.

[26] Cecchetto M, Milani C, Vencato J, Sontas H, Mollo A, Contiero B, Romagnoli S. Clinical use of fetal measurements to determine the whelping day in German shepherd breed bitches. Anim Reprod Sci 2017;184:110–9. https://doi.org/10.1016/j.anireprosci.2017.07.005.

[27] Górka A, Ochota M, Niżański W. Ultrasound biometry for estimating delivery dates in small and medium breed cats. Theriogenology 2025;232:56–69. https://doi.org/10.1016/j.theriogenology.2024.10.030.

[28] Siena G, Di Nardo F, Romagnoli S, Mollo A, Contiero B, Milani C. Relationship between days before parturition and fetal kidney length, cortical thickness, medullary thickness and their ratio in dogs. Theriogenology 2022;194:58–63. https://doi.org/10.1016/j.theriogenology.2022.09.021.

[29] Hussein AK, Sullivan M, Penderis J. Effect of brachycephalic, mesaticephalic, and dolichocephalic head conformations on olfactory bulb angle and orientation in dogs as determined by use of in vivo magnetic resonance imaging. Am J Vet Res 2012;73(7):946–51. https://doi.org/10.2460/ajvr.73.7.946. Jul.

[30] Konje J, Abrams K, Bell S, Taylor D. Determination of gestational age after the 24th week of gestation from fetal kidney length measurements. Ultrasound Obstet Gynecol 2002;19(6):592–7. https://doi.org/10.1046/j.1469-0705.2002.00704.x.

[31] Abonyi EO, Eze CU, Agwuna KK, Onwuzu WS. Sonographic estimation of gestational age from 20 to 40 weeks by fetal kidney lengths' measurements among pregnant women in portharcourt, Nigeria. BMC Med Imag 2019;19(1):72. https://doi.org/10.1186/s12880-019-0371-z.

[32] Murase H, Endo Y, Tsuchiya T, Kotoyori Y, Shikichi M, Ito K, Nambo Y. Ultrasonographic evaluation of equine fetal growth throughout gestation in normal mares using a convex transducer. J Vet Med Sci 2014;76(7):947–53. https://doi.org/10.1292/jvms.13-0259.

[33] Choudhary A, Sibia P, Kaur S, Gupta S, Gambhir P, Kaur R. Evaluation of fetal kidney length as a marker for fetal biometry. Arch Gynecol Obstet 2024;310(3): 1451–9. https://doi.org/10.1007/s00404-024-07374-9.

[34] Fontbonne A. Infertility in bitches and queens: recent advances. Rev Bras Reprod Anim 2011;35(2):202–9.

[35] Kowalewski MP. Luteal regression vs. prepartum luteolysis: regulatory mechanisms governing Canine corpus luteum function. Reprod Biol 2014;14(2):89–102. https://doi.org/10.1016/j.repbio.2013.11.004.

animals

MDPI

Article

Glucose Levels as a Key Indicator of Neonatal Viability in Small Animals: Insights from Dystocia Cases

Raquel Rodríguez-Trujillo , Miguel Batista-Arteaga * and Kseniia Iusupova

Unit of Reproduction, University Institute of Biomedical Research and Health, University of Las Palmas de Gran Canaria, Transmontaña s/n, 35413 Arucas, Las Palmas, Spain; raquel.rodriguez@ulpgc.es (R.R.-T.); ksfedorchak23@gmail.com (K.I.)

* Correspondence: miguel.batista@ulpgc.es

Simple Summary: Neonatal mortality in small animals, such as dogs and cats, can be alarmingly high, especially in situations of difficulty during delivery. This study investigates how glucose levels in newborns can serve as a key indicator of their viability. We analyzed 54 mothers and their 284 neonates to understand the relationship between maternal weight, litter size, and neonatal glucose levels. Our findings indicate that a glucose level below 79.50 mg/dL is associated with a decreased likelihood of survival in newborns. Additionally, neonates with low birth weight and those from smaller litters showed a higher prevalence of hypoglycemia. The results highlight the importance of monitoring glucose levels in neonates, as hypoglycemia can lead to serious health issues. This research emphasizes the need for timely interventions to improve neonatal outcomes and reduce mortality in small animal litters, thereby benefiting veterinarians and pet owners alike.

Abstract: Neonatal mortality rates in small animals can reach alarming figures, with perinatal mortality ranging from 20% to 40%, primarily due to the abrupt transition from intrauterine to extrauterine environments. This study investigates the critical role of glucose levels in neonatal viability, particularly in cases of dystocia and fetal stress during cesarean sections. A cohort of 54 mothers and their 284 neonates was analyzed, focusing on maternal weight, litter size, and corresponding neonatal glucose levels. The results indicated a significant relationship between glucose concentrations and Apgar scores, with a cutoff established at 79.50 mg/dL for optimal neonatal viability. Additionally, a higher prevalence of hypoglycemia was documented in neonates with low birth weight and those from smaller litters. The findings underscore the importance of monitoring glucose levels in neonates, as hypoglycemia is associated with various pathologies, including sepsis and portosystemic shunts. Overall, this study highlights the necessity for prompt assessment of glucose levels to improve neonatal outcomes and reduce mortality in small animals.

Keywords: glucose; neonate; Apgar score; viability; mother

Academic Editor: Monica De Los Reyes

Received: 4 February 2025
Revised: 25 February 2025
Accepted: 21 March 2025
Published: 27 March 2025

Citation: Rodríguez-Trujillo, R.; Batista-Arteaga, M.; Iusupova, K. Glucose Levels as a Key Indicator of Neonatal Viability in Small Animals: Insights from Dystocia Cases. *Animals* **2025**, *15*, 956. https://doi.org/10.3390/ani15070956

1. Introduction

Neonatal mortality during the first years of life in small animals presents an alarming rate, reaching figures as high as 40%, which raises significant concern among both breeders and veterinarians [1]. Perinatal mortality, which ranges from 20% to 40%, is particularly high compared to other domestic species [2]. This phenomenon is attributed to the abrupt transition from the intrauterine to the extrauterine environment, necessitating rapid and effective adaptation by the neonates [3]. This adaptation is intrinsically linked to the development, maturity, and viability of the individuals at the time of birth [2].

The primary cause of neonatal mortality is associated with immaturity at the time of birth, rendering these individuals particularly vulnerable [1]. Mortality can manifest during formation in utero, during expulsion, immediately after birth, or in the first weeks of life, with the first week being the most critical [3]. At the time of birth, the most common causes of mortality include asphyxia, acidosis, glucose imbalance, and hypothermia [2,4,5]. However, during the neonatal period, infections, particularly those of bacterial origin, represent the second most common cause of mortality, following hypoxia [6,7]. Among the most characteristic clinical signs of neonates affected by infections is the neonatal triad, which includes hypothermia, hypoglycemia, and dehydration [6]. The depression and lethargy observed in these individuals can result in the loss of the suckling reflex, which in turn leads to decreased intake of colostrum or milk and, consequently, weight loss [6]. This situation may trigger hypoglycemia, which, according to some authors, occurs in 65.5% of neonates with bacterial infections [6]. Additionally, neonatal hypoxia and hypercapnia are consequences of pulmonary immaturity. However, these conditions can also develop due to umbilical cord occlusion during whelping. If this condition persists, it may lead to respiratory acidosis, accumulation of acid radicals, and subsequently to metabolic acidosis, decreased glucose levels, and bradycardia [8,9]

Neonatal viability refers to the condition and reactivity of the neonate, factors that influence its survival. A neonate is considered viable when it exhibits efficient breathing after birth, emits clear cries, actively seeks the mammary gland, is capable of properly grasping the nipple, and demonstrates normal neonatal reflexes, including the suckling reflex [10]. The Apgar score has been recognized as an effective method for assessing neonatal viability in various species, including humans, horses, cows [11], and dogs. This method allows for the classification of neonatal viability, and based on the obtained score, the prognosis for survival in the first 24–48 h can be determined [12]. Several authors have described classification ranges: a score of 7–10 points indicates a survival rate of 95–100% [10,13,14]; a score of 4–6 points suggests a survival rate of 94–100% [9]; and a score of 0–3 points is indicative of a survival rate of 0–39% [3,10,15].

Newborns have a predisposition to suffer from hypoglycemia, attributed to hepatic immaturity, limited energy reserves, and immaturity in glucose balance [15–17]. This situation, along with the low capacity for gluconeogenesis and glycogenolysis, impairs the ability to maintain glucose levels within the normal range during the first days of life [4]. Additionally, the loss of glucose through urine, low muscle mass, absence of adipose tissue, and limited capacity to utilize free fatty acids as an alternative energy source are factors that increase the risk of hypoglycemia [18].

Hypoglycemia may occur as a consequence of various neonatal pathologies, such as neonatal sepsis or endotoxemia, hepatic dysfunction, low birth weight, as well as maternal factors like placental abnormalities or gestational diabetes [10,19]. This disorder has been identified as one of the leading causes of neonatal mortality and presents characteristic symptoms that include neurological signs such as lethargy, absence of suckling reflex, depression, seizures, coma, and even death [10]. Initially, a cutoff value of 40 mg/dL was established as indicative of neonatal hypoglycemia [19]; however, subsequent studies have suggested different cutoff values. A value of 40 mg/dL was associated with a low Apgar score and decreased reflexes, whereas a value of 28 mg/dL was linked to a higher probability of mortality during the first 7 days of life [20].

Other authors have indicated that values below 37 mg/dL within the first 8 h of life are associated with an increased mortality risk in the first 24 h [21]. Furthermore, additional investigations have evaluated the influence of hyperglycemia in neonates, noting that elevated glucose levels correlate with an increase in neonatal mortality; additionally, lower Apgar scores are associated with a higher risk of mortality alongside higher blood glucose levels [1,22]. Nonetheless, the conclusion of several studies is that glucose measurement does not serve as a useful tool for predicting neonatal mortality [22–24]. During the first weeks of life, different ranges of glucose values can be observed, with values between days 1 and 3 ranging from 76 to 155 mg/dL, between days 8 and 10 from 101 to 161 mg/dL, and between the fourth and fifth week from 121 to 158 mg/dL [10]. Recent studies measuring glucose immediately after birth and before the intake of colostrum report a normal value of 73.16 mg/dL. However, this value may vary depending on the type of delivery, being generally higher in neonates born via cesarean section and in cases of dystocia, reaching levels above 120 mg/dL [10].

Neonatal hypoglycemia may also be associated with various neonatal conditions, such as low birth weight, which typically leads to an increased metabolism and consequently a greater energy requirement. In neonates with low birth weight, the liver is often smaller, implying that hepatic reserves are reduced, thereby predisposing them to hypoglycemia [25]. Additionally, factors such as breed may influence this predisposition, as smaller or "toy" breeds exhibit greater susceptibility compared to larger breeds [25].

The process of parturition generates a state of stress for both the mother and the fetus, which can result in an increase in glucose production due to processes such as glycogenolysis and gluconeogenesis [24]. Glucose levels in neonates subjected to stressful situations correlate directly with maternal glucose levels [24].

Further investigations have explored the concentrations of glucose in amniotic fluid, observing a correlation between these concentrations and an increased probability of mortality. A positive correlation has been indicated between low glucose concentration and a higher likelihood of mortality at the time of birth [12]. However, no correlation was found with other parameters, such as the Apgar score [12]. For this reason, several authors have described that glucose concentrations in amniotic fluid correlate more closely with the metabolic maturity of the neonate [12,23]. Moreover, immaturity at the time of birth has also been investigated in relation to the renal function of the neonate, through urinalysis [5]. These authors suggest that the presence of glucose in the urine may indicate alterations in the functioning of the renal tubules as well as in the reabsorption processes [5].

The objective of the present study was to analyze glucose levels and how they influence neonatal viability. Additionally, this study evaluated how maternal glucose and other factors could alter blood glucose concentrations. Currently, some authors have established cutoff values for neonatal glucose, showing differences among them; however, to the best of the authors' knowledge, no study has measured glucose levels immediately after birth without neonatal food intake and under conditions of dystocia or fetal stress. Immediate glucose measurement is crucial in these neonates, as dystocia and fetal distress induce metabolic responses that can compromise viability. In these cases, hypoxia and activation of the sympathetic system may alter glucose homeostasis, affecting the neonate's ability to adapt to extrauterine life. Evaluating glucose at this critical moment allows the identification of neonates at risk, optimizing clinical decision-making to improve survival rates. Furthermore, there are no authors who have assessed whether there are differences in the cutoff values between brachycephalic and non-brachycephalic neonates. Regarding the evaluation of maternal glucose concentrations, few authors have investigated how these levels change during gestation and how other factors, such as litter size, may influence them.

2. Materials and Methods

2.1. Animals

For this study, a total of 54 mothers along with their respective litters were included, representing a total of 284 neonates. The mothers were categorized according to their breed, age, and weight, and the size of the litter was considered after the procedure was performed.

Before carrying out the surgical procedure, the mothers underwent a physical examination in which parameters such as heart rate, respiratory rate, rectal temperature, and mucosal color were evaluated. Additionally, a complete blood analysis was performed, which included the measurement of glucose concentration.

The procedure was conducted at the Veterinary Clinical Hospital of the University of Las Palmas de Gran Canaria, over a two-year period of sample collection, from 2022 to 2024. The entire protocol was supervised and approved by the corresponding ethics committee, and the owners of the bitches were informed about the procedure prior to its execution.

All bitches included in this study were classified as ASA II (American Society of Anesthesiologists Physical Status Classification System), as they were not considered completely hemodynamically stable due to gestation and the modifications this entails, although they presented no functional limitations at the time of the intervention.

2.2. Experimental Design

The bitches were classified based on their weight and the size of the litter. For weight, two groups were defined: bitches weighing less than 10 kg (n = 22) and bitches weighing more than 10 kg (n = 32). Regarding litter size, three groups were established: litters of 1–2 neonates (n = 12), litters of 3–5 neonates (n = 27), and litters of more than 5 neonates (n = 15). Additionally, from the 54 participating mothers (n = 54), a total of 15 mothers were randomly selected for pre-surgical and post-surgical glucose measurements, with the aim of determining whether significant differences existed in the values after surgery.

A rigorous procedure was followed in the data collection of the mothers and the neonates to ensure that this did not impose additional stress or interfere with neonatal viability.

The neonates were also classified according to the same criteria as the mothers to assess whether there was maternal influence on the obtained results. Two groups were established based on maternal weight: neonates from mothers weighing more than 10 kg (n = 109) and neonates from mothers weighing less than 10 kg (n = 74). Likewise, depending on the size of the litter, three groups were formed: neonates from litters of 1–2 (n = 24), neonates from litters of 3–5 (n = 102), and neonates from litters of more than 5 (n = 158).

For all mothers, the same procedure was followed upon arrival at the clinic. First, after evaluating the physical examination, an ultrasonographic examination (C4-1 Curved Array, ZONE Sonography®, Mindray Zonare Z. One PRO, Mountain View, CA, USA) was performed to determine the presence of fetal stress. Subsequently, a blood sample (3 mL) was taken to conduct a complete blood count (Procyte dx, IDEXX Laboratories S.L, Barcelona, Spain), a biochemical analysis (Catalyst Dx, IDEXX Laboratories S.L, Barcelona, Spain), and to measure progesterone levels (Speed Reader, Speed™ Progesterone, VIRBAC Spain, S.A, Barcelona, Spain). Only those mothers with progesterone levels below 1 ng/dL, who exhibited evident signs of parturition (at least 30 min of uterine contractions without fetal expulsion, suggesting potential dystocia), or who showed signs of fetal stress (fetal heart rate below 180–160 bpm on ultrasound) at the time of examination, were selected for this study.

2.3. Pre-Anesthetic and Pre-Surgical Evaluation

Initially, a simple physical examination was conducted that was designed to avoid generating stress in the pregnant mother. During this examination, heart rate was measured using a stethoscope, respiratory rate was assessed, mucosal color was observed, and a thoracic radiograph (R108, Ralco S.R.L., Biassono, Italy) and an electrocardiogram (MAC600, GE Medical System information technologies Inc., Karnataka, India) were performed. Subsequently, an ultrasonographic examination was conducted to measure the heart rate of at least 50% of the fetuses, along with the collection of blood samples to determine the suitability of the animal for the surgical procedure.

While awaiting the results of the analyses, pre-oxygenation of the bitch was initiated for at least 10 min, and preparations for the surgical procedure commenced. A catheter was placed in the cephalic vein of the forelimb, and the operating room was prepared for surgery. Once it was confirmed that the bitch could safely and stably enter the operating room, intravenous premedication was administered with fentanyl (Fentadon 50 μg/mL, Northwich, UK).

In addition to preparing the surgical area, a recovery zone was established, where special attention was given to temperature control using electric blankets (Carbon Vet, B.Braun VetCare, S.A, Barcelona, Spain), thermometers (Digital Braun PRT1000, B.Braun VetCare S.A, Barcelona, Spain), light lamps, and towels. A stethoscope was also available to monitor heart rate, along with oxygen therapy and the necessary drugs for resuscitation.

2.4. Patient Preparation and Monitoring

Initially, the bitch was induced with propofol at a dose of 4 mg/kg (Propovet, 10 mg/mL, Esteve, Barcelona, Spain). Although the total dose was calculated based on the weight of the bitch, an initial dose of 1 mg/kg was administered, with a one-minute wait between each dose. Following induction, the bitch was intubated and connected to the respiratory machine with automatic ventilation (Ventilator GE Datex Ohmeda Anesthesia Machine, GE Medical System information technologies Inc., Karnataka, India). To maintain the anesthetic plane, sevoflurane at 2% (SevoFlo 250 mL, Zoetis Inc., Tokyo, Japan) was used, which was gradually reduced during the surgery.

Throughout the surgical procedure, automatic monitoring was conducted, which included heart rate, respiratory rate, blood pressure (systolic and diastolic), temperature, oxygen saturation, and carbon dioxide saturation (WATO EX-35Vet, Shenzhen Mindray Animal Medical Technology Co., Ltd., Shenzhen, China). Blood pressure was measured indirectly using a cuff on the limb, selected according to the size of its circumference. A pulse oximeter was placed on the patient's tongue to measure oxygen saturation, while heart rate was monitored using electrodes attached to the pads of the bitch. Finally, temperature was measured with an oral thermometer (uMEC12 Vet, Shenzhen Mindray Animal Medical Technology Co., Ltd., Shenzhen, China), and carbon dioxide saturation, as well as respiratory rate, were evaluated using a capnometer (uMEC12 Vet, Shenzhen Mindray Animal Medical Technology Co., Ltd., Shenzhen, China). All measurements were updated every 5 min throughout the entire duration of the surgery, from start to finish.

During the surgery, fluids (Ringer Lactate® 3 mL/kg/h, Braun, Barcelona, Spain) were administered to maintain blood pressure, along with a continuous rate infusion (CRI) of fentanyl (Fentadon 50 μg/mL, Northwich, UK) for pain control throughout the surgical procedure.

2.5. Surgical Procedure

For the cesarean sections, a standard procedure was performed, beginning with an incision in the medial abdominal area. The subcutaneous tissue was dissected to expose the linea alba, through which access to the abdominal cavity was gained. Subsequently, the uterine horns were exteriorized with care to avoid ruptures, as the uterus exhibits significant friability during gestation. An incision was then made in the body of the uterus, and the uterine horns were alternately massaged until all the neonates were extracted, which were then transported to the recovery area.

To conclude the surgery, the uterus was closed using a continuous simple suture with atraumatic absorbable monofilament suture material (Monosyn® 3/0, HR22, B. Braun Surgical SA, Rubí, Spain), followed by the application of an inverted pattern (Cushing). An abdominal lavage was performed with a warmed saline solution, both within the abdominal cavity and the uterine horns. Finally, the abdominal cavity was closed in layers, and oxytocin (1–4 IU Oxiton®, Ovejero, León, Spain) was administered intravenously to facilitate the expulsion of uterine remnants and promote the descent of milk.

2.6. Neonatal Resuscitation

In the recovery area, the same veterinarians were consistently responsible for the resuscitation process and the collection of results. The same resuscitation protocol was utilized: the ABC protocol (airway/breathing/cardiac). Before commencing, the temperature was monitored using electric blankets (Carbon Vet, B.Braun VetCare, S.A, Barcelona, Spain) and light lamps, and the neonates were dried with towels.

To facilitate ventilation, suction of the contents from the mouths and noses of the neonates was initiated using a bulb syringe or neonatal suction device (Beaba, Paris, France), with the aim of eliminating fetal fluids and meconium. It was verified that the airways were completely cleared, and during this process, the neonates were vigorously rubbed with a towel, which also aided in the expulsion of airway contents. During this process, the head was carefully supported and slightly tilted to promote the elimination of fluids, avoiding any swinging or potential impact on the brain, which could lead to increased intracranial pressure, subdural hemorrhages, or aspiration of stomach contents into the airways (aspiration pneumonia).

Once the neonates exhibited continuous breathing, they were maintained on oxygen therapy during the Apgar evaluation and the determination of other measures (temperature, glucose, lactate, and weight). Additionally, throughout this time, the temperature was cautiously monitored (Digital Braun PRT1000, B.Braun VetCare S.A, Barcelona, Spain) due to the thermal loss that occurs during the first hour of life.

For those neonates who were not breathing adequately or who presented bradycardia (low heart rate), an additional resuscitation protocol was instituted, combining the use of medications and cardiac compressions. Two to three compressions were performed per second, and a catheter was placed in the jugular vein. If necessary, medications were administered in the following order: naloxone (0.05 mg/kg, Naloxona B. Braun 0.4 mg/mL, B. Braun Medical, SA, Barcelona, Spain) and epinephrine (0.2 mg/kg; Adrenalina B.Braun 1 mg/mL, B. Braun Medical, SA, Barcelona, Spain). The resuscitation process was maintained for 45 min, and if no favorable outcome was achieved, it was discontinued. Neonates with congenital malformations incompatible with life were euthanized.

2.7. Apgar Assessment and Data Collection

To determine neonatal viability, the Apgar test was performed. This procedure involves measuring several parameters: heart rate, respiratory rate, mucosal color, mobility, and reflexes/irritability. Heart rate was measured using a stethoscope, while respiratory rate was counted by assessing the number of spontaneous breaths per minute. Mucosal color was evaluated through direct visual examination; mobility was assessed based on spontaneous movements; and reflexes of irritability were determined by the neonate's response to external stimuli, such as compression on the pads or skin. For each parameter, a score of 0, 1, or 2 points was assigned, resulting in a final score ranging from 0 to 10. Based on this score, neonatal viability was classified into three categories: critical neonates

(<3 points), moderate viability (4–7 points), and normal viability (8–10 points). This assessment was conducted immediately after neonatal resuscitation, within the first 5–10 min of life. The order in which neonates were removed from the uterus was recorded, although it was not considered as a variable in this study.

Subsequently, each neonate was weighed individually using a scale, and temperature was measured with a thermometer. To determine glucose and lactate levels, a blood sample was obtained from the pad of the hind limb, which was analyzed using a glucometer (Advocate PetTest, Rafael del Campo, Córdoba, Spain) and a lactate meter (Cera Check Lactato, RAL S.A, Barcelona, Spain).

Once the neonates were stable and measurements had been completed, they were transferred to the incubator (Vetario© S40, Vetario, Weston-super-Mare, UK), where a temperature of 33–34 °C was maintained, and they were immediately fed, provided that their body temperature exceeded 34.5 °C. One of the strengths of this study is the strict control over neonatal feeding prior to glucose measurement. By keeping neonates separate from the mother and standardizing the first feeding through orogastric tube administration with a milk replacer, we minimized potential confounding factors related to maternal colostrum intake. This methodological approach ensures that the glucose values reported truly reflect the neonates' metabolic state immediately after birth, rather than postprandial variations.

2.8. Statistical Analysis

Statistical analyses were conducted using SPSS Statistics and Excel 2019 (SPSS Inc., Chicago, IL, USA). Initially, the normality of the data was assessed using the Shapiro–Wilk test, which yielded statistically significant results, indicating that the data did not follow a normal distribution. Receiver Operating Characteristic (ROC) curve analysis was performed to determine the cutoff values, using the Youden Index to identify the optimal threshold. Sensitivity was calculated as TP/(TP + FN), where TP (true positive) represents the number of correctly identified positive cases, and FN (false negative) represents the number of positive cases incorrectly classified as negative. Similarly, 1-specificity (false positive rate) was obtained as 1 minus the specificity value, where specificity was calculated as TN/(TN + FP), with TN (true negative) representing the correctly identified negative cases and FP (false positive) representing the negative cases incorrectly classified as positive. All values were derived from the ROC curve analysis. Given the non-normal distribution of the data, Spearman's rank correlation was used for correlation analyses. Variables with a p-value ≤ 0.05 were considered statistically significant.

To ensure that our sample size was adequate for detecting meaningful differences in glucose levels across groups, a power analysis was performed using a two-tailed t-test with equal variance assumed. The analysis indicated that a sample size of 26 neonates per group would be required to achieve a power of 0.8 with an effect size of 0.8 and a significance level of 0.05. Given that our study included 284 neonates, the sample size was sufficient to detect statistically significant differences in glucose levels.

3. Results

Mother and Neonate Glucose

This study involved 54 mothers and 284 neonates, classified by maternal weight into two groups: under 10 kg (n = 22) and over 10 kg (n = 32). Mothers were also categorized by litter size: 1–2 neonates (n = 12), 3–5 neonates (n = 27), and more than 5 neonates (n = 15). Similarly, neonates were classified based on maternal weight (74 from mothers < 10 kg and 109 from mothers > 10 kg) and litter size (24 neonates from 1–2, 102 from 3–5, and 158 from more than 5). Neonatal mortality was recorded at 6% (n = 17), with 2.5% ($n = 7$) of live-born neonates dying within the first hours and 3.5% ($n = 10$) being stillborn. Maternal mortality was 0%.

Table 1 compares the mean blood glucose levels among the mothers, along with the standard deviation for those weighing more than 10 kg and for those weighing less than 10 kg. Additionally, the mean glucose levels of the neonates were presented along with their corresponding standard deviation, based on the mothers' weight. When comparing the glucose levels of the mothers according to their weight, no significant differences were observed between the groups. However, concerning the neonates, significant differences ($p < 0.05$) were found between both groups.

Table 1. Mean (± SD) maternal and neonatal blood glucose concentrations depending on the weight.

	More Than 10 kg	**Less Than 10 kg**
Maternal glucose concentration	113.90 ± 20.80	108.79 ± 25.64
Neonatal glucose concentration	110.59 ± 7.69 [a]	130.40 ± 15.37 [b]

[ab] Different letters in the same column denote significant differences in glucose concentration according to maternal weight ($p < 0.05$).

Additionally, a comparison was made between groups based on litter size regarding the mean blood glucose levels in the mothers (Table 2). The three groups were compared using the Kruskal–Wallis test for independent variables, and it was observed that there were no significant differences ($p > 0.05$) in the glucose levels of the mothers according to litter size. However, it was noted that the mean glucose level was slightly lower in the bitches with 3–5 neonates compared to the other two groups.

Table 2. Mean (± SD) maternal and neonatal blood glucose concentrations according to litter size.

	Group	**Mean**	**Std Err**	**Lower**	**Upper**
Mother	1–2 neonates ($n = 13$)	114.73 ± 8.30	8.30	98.05	131.40
	3–5 neonates ($n = 26$)	110.04 ± 5.40	5.40	99.19	120.89
	>5 neonates ($n = 15$)	113.93 ± 7.35	7.35	99.15	128.71
Neonates	1–2 neonates ($n = 13$)	123.04 ± 10.36	10.36	102.66	143.43
	3–5 neonates ($n = 26$)	114.38 ± 5.02	5.024	104.49	124.27
	>5 neonates ($n = 15$)	114.98 ± 4.05	4.049	107.02	122.96

Regarding the glucose levels in the neonates, an analysis was conducted to determine if there were differences based on litter size (Table 2). The mean glucose level was slightly higher in bitches with 1–2 neonates compared to the other two groups. However, the differences observed between groups were not significant ($p > 0.05$), according to the Kruskal–Wallis test.

On the other hand, an investigation was conducted to determine whether there were differences in the glucose levels of the mothers before and after the surgical procedure. The results indicated that following surgery, the mean glucose level was slightly higher (120.93 ± 33.54) compared to the levels before surgery (114.79 ± 31.84). However, no significant differences were observed between the groups.

Of the total neonates, those that were brachycephalic ($n = 84$) were selected to establish a cutoff for glucose based on the obtained Apgar score. The ROC curve showed a good capacity of the model to distinguish between positive cases (Apgar > 7 points) and negative cases (Apgar < 7 points), as the curve was situated above the reference line (diagonal line). The curve exhibited a rapid ascent, indicating a high true positive rate (Figure 1). The ROC curve had an area under the curve (AUC) of 0.882, indicating good model performance. This value suggested that the model was capable of effectively distinguishing between positive and negative classes. The standard deviation was 0.060, and the significance level was <0.001, indicating that the results were statistically significant. The 95% confidence interval for the AUC ranged from 0.765 to 0.998, demonstrating that the model was consistently effective across different samples. Based on these data, a cutoff of 89.50 mg/dL was established (sensitivity 98.1%; specificity 81.5%).

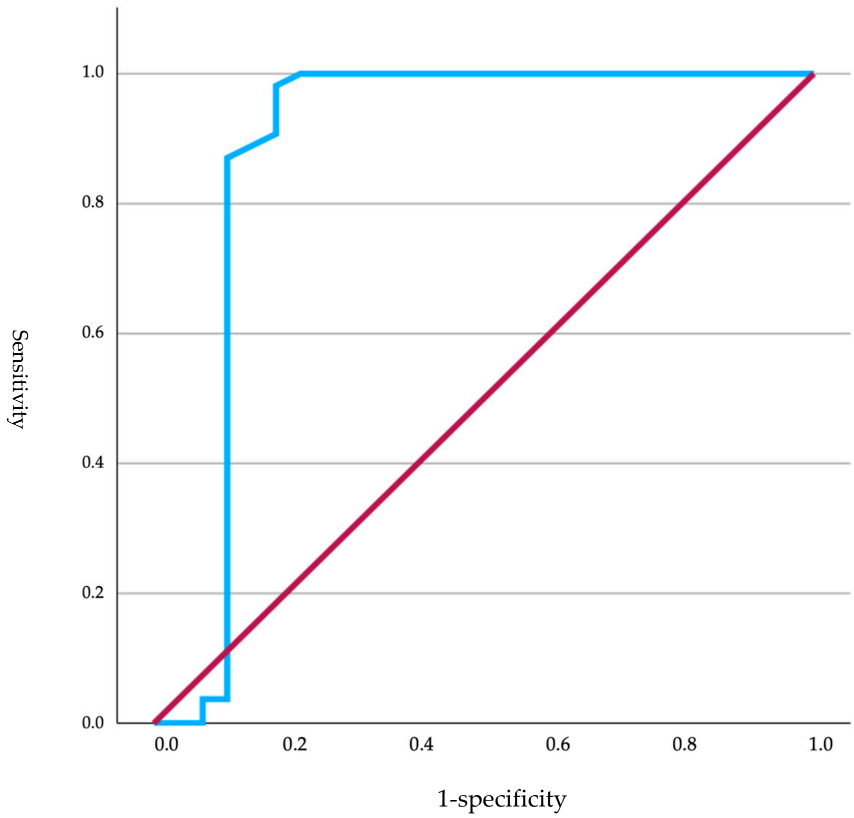


Figure 1. ROC curves analyzing glucose levels in relation to Apgar scores in brachycephalic neonates, where the red line represents the reference line (random performance) and the blue line represents the actual ROC curve reflecting the model's performance.

Regarding Figure 2, a precision–recall curve was presented, indicating the model's performance and its classification capability based on both parameters. Sensitivity measured the proportion of positive cases correctly identified by the model, while precision indicated the accuracy in predicting positives. The curve started at the origin and moved towards the upper right corner, reflecting good overall model performance. However, a sharp decline in precision was observed near the far right end, suggesting that, in attempting to maximize sensitivity, precision was slightly compromised.

On the other hand, the ROC curve (Figure 3) was represented for non-brachycephalic neonates (n = 111) concerning glucose levels based on neonatal Apgar scores. The obtained area under the curve (AUC) was 0.842, indicating good model performance in classifying positive cases (Apgar > 7 points) and negative cases (Apgar < 7 points). The curve rose rapidly towards the vertical axis, suggesting a high true positive rate and a low number of false positives. The standard deviation was 0.064, and the significance level was <0.001, indicating that the results were skmtatistically significant. The 95% confidence interval ranged from a minimum of 0.717 to a maximum of 0.968, reinforcing the reliability of the model. Based on these data, a cutoff of 87.50 mg/dl was established (sensitivity 97.5%; specificity 83.9%).

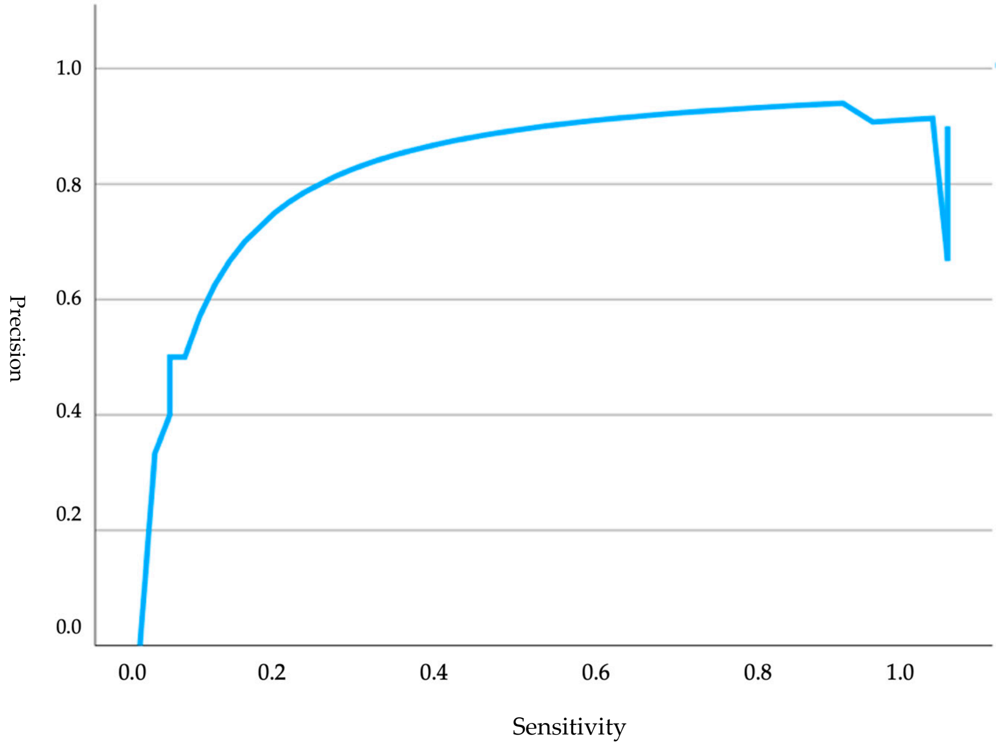


Figure 2. Precision curves analyzing glucose levels in relation to Apgar scores in brachycephalic neonates.

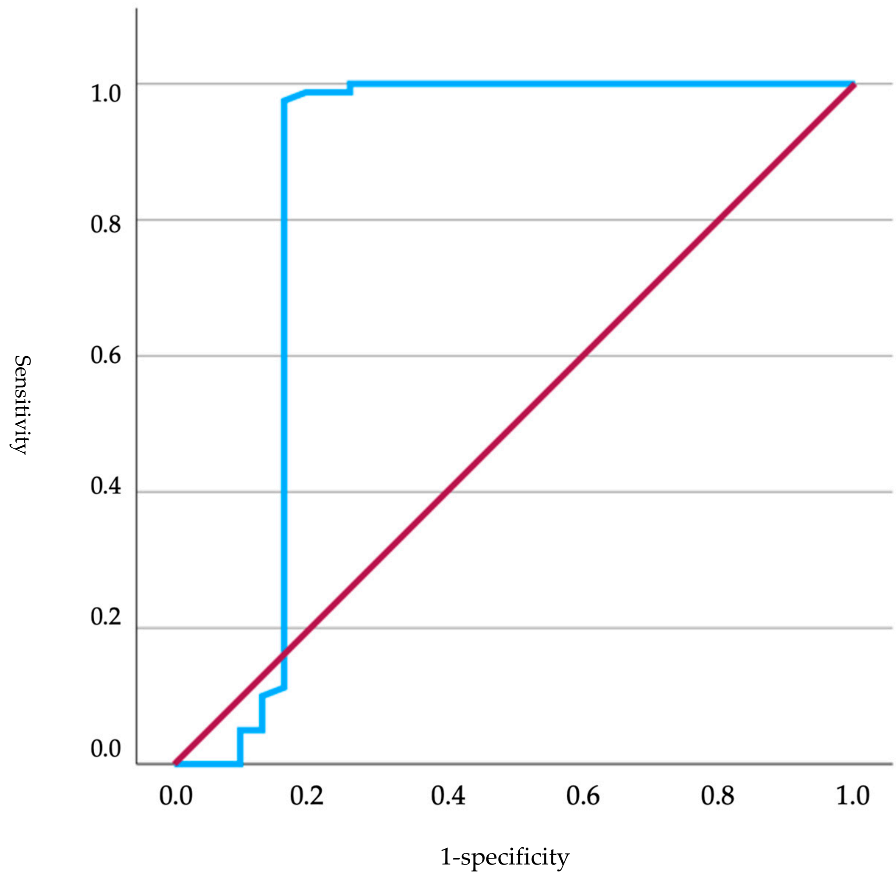


Figure 3. ROC curves analyzing glucose levels in relation to Apgar scores in non-brachycephalic neonates, where the red line represents the reference line (random performance) and the blue line represents the actual ROC curve reflecting the model's performance.

Additionally, the sensitivity–precision curve (Figure 4) was represented, starting near the origin and rising, indicating that the model improved its performance as sensitivity increased. The curve stabilized at a high level of precision, suggesting that the model maintained a good balance in correctly identifying positive cases and minimizing false positives. However, towards the end, a significant drop occurred, which may have implied that precision was compromised when attempting to maximize sensitivity.

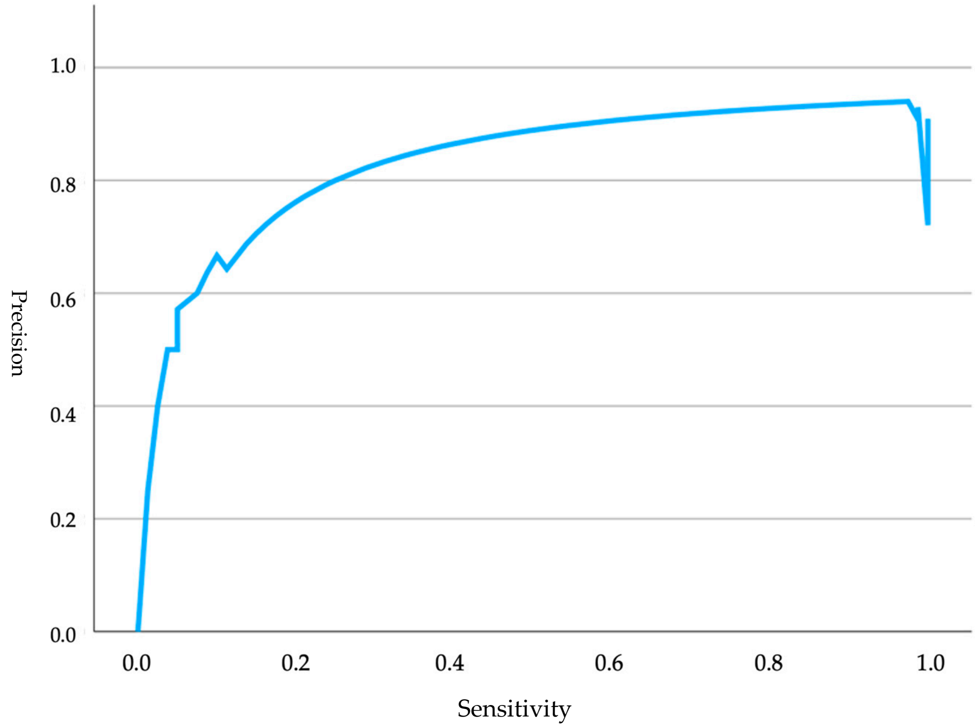


Figure 4. Precision curves analyzing glucose levels in relation to Apgar scores in non-brachycephalic neonates.

In relation to Figure 5, the ROC curve was presented to determine the cutoff point for the total neonates in this study (*n* = 282). The model showed a good capacity to distinguish between classes, as the curve was positioned above the reference line (diagonal line). The curve exhibited a rapid initial growth, suggesting a high true positive rate and a low number of false positives. The area under the curve (AUC) was 0.710, indicating moderate model performance between the classes. The standard deviation was 0.045, and the significance level was <0.001, indicating that the results were statistically significant. The 95% confidence interval ranged from 0.622 to 0.799, implying that although the model performed better than a random one, there was room for improvement in its precision and discrimination. The obtained cutoff was 79.50 mg/dL (sensitivity 99.2%; specificity 59.2%).

On the other hand, a precision–recall curve (Figure 6) was constructed for the same model, showing that the curve started at the origin and rose rapidly, indicating that the model improved its precision with an initial increase in sensitivity. As it progressed, the curve stabilized at a precision of 0.8, suggesting that the model exhibited better precision in that region. However, as it approached higher sensitivity levels, there was a decline in precision, indicating that the model struggled to maintain prediction quality.

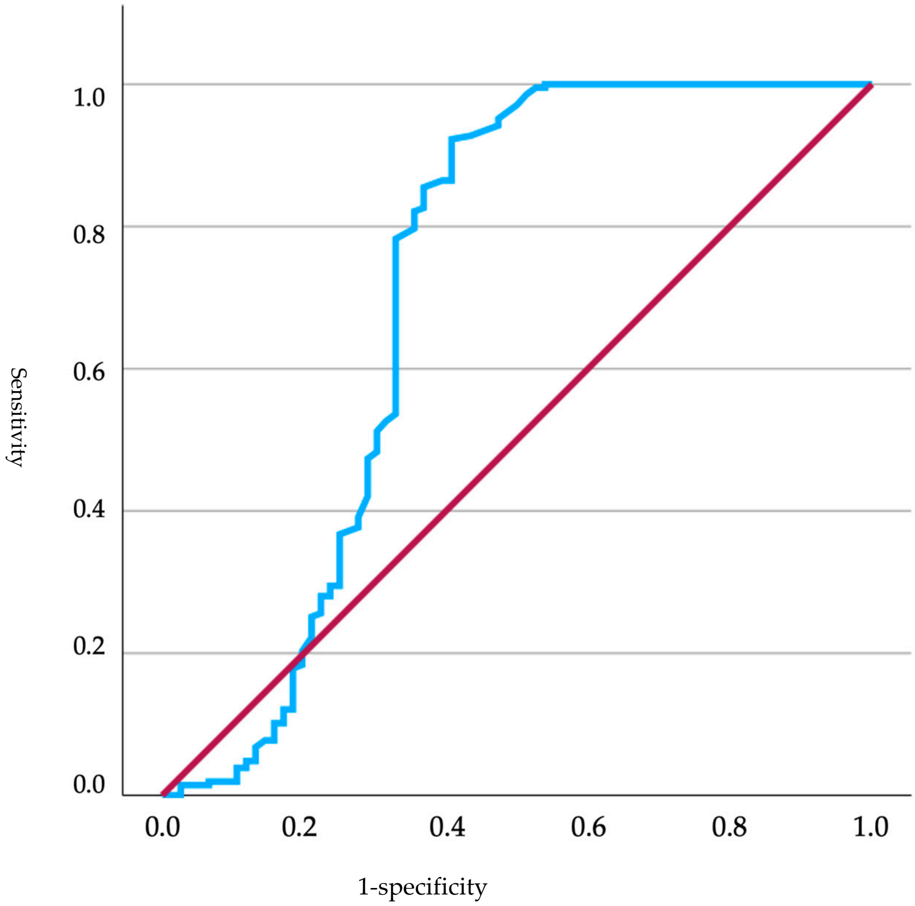


Figure 5. ROC curves analyzing glucose levels in relation to Apgar scores in neonates, where the red line represents the reference line (random performance) and the blue line represents the actual ROC curve reflecting the model's performance.

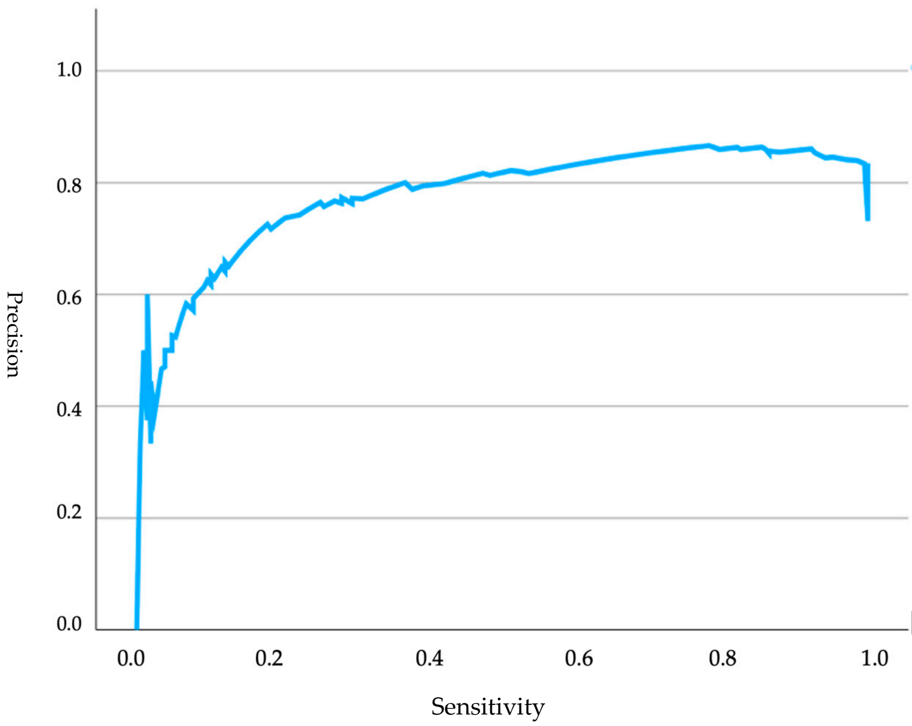


Figure 6. Precision curves analyzing glucose levels in relation to Apgar scores in neonates.

Neonatal glucose was correlated with other neonatal parameters, yielding the following results. When glucose was correlated with the Apgar score, a weak correlation was observed (*rs* = 0.3), which was statistically significant ($p < 0.0001$). However, when glucose was correlated with lactate and temperature, a very weak correlation was found (*rs* = 0.12 for both parameters), which did not reach statistical significance. Finally, regarding the neonate's weight, no correlation was observed between the two parameters (*rs* = −0.004), which was also not statistically significant.

4. Discussion

Neonatal mortality has been the subject of study by various authors, showing differences among different studies, although it commonly hovers around 20% [21]. This mortality rate tends to increase in the first weeks of life. In our study, neonatal mortality was 6%, of which 3.5% corresponded to neonates that were stillborn and 2.5% to those that were born alive but died within the first hours of life. This discrepancy in percentages, with a higher proportion among stillborn neonates, may be related to the fact that all cesarean sections performed were urgent and not scheduled, and some presented dystocia upon arrival at the Veterinary Clinical Hospital. Conversely, other authors have reported lower mortality rates, around 11–13% [16] or even as low as 4% [26]. Neonatal mortality is a significant challenge for veterinarians and breeders. This study aimed to analyze the impact of neonatal markers like glucose, lactate, and birth weight on births during dystocia cesarean sections or fetal stress. It also established cutoff points based on the Apgar score and examined the influence of glucose on other neonatal and maternal factors.

Neonates are particularly susceptible to hypoglycemia due to limitations in their glycogen energy reserves and their minimal capacity for gluconeogenesis [6,8]. Their ability to maintain normoglycemia is limited, and hepatic immaturity in neonates is not sufficiently efficient to generate energy [27,28]. For this reason, some authors have indicated that low glucose levels (<92 mg/dL) are associated with a higher risk of mortality within the first 24 h of life [6]. In our study, the mean glucose level of the neonates ($n = 282$) was 115.6 mg/dL. This result could explain our low neonatal mortality both in the first hours and during the first week of life. In our study, glucose was measured immediately after birth, as neonatal glucose levels tend to drop rapidly within the first 24 h of life [22]. This immediate measurement is particularly relevant in neonates born via cesarean section due to dystocia, as the metabolic transition in these individuals may be altered by factors such as fetal hypoxia, oxidative stress, and an exacerbated endocrine response. Studies in human neonates have shown that perinatal stress can induce transient hyperglycemia or, in more severe cases, persistent hypoglycemia, compromising neonatal viability [29,30]. Our findings reinforce the importance of assessing glucose at this critical moment to detect metabolic alterations early and improve clinical intervention strategies. However, other authors [21] have reported a mean value of 97 mg/dL in measurements taken between the first 10 min and 8 h of life of the neonates. The author notes that these results are inconclusive, as the measurements were made on the total number of neonates without differentiating those who had taken milk. This slight increase in the mean glucose in our study may be related to the type of delivery, in this case, emergency or dystocia cesarean sections. Such circumstances may lead to increased hypoxia, which in turn elevates catecholamines, promoting the production of epinephrine and norepinephrine, which suppress insulin production and stimulate hepatic glucose release [24,31]. Therefore, it is plausible that there is an increase in the mean glucose level in our study compared to other authors.

Despite the mean glucose level in our study being 115.6 mg/dL, the results obtained when establishing the cutoff points according to the Apgar score showed significant differences. The Apgar score has been the subject of study by several authors, who have associated the obtained score with neonatal viability, suggesting that a higher score correlates with a greater probability of survival [32]. Some studies indicate that a score below 5 points compromises neonatal viability [13], while others establish a cutoff at 6 points as the optimal score to predict neonatal survival [21]. In this context, and considering a cutoff of 6 points, the present study determined the glucose cutoff for brachycephalic neonates, non-brachycephalic neonates, and the total neonates.

In the case of brachycephalic neonates (n = 84), it was observed that the cutoff for achieving an Apgar score greater than 7 points, and consequently better neonatal viability, is set at 89.5 mg/dL. On the other hand, in non-brachycephalic neonates (n = 111), the cutoff was established at 87.50 mg/dL. Although numerous studies address the mean glucose levels at birth [10,21,24], none have determined a cutoff based on neonatal viability as represented by the Apgar score. However, the overall result (both brachycephalic and non-brachycephalic neonates; n = 282) was 79.50 mg/dL.

The observed differences between groups could be explained by several theories. Firstly, the discrepancy in results between brachycephalic and non-brachycephalic neonates may be related to the level of hypoxia at birth. Lower Apgar scores are often correlated with reduced oxygen saturation, which tends to resolve as spontaneous breathing stabilizes [3]. Brachycephalic neonates face greater difficulties in achieving optimal scores due to their physiological conditions [33], which could justify a slightly higher glucose cutoff; by using the same scale for all, brachycephalic neonates may differ in the scores obtained. Additionally, other authors have noted specific modifications in the scale (such as mucosal color and heart rate), adapting it to the characteristics of brachycephalic neonates [13,32].

On the other hand, the authors of [20] have indicated that neonates with glucose levels below 40 mg/dL typically present lower Apgar scores and decreased reflexes [20]. However, according to the results obtained in our study, we consider that a glucose value lower than 79.50 mg/dL is associated with a significantly lower Apgar score compared to the rest of the neonates, which impairs their ability to suckle or respond to external stimuli, thus compromising their survival. Furthermore, other authors [3], reported that their mean glucose value was even lower, observing that neonates with a score below 7 had glucose levels of less than 30.5 mg/dL. Additionally, some studies indicate that a glucose concentration below 40 mg/dL begins to manifest visible clinical symptoms in the neonate [20]. A possible difference between the values reported by other authors and those obtained in our study may be related to the sample size. Another theory to consider is fetal stress; studies in humans have shown that neonates experiencing fetal stress at the time of birth present elevated glucose concentrations [3,34]. This may explain why our values are higher, given that the surgeries performed were urgent or dystocia-related.

Additionally, no significant correlation was observed between glucose concentration and neonatal mortality, as there were neonates who died with glucose levels below 50 mg/dL and others who died with concentrations exceeding 230 mg/dL. Therefore, as noted by other authors [35], no correlation is established between these two factors. However, glucose can be an important indicator of neonatal viability, as a neonate with hypoglycemia may indicate underlying pathologies such as sepsis or portosystemic shunts [10,15]. Nonetheless, the absence of correlation in our study may be attributed to the low mortality observed throughout this study, as described by Greghi et al. [35].

During gestation, fetuses maintain their glucose levels through continuous infusion via the placenta, without relying on gluconeogenesis [36]. A correlation has been described between maternal glucose and the glucose in the amniotic fluid of fetuses; furthermore, the glucose in the amniotic fluid has also been correlated with the blood glucose of the neonate obtained through the umbilical cord [32]. However, no direct correlation has been observed between maternal glucose and neonatal glucose. Therefore, one of the objectives of this study was to determine how maternal parameters (maternal glucose and maternal weight) influence neonatal glucose.

In our study, maternal glucose levels were measured before surgery, and neonatal glucose was assessed immediately after birth. We acknowledge that maternal glucose levels may fluctuate rapidly due to perioperative stress, anesthesia, and surgical interventions, potentially creating a temporal mismatch between maternal and neonatal glucose values. While our findings provide valuable insights into neonatal glucose dynamics, future studies should incorporate continuous maternal glucose monitoring before, during, and after surgery to better assess the relationship between maternal and neonatal glucose levels. Additionally, measuring neonatal glucose at multiple time points post-birth could help elucidate how glucose metabolism evolves in the immediate neonatal period. However, our results could be explained by several reasons. Firstly, other authors have indicated that neonatal glucose levels depend exclusively on the metabolic maturity of the neonate [12]. As mentioned earlier, glucose levels at birth depend on metabolic reserves and the neonate's capacity for gluconeogenesis [6], which constitutes the primary reason for variation in glucose concentrations without direct influence from maternal glucose. Secondly, other neonatal factors, such as hypoxia, can influence glucose levels [31]. Hypoxia or asphyxia at the time of birth depends on the fetal stress experienced but is not directly related to maternal parameters. Therefore, this factor will influence the neonate's glucose concentrations independently of the levels in the mother. A limitation of our study was not determining the oxygen saturation at the time of birth, which would have allowed us to observe if there was a correlation between both parameters.

Amniotic fluid analyses have begun to be a subject of study in veterinary medicine [12,32], similar to research in human medicine [15]. The conclusions of these studies indicate that there is a correlation between glucose levels and neonatal survival, as lower concentrations of glucose in the amniotic fluid are associated with reduced survival rates [15]. However, the glucose present in the amniotic fluid is primarily related to maternal metabolism and, to a lesser extent, to fetal metabolism, which is more influenced during advanced stages of development [15]. This could explain why there are studies that have demonstrated a correlation between maternal glucose and glucose in the amniotic fluid, but not with neonatal glucose levels at the time of birth.

Regarding maternal glucose at the time of delivery, some authors have described that glucose concentration is lower in small-breed bitches compared to large breeds [37]. In our case, no significant differences were found; however, the concentration was slightly higher in large breeds compared to small ones. Furthermore, glucose concentration at the time of delivery is related to the type of diet [37,38]. Other studies have observed correlations between neonatal glucose and maternal age [15] or have noted the possibility of variations in glucose concentration among breeds, similar to what occurs with other biochemical parameters, such as creatinine or ALT (alanine aminotransferase) [32].

In this study, we also attempted to evaluate whether litter size influenced glucose concentrations in the mother or neonate. No differences in concentrations were found among the three groups analyzed. To the best of the authors' knowledge, there are no previous studies that have sought to identify differences in glucose concentration based on litter size. Only one study noted that mothers with large litters had a higher probability of hypoglycemia due to inadequate nutrient intake, which was attributed to the pressure exerted by the uterus on the stomach and its inability to distend properly [37,39].

The authors of [24] have determined the differences in cortisol and glucose levels in mothers based on the type of delivery, observing that levels were slightly higher in those undergoing cesarean sections. In our study, glucose concentrations were evaluated both before and after the surgery, and these values were slightly higher compared to those determined in mothers with natural births [24]. However, to the best of the author's knowledge, no studies have evaluated maternal glucose concentrations before and after surgery. In our case, no significant differences were observed, although it was noted that the concentration was slightly higher after the surgical procedure. This could be attributed to the stress situation associated with cesarean sections, which leads to an increase in cortisol levels and, consequently, glucose levels [24,34].

Regarding other markers of neonatal viability (temperature, birth weight, Apgar score, or lactate), correlations with glucose at the time of birth were performed. In our case, the correlations were weak or not statistically significant. Other authors have sought correlations between glucose and birth weight, Apgar score, or lactate [1,3,6], but not with temperature. In relation to birth weight and lactate, consistent with our results, no correlation with glucose concentration was observed [1,6]. Regarding the Apgar score, we found a weak correlation; in contrast, the authors of [3] indicated a negative correlation between the two variables (higher glucose and lower Apgar score) [3]. However, other authors state that it is not a definitive indicator of neonatal morbidity or viability [24,25].

Currently, there are no studies correlating temperature with glucose, although all authors note that the occurrence of hypothermia and hypoglycemia is common. In our study, no relationship between these two factors was found at the time of birth [6,21,32]. This can be explained by the following reasons: hypothermia often causes neonatal depression, leading to the loss of the suckling reflex, decreased reflexes, and alterations in the cardiovascular system. Consequently, this prevents the neonate from ingesting milk, favoring the subsequent onset of hypoglycemia, which could lead to both conditions presenting simultaneously in critical neonates [6,10,33,40]. However, at the time of neonatal resuscitation, our study primarily monitored temperature, as neonates are unable to thermoregulate at birth. Therefore, this could be a limitation, as the temperature recorded during the measurement depended on the ambient temperature provided (e.g., heating pads, towels, light lamps, etc.).

5. Conclusions

This study highlights the critical role of glucose levels in neonatal viability, particularly in small animals undergoing cesarean sections due to dystocia. The findings reveal that neonatal hypoglycemia is a significant contributor to mortality within the first hours and days of life, underscoring the necessity for vigilant monitoring of glucose levels in neonates.

The established cutoff values for glucose, based on Apgar scores, demonstrate that lower glucose concentrations correlate with poorer neonatal outcomes, thereby emphasizing the importance of timely interventions in cases of hypoglycemia. Specifically, a glucose level below 79.50 mg/dL is associated with a marked decrease in viability, which can severely impair a neonate's ability to suckle and respond to environmental stimuli.

Furthermore, this study indicates that maternal factors, such as weight and glucose levels, influence neonatal glucose status, though a direct correlation between maternal and neonatal glucose levels was not established. This suggests that while maternal health is pivotal, neonatal metabolic maturity and response to stressors also play a crucial role in determining glucose levels at birth.

Overall, these findings advocate for the implementation of routine glucose assessments in neonates immediately after birth, particularly in high-risk situations such as cesarean deliveries or when signs of fetal distress are present. By addressing hypoglycemia proactively, veterinarians can enhance neonatal survival rates and improve overall outcomes for small animal litters. Future research should continue to explore the intricate relationships between maternal and neonatal health parameters to further refine strategies aimed at reducing neonatal mortality in small animals.

Author Contributions: Conceptualization, M.B.-A.; methodology, R.R.-T., M.B.-A. and K.I.; formal analysis, M.B.-A., R.R.-T. and K.I.; writing—original draft preparation, R.R.-T. and M.B.-A.; writing—review and editing, M.B.-A. and R.R.-T.; visualization, M.B.-A. and R.R.-T., supervision, M.B.-A. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external or internal funding.

Institutional Review Board Statement: The present study did not require ethical approval. All the veterinary activities were carried out according to the Spanish legislation about animal care (L7/2023, 28 March 2023) and the European Guidelines on Animal Welfare (Directive 2010/63/EU).

Informed Consent Statement: A written informed consent was signed by each owner to submit the bitches to ovariohysterectomy and cesarean section, allow all the needed clinical procedures on females and newborns, and allow the use of the clinical records for research purposes.

Data Availability Statement: Data will be available for all readers upon request.

Acknowledgments: Miguel Batista acknowledges the excellent technical work carried out by the veterinarian assistant at the Veterinary Hospital of the University of Las Palmas de Gran Canaria. The authors acknowledge support from the Veterinary Hospital of the University of Las Palmas de Gran Canaria for the facilities and resources in this study.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Lezama-García, K.; Martínez-Burnes, J.; Baqueiro-Espinosa, U.; Olmos-Hernández, A.; Hernández-Ávalos, I.; Domínguez-Oliva, A.; Mota-Rojas, D. Assessment of Vitality, Blood Profile, and Degree of Meconium Staining on the Skin in Newborn Dogs According to Its Birth Weight. *Vet. Sci.* **2023**, *10*, 453. [CrossRef] [PubMed]
2. Veronesi, M.C.; Bucci, R.; Probo, M.; Faustini, M.; Fusi, J. Apgar Score for Newborn Dog Viability Assessment: Differences between English and French Bulldogs Born via Cesarean Section. *Animals* **2023**, *13*, 3318. [CrossRef] [PubMed]
3. Antończyk, A.; Ochota, M.; Niżański, W. Umbilical cord blood gas parameters and Apgar scoring in assessment of new-born dogs delivered by cesarean section. *Animals* **2021**, *11*, 685. [CrossRef] [PubMed]
4. Molina, C.; Bosch, L.; Rigau, T.; Yeste, M.; Torrente, C.; Del Alamo, M.M.R. Urine glucose concentration: A useful parameter as a surrogate for glycaemia on the first day of life in canine neonates. *Res. Vet. Sci.* **2020**, *133*, 59–62. [CrossRef]
5. Melandri, M.; Veronesi, M.C.; Alonge, S. Urinalysis in Great Dane puppies from birth to 28 days of age. *Animals* **2020**, *10*, 636. [CrossRef]
6. Pereira, K.H.N.P.; Fuchs, K.D.M.; Corrêa, J.V.; Chiacchio, S.B.; Lourenço, M.L.G. Neonatology: Topics on puppies and kittens neonatal management to improve neonatal outcome. *Animals* **2022**, *12*, 3426. [CrossRef]
7. AregaTafere, G.K.; Ayele, M.B.C.T. Review on Dog Neonatal Mortality. *Adv. Life Sci. Technol.* **2018**, *65*, 19–26.
8. Fuchs, K.D.M.; Pacífico Pereira, K.H.N.; de Sousa, G.C.; Xavier, G.M.; Camargo, G.D.A.; de Souza, F.F.; Lourenço, M.L.G. Hypoglycaemia management with a hypercaloric supplementation in neonatal puppies delivered by caesarean section. *Reprod. Domest. Anim.* **2023**, *58*, 1345–1351. [CrossRef]
9. Pinchefsky, E.F.; Schneider, J.; Basu, S.; Tam, E.W.Y.; Gale, C.; Guidelines, N.B.S.; Committee, P. Nutrition and management of glycemia in neonates with neonatal encephalopathy treated with hypothermia. *Semin. Fetal Neonatal Med.* **2021**, *26*, 101268. [CrossRef]
10. Veronesi, M.C. Assessment of canine neonatal viability—The Apgar score. *Reprod. Domest. Anim.* **2016**, *51*, 46–50. [CrossRef]
11. Vannucchi, C.I.; Rodrigues, J.A.; Silva, L.C.G.; Lúcio, C.F.; Veiga, G.A.L.; Furtado, P.V.; Oliveira, C.A.; Nichi, M. Association between birth conditions and glucose and cortisol profiles of periparturient dairy cows and neonatal calves. *Vet. Rec.* **2015**, *176*, 358. [CrossRef]
12. Bolis, B.; Scarpa, P.; Rota, A.; Vitiello, T.; Veronesi, M.C. Association of amniotic uric acid, glucose, lactate and creatinine concentrations and lactate/creatinine ratio with newborn survival in small-sized dogs–preliminary results. *Acta Vet. Hung.* **2018**, *66*, 125–136. [CrossRef] [PubMed]

13. Vilar, J.M.; Batista, M.; Pérez, R.; Zagorskaia, A.; Jouanisson, E.; Díaz-Bertrana, L.; Rosales, S. Comparison of 3 anesthetic protocols for the elective cesarean-section in the dog: Effects on the bitch and the newborn puppies. *Anim. Reprod. Sci.* **2018**, *190*, 53–62. [CrossRef] [PubMed]
14. Titkova, R.; Fialkovicova, M.; Karasova, M.; Hajurka, J. Puppy Apgar scores after vaginal delivery and caesarean section. *Vet. Med.* **2017**, *62*, 488–492. [CrossRef]
15. Fusi, J.; Bolis, B.; Probo, M.; Faustini, M.; Carluccio, A.; Veronesi, M.C. Clinical trial on the usefulness of on-site evaluation of canine fetal fluids by reagent test strip in puppies at elective Caesarean section. *Biology* **2021**, *11*, 38. [CrossRef]
16. Gill, M.A. Perinatal and Late Neonatal Mortality in the Dog. Doctoral Dissertation, The University of Sydney, Camperdown, NSW, Australia, 2001.
17. Fox, M.W. The pathophysiology of neonatal mortality in the dog. *J. Small Anim. Pract.* **1965**, *6*, 243–254. [CrossRef]
18. Fitzgerald, K.T.; Newquist, K.L. Husbandry of the neonate. *Small Anim. Pediatr.* **2010**, 44–52. [CrossRef]
19. Lawler, D.F. Neonatal and pediatric care of the puppy and kitten. *Theriogenology* **2008**, *70*, 384–392. [CrossRef]
20. Vassalo, F.G.; Simões, C.R.B.; Sudano, M.J.; Prestes, N.C.; Lopes, M.D.; Chiacchio, S.B.; Lourenço, M.L.G. Topics in the routine assessment of newborn puppy viability. *Top. Companion Anim. Med.* **2015**, *30*, 16–21. [CrossRef]
21. Mila, H.; Grellet, A.; Delebarre, M.; Mariani, C.; Feugier, A.; Chastant-Maillard, S. Monitoring of the newborn dog and prediction of neonatal mortality. *Prev. Vet. Med.* **2017**, *143*, 11–20. [CrossRef]
22. Castagnetti, C.; Cunto, M.; Bini, C.; Mariella, J.; Capolongo, S.; Zambelli, D. Time-dependent changes and prognostic value of lactatemia during the first 24 h of life in brachycephalic newborn dogs. *Theriogenology* **2017**, *94*, 100–104. [CrossRef] [PubMed]
23. Sahraei, H.; Mogheiseh, A.; Nazifi, S.; Divar, M.R.; Iraji, F. Canine and feline foetal fluids: Volume, hormonal and biochemical characterization during pregnancy. *Vet. Med. Sci.* **2024**, *10*, e1452. [CrossRef] [PubMed]
24. Lúcio, C.F.; Silva, L.C.G.D.; Vannucchi, C.I. Perinatal cortisol and blood glucose concentrations in bitches and neonatal puppies: Effects of mode of whelping. *Domest. Anim. Endocrinol.* **2021**, *74*, 106483. [CrossRef]
25. Vanspranghels, R.; Houfflin-Debarge, V.; Deken, V.; Rakza, T.; Maboudou, P.; Storme, L.; Ghesquiere, L.; Garabedian, C. Umbilical Cord Arterial and Venous Gases, Ionogram, and Glucose Level for Predicting Neonatal Morbidity at Term. *Europ. J. Obstet. Gynec. Reprod. Biol.* **2020**, *252*, 181–186. [CrossRef]
26. Rodríguez-Trujillo, R.; Batista-Arteaga, M.; Iusupova, K.; Rosario-Medina, I.; Alamo-Santana, D. Comparison of Propofol and Alfaxalone as Anesthesic Drugs in Bitches Undergoing Ovariohysterectomies (Healthy Bitches and with Pyometra) and Cesarean Sections. *Animals* **2024**, *14*, 1343. [CrossRef] [PubMed]
27. Grundy, S.A. Clinically relevant physiology of the neonate. *Vet. Clin. Small Anim. Pract.* **2006**, *36*, 443–459. [CrossRef]
28. Münnich, A.; Küchenmeister, U. Causes, diagnosis and therapy of common diseases in neonatal puppies in the first days of life: Cornerstones of practical approach. *Reprod. Domest. Anim.* **2014**, *49*, 64–74. [CrossRef]
29. Stanescu, D.L.; Stanley, C.A. Advances in understanding the mechanism of transitional neonatal hypoglycemia and implications for management. *Clin. Perinatol.* **2022**, *49*, 55–72. [CrossRef]
30. Hay, W.W.; Rozance, P.J. Neonatal hyperglycemia—Causes, treatments, and cautions. *J. Pediatr.* **2018**, *200*, 6–8. [CrossRef]
31. Reyes-Sotelo, B.; Mota-Rojas, D.; Mora-Medina, P.; Ogi, A.; Mariti, C.; Olmos-Hernández, A.; Gazzano, A. Blood biomarker profile alterations in newborn canines: Effect of the mother's weight. *Animals* **2021**, *11*, 2307. [CrossRef]
32. Veronesi, M.C.; Bolis, B.; Faustini, M.; Rota, A.; Mollo, A. Biochemical composition of fetal fluids in at term, normal developed, healthy, viable dogs and preliminary data from pathologic littermates. *Theriogenology* **2018**, *108*, 277–283. [CrossRef]
33. Uchańska, O.; Ochota, M.; Eberhardt, M.; Niżański, W. Dead or alive? A review of perinatal factors that determine canine neonatal viability. *Animals* **2022**, *12*, 1402. [CrossRef]
34. Groppetti, D.; Martino, P.A.; Ravasio, G.; Bronzo, V.; Pecile, A. Prognostic potential of amniotic fluid analysis at birth on canine neonatal outcomes. *Vet. J.* **2015**, *206*, 423–425. [CrossRef] [PubMed]
35. Greghi, J.R.; Favaron, P.O.; Trautwein, L.G.C.; da Silva, C.G.B.; de Lemos, G.A.A.; Martins, M.I.M. Emergency cesarean section in dogs: Usefulness of amniotic fluid biochemical parameters and placental morphology as indicators of neonatal viability. *Theriogenology* **2023**, *211*, 115–124. [CrossRef]
36. Kliegman, R.M.; Miettinen, E.L.; Morton, S.K. Hepatic and cerebral energy metabolism after neonatal canine alimentation. *Pediatr. Res.* **1983**, *17*, 285–291. [CrossRef] [PubMed]
37. Fuchs, K.D.M.; Pereira, K.H.N.P.; Xavier, G.M.; Mendonça, J.C.; Barreto, R.O.; Silva, R.C.; de Souza, F.F.; Lourenço, M.L.G. Neonatal hypoglycemia in dogs—Pathophysiology, risk factors, diagnosis and treatment. *Front. Vet. Sci.* **2024**, *11*, 1345933. [CrossRef] [PubMed]
38. Romsos, D.R.; Palmer, H.J.; Muiruri, K.L.; Bennink, M.R. Influence of a low carbohydrate diet on performance of pregnant and lactating dogs. *J. Nutr.* **1981**, *111*, 678–689. [CrossRef]

39. Balogh, O.; Bruckmaier, R.; Keller, S.; Reichler, I.M. Effect of maternal metabolism on fetal supply: Glucose, non-esterified fatty acids and beta-hydroxybutyrate concentrations in canine maternal serum and fetal fluids at term pregnancy. *Anim. Reprod. Sci.* **2018**, *193*, 209–216. [CrossRef]
40. Kutzler, M.A. Canine neonatal mortality. *Clin. Theriogenology* **2010**, *2*, 513–530. [CrossRef]

Theriogenology 241 (2025) 117437

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Original Research Article

Neonatal blood lactate as a predictor of early survival in puppies delivered by emergency cesarean section

Rodríguez Raquel, Batista Miguel *, Iusupova Kseniia, Alamo Desirée

Unit of Reproduction, Universitary Institute of Biomedical Research and Health, University of Las Palmas de Gran Canaria, Transmontaña s/n 35413, Arucas, Las Palmas, Spain

ARTICLE INFO

Keywords:
Cesarean section
Lactate
Viability biomarker
Bitches
Neonate
Dog

ABSTRACT

This study evaluated blood lactate as a biomarker of neonatal viability in puppies delivered by emergency cesarean section. A total of 28 bitches and 118 neonates were assessed for weight, temperature, plasma lactate and glucose levels, and Apgar scores immediately after birth. Eighteen neonates (15.25 %) did not survive: 14 (11.86 %) were stillborn, and 4 (3.39 %) died within 30 min postpartum. A moderate negative correlation between neonatal lactate and Apgar scores ($p < 0.005$) indicated that higher lactate levels were associated with increased neonatal stress and reduced viability. Lactate was positively correlated with glucose ($p < 0.005$) and negatively correlated with body temperature at birth ($p < 0.05$). No significant association was found between maternal and neonatal plasma lactate, but a negative correlation ($p < 0.01$) was observed between neonatal lactate and maternal glucose. A lactate threshold of 9.96 mmol/L was identified, significantly associated with Apgar scores above seven at one and 5 min postpartum, suggesting improved neonatal viability. These findings support the use of neonatal blood lactate as an early marker of metabolic stress and perinatal hypoxia. However, its predictive value should be interpreted alongside other physiological parameters to optimize neonatal care.

1. Introduction

Parturition is a natural event that presents a challenge for both the mother and the neonates. In dogs, neonatal viability depends on a successful transition from intrauterine to extrauterine life, a process that requires rapid adaptations in respiration, circulation and metabolism [1,2]. To supervise the moment of delivery, several parameters may be assessed, including changes in body temperature, decreasing progesterone levels, or the presence of clear signs of labor. These indicators help distinguish between normal delivery and dystocia, which is crucial for minimizing fetal and maternal stress and reducing maternal and neonatal mortality [3].

Neonatal mortality in dogs varies between 5 % and 35 % [1,2,4], depending on multiple factors that can influence survival before, during, and after birth [5]. While perinatal mortality is influenced by factors such as litter size, mode of delivery, and maternal health [4], neonatal viability at birth is primarily determined by the neonate's ability to transition from intrauterine to extrauterine life. This transition is particularly critical in altricial species such as canines, meaning they are born in a vulnerable state with limited thermoregulatory capacity and an immature respiratory system [1,6]. Identifying early indicators of neonatal viability in the first moments after birth is essential to provide immediate support and improve survival rates [7].

In human medicine, the mode of delivery has been shown to influence neonatal adaptation, particularly in terms of pulmonary function. Infants born via cesarean section exhibit impaired lung function due to reduced pulmonary fluid reabsorption, which can impact their respiratory transition at birth [8]. Similar findings have been observed in dogs, where neonates born naturally show better cardiorespiratory and metabolic adaptation than those delivered by cesarean section. Additionally, bitches that undergo vaginal delivery experience lower stress levels compared to those undergoing cesarean section [8]. Beyond these immediate effects, the type of delivery plays a fundamental role in both neonatal and maternal physiological and metabolic changes. Furthermore, complications arising during the first week of life have been linked to the duration of labor, with prolonged labor and dystocia being associated with higher rates of adverse outcomes, including increased neonatal mortality [8].

One of the most widely used methods for evaluating neonatal viability at birth is the Apgar Score, which assesses five parameters:

* Corresponding author.
E-mail addresses: Raquel.rodriguez@ulgpc.es (R. Raquel), miguel.batista@ulpgc.es, migueljbatart@gmail.com (B. Miguel).

https://doi.org/10.1016/j.theriogenology.2025.117437
Received 20 February 2025; Received in revised form 28 March 2025; Accepted 6 April 2025
Available online 7 April 2025

heart rate, respiratory rate, reflex irritability, mucosal coloration, and mobility [1,9–11]. While this tool provides a rapid and practical assessment, it remains subjective, as it relies on observer interpretation. Additionally, the Apgar score does not provide direct metabolic information, making it necessary to integrate complementary biomarkers to improve neonatal assessment [12]. However, for the accurate application of the Apgar score, certain breed-specific adaptations have been necessary. For instance, modifications have been made for breeds such as the French and English Bulldog, whereas differences have been observed in breeds like the Chihuahua. Nevertheless, authors emphasize the need for further research to explore the Apgar score's variability across different dog breeds and refine its application accordingly [11, 12].

In humans, lactate measurement is a key parameter for assessing fetal distress and the degree of hypoxia during delivery, as elevated lactate levels indicate increased anaerobic metabolism, suggesting potential tissue hypoperfusion and cellular hypoxia [13–15]. In veterinary medicine, lactate has been studied as a potential indicator of neonatal viability, particularly in equine neonates, where it has been associated with perinatal hypoxia and survival outcomes [16,17]. In dogs, there is no consensus regarding the specific lactate thresholds predictive of neonatal survival, and its reliability as an independent viability marker remains uncertain. Previous studies have suggested that elevated lactate levels (>13 mmol/L) are associated with increased neonatal mortality within the first 24 h of life, whereas normal lactate levels range around 5 mmol/L [1]. However, these measurements must be taken as soon as possible, ideally within the first 5 min after birth, to provide relevant prognostic information [1,2].

In addition to lactate, other biochemical and physiological parameters have been studied as indicators of neonatal viability, including glucose levels, temperature and birth weight [7]. Neonatal glucose is a critical factor, as hypoglycemia is one of the leading causes of neonatal mortality. Blood glucose concentrations below 40 mg/dL have been associated with poor Apgar scores [18], while levels below 37 mg/dL within the first 8 h of life have been linked to increased mortality [19]. Similarly, neonatal temperature plays a crucial role in survival, with hypothermic neonates being more susceptible to poor resuscitation outcomes and higher mortality rates [1]. Puppies with body temperatures below 35 °C in the first hour of life are considered hypothermic, and temperatures below 33.9 ± 1.2 °C in neonates younger than seven days have been associated with increased mortality [18].

Furthermore, maternal factors may also influence neonatal biochemical parameters at birth. Maternal hypoglycemia, metabolic status, and anesthetic protocols could potentially impact neonatal lactate concentrations, yet their specific influence remains poorly understood [20,21] Additionally, anesthetic agents used during cesarean sections could affect neonatal viability by crossing the placental barrier and altering neonatal respiratory function [21–23]. While some studies suggest that alfaxalone may result in higher Apgar scores compared to propofol, its direct impact on neonatal lactate levels has not been fully elucidated [24,25].

This study aimed to assess whether neonatal blood lactate can serve as an early biomarker of viability in puppies delivered via emergency cesarean section, but not to assess lactate as a long-term predictor of neonatal survival. Additionally, we assess the relationship between lactate levels with Apgar scores, glucose levels, and temperature to determine its clinical utility. Finally, this study aimed to value whether different maternal factors—including maternal weight, glucose levels, lactate levels, and age—could influence neonatal lactate concentration.

2. Material and methods

2.1. Animals

This study included 28 bitches (*Canis lupus familiaris*) diagnosed with dystocia and admitted to the Reproduction Service of the Veterinary Clinical Hospital (University of Las Palmas de Gran Canaria). A total of 118 neonates were recorded, of which 18 (15.25 %) did not survive (14 stillborn, 4 died within 30 min post-partum). Only dystocic bitches requiring emergency cesarean section were included in the study. The bitches weighed between 1.5 kg and 58 kg; both primiparous and multiparous females were included in the study. The population consisted of various breeds, including Chihuahua, American Bully, Teckel, Presa Canario, and mixed-breed dogs, among others. The age of the bitches ranged from 2 to 8 years, with the majority being between 3 and 4 years old.

Dystocia was diagnosed based on a history of labor for more than 1 h without fetal expulsion or a resting phase exceeding 5 h. On the other hand, ultrasonographic evidence of fetal distress (fetal heart rate <180 bpm) was also defined. Bitches diagnosed with severe systemic diseases (e.g., sepsis, metabolic disorders) or those undergoing elective cesarean sections were excluded from the study.

Data collection was carried out between 2023 and 2024. This study was conducted in accordance with the ethical guidelines established by the Bioethics Committee of the Veterinary Hospital of Las Palmas. Since all animals were clinical patients requiring emergency cesarean section, no additional ethical approval number was necessary. All procedures followed standard emergency protocols, and owners provided informed consent prior to any intervention.

To establish a clear inclusion criterion, only bitches that met at least one of the following conditions were selected: (1) failure to deliver a fetus after at least 1 h of active labor, despite having a progesterone level below 1 ng/mL; (2) after delivering one or more fetuses naturally, the resting period exceeded 6 h without resumption of labor; (3) ultrasound examination showed at least one fetus with a heart rate below 180 bpm.

In cases where some neonates were born naturally, those neonates were excluded from the study, and only those born via cesarean section under the previously mentioned conditions were included. Additionally, the following cases were excluded from the study: (1) stillborn neonates (not counted in the study data); (2) bitches with uterine abnormalities (e.g., torsion, rupture, or poor uterine condition); (3) neonates with congenital malformations; (4) bitches diagnosed with other concurrent pathologies beyond dystocia; (5) cases of elective cesarean sections.

2.2. Experimental design

This study was designed as a prospective observational study, including bitches diagnosed with dystocia and requiring emergency cesarean section. The animals (Table 1) were randomly assigned into two groups based on the anesthetic protocol used for induction. Group 1 received alfaxalone (2 mg/kg IV), while Group 2 received propofol (4 mg/kg IV). Additionally, the bitches were categorized according to maternal weight, with two groups established: those weighing less than 10 kg and those weighing more than 10 kg. Litter size was also classified into three categories: small litters (<3 neonates), medium litters (3–5 neonates), and large litters (>5 neonates). All neonates were evaluated at three specific time points: immediately at birth (T0), 5 min postpartum (T5), and 60 min postpartum (T60) as part of routine neonatal monitoring. Blood samples were collected at each time point for glucose and lactate analysis. However, for this study, only the data obtained at T5 were considered for analysis (Table 2).

Table 1
Groups classification for the experimental design.

Category	Subgroup	N (bitches)	N (Neonates)
Anesthetic group	Alfaxalone	14	60
	Propofol	14	58
Maternal weight	<10 Kg	12	69
	>10 Kg	16	49
Litter Size	Few (<3 neonates)	5	10
	Moderate (3–5 neonates)	13	52
	Many (>5 neonates)	10	56

Table 2
Apgar score for dog newborn viability evaluation.

PARAMETERS	SCORE		
	0	1	2
Mucus colour	Cyanotic	Pale	Pink
Heart rate (bpm)	<180	180–220	>220
Reflex irritability	Absent	Grimace	Vigorous
Motility	Flaccid	Some flexions	Active motion
Respiratory efforts	No crying/<6	Mild crying/6-15	Clear crying/>15

2.3. Preoperative evaluation and anesthetic induction

Upon admission, all bitches underwent a complete clinical examination, including heart rate (bpm), respiratory rate (breaths per minute), blood pressure (mmHg), and rectal temperature (°C) measurements. A venous blood sample was collected from the cephalic vein before anesthesia to assess maternal hematological and biochemical parameters, including hematocrit (%), total protein (g/dL), urea (mg/dL), and creatinine (mg/dL) (Catalyst Dx, IDEXX Laboratories, S.L, Spain). Glucose and lactate concentrations were measured using a PetTest Glucose Monitoring System (PetTest, USA) and a Cera Check Lactate Analyzer (RAL, Spain), respectively.

All bitches underwent a preoperative ultrasonographic examination using a microconvex transducer (C4-1 Curved Array, ZONE Sonography®, Mindray Zonare Z.One PRO) to evaluate fetal viability based on heart rate (bpm) and fetal movements. Fetal distress was defined as a heart rate below 180 bpm. Blood samples were taken to measure progesterone levels using the Speed™ Reader for Speed™ Progesterone (Virbac, Spain). Additionally, thoracic radiographs and an electrocardiogram were obtained as part of the routine pre-surgical protocol to assess anesthetic stability and to estimate the number of neonates, allowing for appropriate organization of the resuscitation team.

2.4. Surgical procedure

For anesthesia, all bitches received premedication with intravenous fentanyl (Fentadon 50 μg/mL, Eurovet Animal Health B.V., Netherlands; 5 μg/kg) diluted in lactated Ringer's solution. Induction was performed using: Alfaxalone (2 mg/kg, IV; Alfaxane 10 mg/mL, Jurox, Ireland) in Group 1; Propofol (4 mg/kg IV; Propovet, 10 mg/mL, Esteve, Spain) in Group 2. Anesthesia was maintained with sevoflurane (2 %) in 100 % oxygen (1–2 L/min) using mechanical ventilation. Throughout the procedure, continuous monitoring was performed, including electrocardiography (ECG), pulse oximetry (SpO_2, %), capnography (end-tidal CO_2, mmHg), and non-invasive blood pressure measurements at 5-min intervals. To minimize fetal exposure to anesthetic agents, the time from induction to fetal extraction was recorded and kept as short as possible (always less than 15 min).

All cesarean sections were performed by the same surgical team following a standardized protocol. A midline abdominal incision was performed, with the length adjusted according to uterine size; the uterus was exteriorized, and fetal extraction was performed via a single hysterotomy at the uterine body. The uterus was closed using a double-layer continuous suture technique with Monosyn 3/0 (B. Braun Surgical SA, Spain). The abdominal wall was closed routinely, and postoperative analgesia was provided with methadone at a dose of 0.2 mg/kg (Semfortan 10 mg/dl, Dechra Veterinary Products S.L.U, Barcelona, Spain).

2.5. Neonatal reanimation

Immediately after birth, each neonate was transferred to a resuscitation area, where a standardized protocol was implemented. Airway clearance was ensured by removing fetal membranes and aspirating fluids from the mouth and nose using a bulb syringe. Neonates were vigorously dried and stimulated to encourage respiration.

Neonatal viability was assessed using the Apgar scoring system (Table 2). The Apgar score, assessed 5 min after birth, included heart rate (stethoscope), respiratory rate (breaths per minute), mucosal color (tongue and perinasal region), mobility (spontaneous movements), and irritability reflex (gentle pad pressure). Neonates were classified as normal vitality (7–10 points), moderate vitality (4–6 points), or poor vitality (0–3 points). To assess metabolic adaptation, rectal temperature (°C), body weight (g), blood glucose (mg/dL), and plasma lactate (mmol/L) levels were recorded: T5 (5 min postpartum) and T60 (60 min postpartum).

Neonates classified as severely compromised (Apgar score <4 at T5) received extended resuscitation, which included: jugular catheter placement for rapid fluid administration, naloxone (0.02 mg/kg IV) in cases of opioid-related respiratory depression and heptaminol (2 mg/kg IV) in cases of persistent bradycardia. Neonatal survival was monitored for the first 24 h postpartum, and mortality within this period was recorded for further analysis. The decision to administer pharmacological treatment was based on both heart rate (less than 80 bpm) and the duration of resuscitation efforts. Heart rate was always assessed prior to drug administration to avoid any influence on the recorded values and ensure an objective evaluation of neonatal viability.

2.6. Parameter testing

For each neonate, Apgar score, lactate (Cera Check Lactato, RAL, Barcelona, Spain), glucose (PetTest Glucose Monitoring System, PetTest, USA), rectal temperature, and birth weight were recorded. Blood samples for glucose and lactate analysis were collected via capillary puncture from the digital pad using a calibrated handheld analyzer and rectal temperature was measured with a digital thermometer.

2.7. Statistical analysis

Statistical analyses were performed using SPSS AMOS 29.0 (SPSS Inc., Chicago, IL, USA). Categorical variables, including neonatal viability (live births, neonatal mortality), anesthetic protocol (alfaxalone, propofol), maternal weight categories (<10 kg, >10 kg), and litter size (<3, 3–5, >5 puppies), were expressed as frequency and proportion. Continuous variables, such as lactate levels and Apgar scores, were presented as mean ± standard deviation. Normality was assessed using the Shapiro–Wilk test. Neonatal viability was analyzed using general linear models (GLM), considering the effects of anesthetic protocol, maternal weight, and litter size, as well as their interactions. The correlation between lactate levels and Apgar scores was analyzed using Spearman's rho correlation test. A statistically significant relationship was considered when $p < 0.05$. Receiver operating characteristic (ROC) curves were generated to assess the predictive value of the Apgar score and lactate levels in neonatal risk identification. The optimal cut-off point was determined using Youden's index to improve classification accuracy. A p-value <0.05 was considered statistically significant.

3. Results

A total of 28 bitches were included in the study and all survived the surgery. A total of 118 neonates were recorded; 14 (11.86 %) were stillborn and were not included in further viability analyses, as their exact time of death could not be determined. The remaining 104 liveborn neonates were monitored for survival outcomes and 4 puppies (3.39 %) died within 15–30 min postpartum. All data analyses and reported results in this study were based on the measurements obtained at the 5-min postpartum evaluation.

Table 3 summarizes neonatal viability based on the anesthetic induction agent, litter size, and maternal weight. Regarding **dam's weight**, mortality was significantly higher in dams weighing **more than 10 kg (32.6 %)** compared to those **under 10 kg (2.9 %)** ($p = 0.006$).

Table 3
Neonatal mortality based in the anesthetic protocol, bitch weight and litter size.

		Total Neonatal mortality
Anesthetic Induction	Propofol	20.6 % (13/60)[a]
	Alfaxalone	9.1 % (5/58)[b]
Mothers's weight	<10 kg	2.9 (2/69)[a]
	>10 kg	32.6 % (16/49)[b]
Litter Size	1-2 neonates	10.0 % (1/10)[a]
	3-5 neonates	9.6 % (5/52)[a]
	>5 neonates	21.4 % (12/56)[b]

[ab]: Different letters in the same row and category denote significant differences ($p < 0,05$).

Regarding **litter size,** the highest rate of mortality (**21.4 %**) was observed in litters >5 **neonates** ($p = 0.031$), while few litters (**1–2 neonates: 10.0 %, 3–5 neonates: 9.6 %)** showed lower mortality rates. Table 4 presents the mean values (± standard deviation) of different neonatal physiological parameters based on the anesthetic induction protocol, excluding deceased neonates. No significant differences were observed in blood glucose (mg/dL), blood lactate (mmol/L), birth weight (g), or rectal temperature (°C) between groups. However, the Apgar score was significantly higher in neonates induced with alfaxalone ($p = 0.0014$) compared to those induced with propofol.

Table 5 categorizes neonatal blood lactate concentrations by maternal weight and litter size. While maternal weight did not significantly influence neonatal lactate levels ($p = 0.03$), significantly lower lactate levels were detected in litters of 3–5 neonates compared to many litters ($p = 0.023$). A cut-off value of 9.96 mmol/L for blood lactate concentration at 5 min postpartum was identified as a potential predictor of neonatal viability in emergency cesarean sections. This threshold was associated with Apgar scores >7, with an Area Under the Curve (AUC) of 0.732, indicating a good predictive capability. The sensitivity and specificity for this threshold were 79.3 % and 78.9 %, respectively (Fig. 1).

A significant moderate negative correlation was observed (Table 6) between neonatal lactate concentrations and Apgar scores (Spearman's *rho* = −0.47, $p = 0.0008$), suggesting that higher lactate levels were associated with lower Apgar scores. A moderate positive correlation was observed between neonatal lactate and glucose levels (Spearman's *rho* = 0.53, $p = 0.0001$), which may reflect metabolic adaptations to perinatal stress. Conversely, a moderate positive correlation was observed between lactate and neonatal glucose levels ($p = 0.00003$). However, no statistically significant correlation was found between lactate levels and birth weight ($p = 0.046$).

Maternal parameters were analyzed to determine their influence on neonatal lactate levels (Table 7). A weak, non-significant positive correlation was observed between maternal and neonatal lactate

Table 4
Apgar scores, birth weight, temperature and plasma levels of glucose and lactate of neonates based on the induction anesthetic agent (mean ± standard deviation).

	Induction anesthetic agent			
	Propofol	Alfaxane	Total	Range
Lactate (mmol/l)	8,71 ± 0,39	8,47 ± 0,467	8,59 ± 0,43	3,20-12
Glucose (mg/dl)	126,20 ± 9,28	117,88 ± 5,8	122,04 ± 7,82	40–417
Temperature (°C)	32,63 ± 0,08	32,73 ± 0,09	32,68 ± 0,09	31,20–34.50
Apgar score (0-10)	7,12 ± 0.31[a]	8,32 ± 0,29[b]	7,72 ± 0,31	3.10
Weight (Kg)	0,40 ± 0,03	0,35 ± 0,02	0,37 ± 0,02	70–900

[ab]: different letters in the same row and category denote significant differences ($p < 0,05$).

Table 5
Average lactate levels (mmol/l) based on litter size and mother size.

		Mean	Std Err	Range
Dam's weight	<10 kg	8,47	0,44	3,42-12
	>10 kg	8,80	0,43	3,17-12
Litter size	1-2 neonates	9,85	0,84	6,09-12
	3-5 neonates	7,90	0,45	3,17-12
	>5 neonates	9,10	0,45	4,33-12

[ab]: different letters in the same row and category denote significant differences ($p < 0.05$).

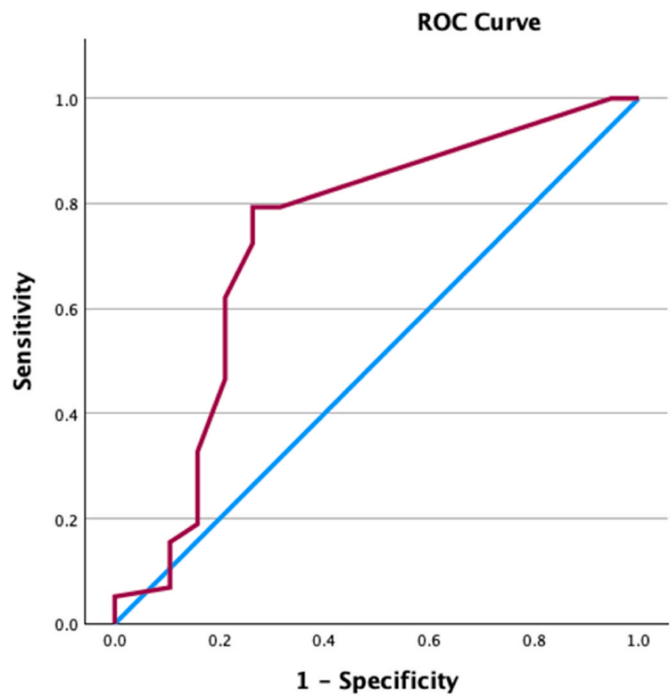


Fig. 1. Receiver operating characteristic (ROC) curve for neonatal blood lactate as a predictor of viability.

Table 6
Correlation coefficients between plasmatic neonatal lactate and neonatal parameters (Apgar score, neonatal weight, neonatal temperature and neonatal glucose level).

Neonatal parameters	Correlation coeficient
Neonatal lactate-Apgar score	−0.471***
Neonatal lactate-Neonatal glucose	−0.531***
Neonatal lactate-Neonatal weight	0.074[ns]
Neonatal lactate-Neonatal temperature	−0.401***

***Correlation was significant at 0,5 % ($p < 0.005$).
[ns] Correlation was not significant.

Table 7
Correlation coefficient of plasma neonatal lactate and maternal parameters (lactate plasma levels, glucose plasma levels, age).

Neonatal parameters	Correlation coeficient
Neonatal lactate-Mother's lactate	0.147 [ns]
Neonatal lactate- Mother's glucose	−0.300**
Neonatal lactate-Bitch weight	0.020[ns]

** Correlation is significant at 1 % level ($p < 0.01$).
[ns] Correlation is not significant.

concentrations (Spearman's *rho* = 0.15, $p = 0.027$). In contrast, a significant moderate negative correlation was detected between maternal glucose levels and neonatal lactate concentrations (Spearman's $rho = -0.30$, $p = 0.0016$). Regarding maternal age, no significant correlation with neonatal lactate levels was identified ($p = 0.081$).

4. Discussion

The primary objective of this study was to evaluate neonatal blood lactate concentration at birth in puppies delivered via emergency cesarean section and assess its potential as a biomarker of neonatal viability. Since lactate is a product of anaerobic metabolism and reflects both tissue oxygenation and metabolic stress, its measurement immediately after birth could provide valuable insights into neonatal adaptation and survival. While previous studies have investigated lactate concentration in amniotic fluid [5,26], data on direct neonatal blood lactate measurement and its relationship with viability parameters remain limited.

In this study, neonatal lactate levels showed a significant correlation with well-established viability indicators, such as Apgar scores, neonatal temperature, and glucose concentration, reinforcing its potential as a prognostic biomarker [1]. These findings align with previous research suggesting that elevated lactate levels are associated with increased neonatal stress and perinatal hypoxia [18]. The observed neonatal mortality rate was 15.25 %, with 11.86 % of neonates stillborn and 3.39 % dying within the first few hours postpartum. These results are consistent with previous reports, where perinatal mortality rates range from 11.3 % within the first 48 h [2] to as high as 25 % in some studies [5]. In other species, such as foals, lactate has been recognized as a valuable health indicator, with elevated concentrations being associated with bacterial infections and systemic inflammatory response syndrome (SIRS) [27,28].

Previous studies have reported an association between elevated lactate concentrations (>12.2 mmol/L at 48 h) and increased neonatal mortality risk [1]. However, the predictive value of lactate may vary depending on perinatal conditions, resuscitation efforts, and postnatal management [29]. In the present study, neonates delivered via dystocia cesarean section had an average lactate concentration of 8.59 mmol/L. While this value is above the previously reported survival threshold (6.55 mmol/L), it remains below the critical level associated with increased mortality risk (12.2 mmol/L). Notably, all neonates survived the initial 4 h post-birth during hospitalization. Given that dystocia is associated with fetal distress and perinatal hypoxia, the elevated lactate levels observed in this study may reflect the metabolic response to birth-related stress [21]. Other authors have reported biochemical alterations in calves and lambs, including changes in lactate levels. Their findings indicate that lactate concentrations are significantly higher in non-surviving neonates (16 mmol/L) compared to those that survive (5.6 mmol/L) [27].

Several studies have proposed different lactate cut-off values for neonatal prognosis. Some authors suggest that concentrations around 5 mmol/L may be indicative of favorable outcomes [2], while others report that values exceeding 8 mmol/L could be associated with increased neonatal risk. However, variations in study populations, resuscitation protocols, and measuring devices must be considered when interpreting these thresholds [1]. However, considerable variability exists among reports regarding these threshold values, highlighting the need for further research to establish a clear consensus. In the present study, although the mean lactate concentration surpassed the 8 mmol/L threshold, no neonatal deaths were observed within the first 4 h of life. A lactate threshold of 9.96 mmol/L was established as a predictor of neonatal viability, as it was significantly associated with Apgar scores above seven at one and 5 min postpartum. This discrepancy may be attributed to the increased fetal stress and subsequent tissue hypoxia experienced by neonates born from emergency dystocic cesarean sections. While this threshold provides a valuable reference for clinical decision-making, it should be interpreted with caution until validated in larger, multicentric studies and further research is needed to confirm its applicability across different populations, breeds, and clinical settings. Although our study did not include lactate measurements in neonates born via elective cesarean section or natural birth, previous studies have reported no significant differences between delivery methods. However, higher lactate concentrations have been observed in neonates delivered by emergency cesarean section, whereas the lowest levels are typically found in those born via elective cesarean section [2,17].

Although the main objective of this study was to assess how lactate levels can serve as an indicator of neonatal viability immediately after birth, other authors have explored its utility during the first days of life. Several studies have reported that lactate concentrations are highest immediately after birth and gradually decrease as the neonate develops [17,30]. Additionally, lactate was not evaluated as a long-term marker due to the inability to control neonatal management at home, which could introduce other causes of mortality unrelated to lactate concentrations. Additionally, previous studies have reported that Apgar scores may vary depending on neonatal weight, which could influence the overall results. For instance, significant differences have been observed between large and small neonates, potentially affecting their scores [11]. However, further research is needed to better understand the influence of neonatal size and breed on Apgar scoring, as only a limited number of studies have addressed this aspect. Refining these parameters could help establish more precise cut-off values for neonatal viability assessment at birth.

Recent studies have reported variability in Apgar scores depending on the breed. For instance, in Chihuahuas, the survival cut-off after cesarean section has been established at 4 points [31], whereas in our study, we considered a minimum score of 7 points for good viability. Additionally, other studies analyzing multiple breeds have suggested a cut-off of 6 points to identify adequate neonatal viability [32]. Given that this is a preliminary study aiming to establish a cut-off for neonates born via emergency cesarean section, as well as to compare it with other parameters that have not yet been fully explored, further research is needed to assess potential differences between breeds and delivery methods.

The relationship between lactate concentration and the Apgar score has been previously investigated, with some studies reporting no significant correlation within the first 10 min of life and even suggesting the absence of correlation during the first 8 h postpartum [1,32]. Contrary to these findings, our study identified a moderate yet statistically significant negative correlation between Apgar scores and lactate levels, demonstrating that lower Apgar scores were associated with higher lactate concentrations. Similar results have been described in previous studies, suggesting that elevated lactate concentrations may reflect increased tissue hypoxia and reduced neonatal viability [18]. Given the moderate strength of this correlation, it is essential to consider additional parameters when assessing neonatal viability rather than relying exclusively on lactate and Apgar scores. However, since the association is statistically significant, lactate may still serve as a potential predictive model for neonatal condition. Therefore, it could be a useful tool for determining the level of postnatal care required, helping to identify neonates who may need closer monitoring and medical intervention.

Neonatal glucose measurement is a commonly used parameter at birth, providing insight into the energy reserves of neonates. Due to hepatic immaturity, neonates have limited glycogen stores, making them susceptible to hypoglycemia [33,34]. Several factors, including birth weight and maternal condition, have also been implicated in neonatal glucose fluctuations [1]. While some studies have reported an association between low glucose levels, poorer Apgar scores, and increased mortality risk [18], others have indicated that excessively high glucose concentrations may also correlate with increased neonatal mortality [35]. However, glucose alone has been deemed an unreliable predictor of neonatal mortality [36,37]. In the present study, a moderate yet statistically significant positive correlation was observed between neonatal glucose and lactate concentrations at birth. This relationship could be attributed to perinatal stress, which activates fetal and neonatal adaptive responses, particularly in cases of prolonged or complicated deliveries such as dystocic cesarean sections. Neonatal hypoxia, a common consequence of dystocia, promotes anaerobic metabolism, leading to increased lactate production. Simultaneously, stress-induced catecholamine release may elevate neonatal glucose levels, potentially explaining the observed correlation [5]. Few studies have explored the direct correlation between blood glucose and neonatal lactate levels, although an association between amniotic fluid glucose and blood lactate has been described [1]. Previous studies have reported that neonates born from dystocic deliveries exhibit higher blood glucose levels compared to those delivered eutocically [5]. These findings align with our results, further supporting the hypothesis that neonatal metabolic adaptations to birth stress influence both glucose and lactate dynamics. Future investigations should focus on refining the predictive value of combined glucose and lactate measurements in neonatal viability assessment, particularly in high-risk deliveries. In other species, such as pigs, a potential relationship between glucose and lactate has been described, linked to metabolic cycles. Authors have reported that gluconeogenesis occurs simultaneously with other energy-demanding processes, leading to lactate release from muscle cells and, consequently, lactic acidosis. These metabolic pathways, which remain poorly studied in neonates, could also explain the positive correlation observed between glucose and lactate in our study. Further research is needed to elucidate the physiological mechanisms underlying this association and to determine its clinical relevance in neonatal viability assessment [38].

Birth weight has been widely recognized as a risk factor for neonatal survival, as low-birth-weight neonates are more susceptible to oxygen restrictions and the secondary effects of hypoxia [39,40]. Several studies have reported a significant correlation between low birth weight and increased mortality within the first 2–4 days of life [41–43]. However, in the present study, a weak and statistically insignificant positive correlation was observed between lactate levels and birth weight.

Neonatal temperature is a critical viability parameter, as hypothermia has been associated with reduced neonatal responsiveness and lower success rates in resuscitation efforts [1,18,31]. Moreover, hypoxia in neonates has been linked to bradycardia, respiratory depression, and a diminished suckling reflex, ultimately contributing to lower Apgar scores [1]. For the first time, this study identified a statistically significant moderate negative correlation between neonatal temperature and lactate concentration at birth. This finding suggests that neonates with lower body temperatures tend to exhibit lower heart and respiratory rates, leading to an accumulation of lactate due to insufficient oxygen delivery to tissues [44]. Neonates with lower Apgar scores often struggle with respiration and circulation, linking hypothermia to higher lactate levels and increased metabolic distress. This highlights the need for immediate thermoregulation to reduce hypoxia and acidosis. Further research is needed to assess temperature monitoring in predicting viability and improving early interventions.

One of the objectives of this study was to assess whether maternal weight and litter size influence neonatal lactate concentrations at birth. Previous research has examined these parameters, reporting a non-significant negative correlation between maternal weight and neonatal lactate, as well as a non-significant positive correlation between maternal age and lactate levels [44]. In the present study, no significant correlation was observed between neonatal lactate concentration and maternal weight. A plausible explanation for this finding is that lactate is primarily a metabolite associated with tissue hypoxia and neonatal stress [1], both of which are influenced by perinatal conditions rather than maternal body size.

No significant differences in neonatal lactate levels were observed between different litter sizes. While the impact of litter size on neonatal biochemical parameters in cesarean section deliveries has not been extensively studied, previous research has demonstrated its influence on gas exchange and other physiological parameters in natural births, particularly when comparing the first and last neonates born [40,45]. A possible explanation for the lack of significant differences in lactate levels based on litter size in this study is the timing of delivery. Unlike natural births, where prolonged delivery increases intrauterine hypoxia, emergency cesarean sections allow for rapid fetal extraction, potentially homogenizing neonatal lactate levels regardless of litter size. While litter size may influence perinatal stress in natural births, its effect on lactate levels in cesareans appears reduced due to shorter delivery times. Further research comparing lactate dynamics across different delivery methods is needed to clarify this relationship.

Maternal parameters were evaluated in relation to neonatal lactate levels. A weak, non-significant positive correlation was observed between maternal and neonatal lactate concentrations. This finding aligns with previous literature indicating that the amount of lactate crossing the placenta during labor is minimal, with most of the neonatal lactate concentration being the product of glycolysis produced by the neonate itself [46]. A moderate negative correlation was found between maternal glucose and neonatal lactate levels, suggesting that lower maternal glucose is linked to higher neonatal lactate. This may be due to fetal reliance on maternal glucose; in hyperglycemia, increased fetal insulin promotes aerobic metabolism, reducing lactate. However, as gestation progresses, metabolic changes can impair glucose homeostasis, predisposing bitches to hypoglycemia [20]. This maternal hypoglycemia may negatively impact uterine function, potentially resulting in uterine inertia or weak contractions. Additionally, hypoglycemia can impair neuromuscular function, reducing coordination and contraction strength, which may impede the normal progression of labor [21].

Anesthesia in cesarean sections is a critical factor, particularly because some anesthetic agents can induce respiratory depression in neonates [21,23]. This study assessed the impact of the induction agent (alfaxalone vs. propofol) on neonatal lactate levels, finding no significant differences between protocols. This aligns with existing literature, which suggests that while some anesthetics cross the placenta, their effect on neonatal viability is limited [4,44,47]. In this study, no significant differences in neonatal lactate levels were found, and no neonatal mortality occurred at birth, supporting the safety of the anesthetic protocol. This aligns with previous research showing that while alfaxalone may slightly improve Apgar scores, differences from propofol are not statistically significant, with both agents considered safe for cesarean sections [24,25,48]. It is important to note that although all drugs cross the placental barrier [21], this study specifically evaluated propofol and alfaxalone. Future studies should also consider the potential influence of other drugs, such as fentanyl, to determine whether there is a correlation between their use and lactate levels in cesarean sections.

This study provides relevant insights into neonatal blood lactate as a potential biomarker of viability. Although the power analysis conducted indicates that the sample size (28 bitches and 118 neonates) was sufficient for statistical analysis, a larger cohort is required to strengthen conclusions and improve external validation. This is a preliminary study, and we aim to continue advancing research in this area to further refine lactate thresholds and explore additional neonatal viability biomarkers. Lactate measurements were performed immediately postpartum, but serial assessments over the first 24–48 h could offer a more comprehensive understanding of lactate dynamics and its role in predicting neonatal survival beyond the immediate postnatal period. Additionally, this study focused on emergency cesarean sections due to dystocia, a high-risk neonatal scenario. Further research should explore lactate thresholds in elective cesareans and natural deliveries to establish broader reference values.

5. Conclusion

Plasmatic neonatal lactate may serve as a valuable parameter for assessing metabolic stress and hypoxic conditions in neonates, particularly in high-risk populations such as those delivered via emergency cesarean section. However, its use as a standalone biomarker should be approached with caution, as lactate levels can be influenced by multiple perinatal factors, including delivery conditions, neonatal thermoregulation, and maternal metabolic status. Considering these complexities, lactate assessment should be incorporated into a broader diagnostic framework that includes additional biochemical and clinical parameters. A multimodal approach would allow for a more comprehensive evaluation of neonatal viability, facilitating more accurate clinical decision-making during the perinatal period. Further studies should aim to refine neonatal lactate thresholds and evaluate its integration with other biomarkers to enhance the accuracy of viability assessments in newborn puppies.

CRediT authorship contribution statement

Rodríguez Raquel: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Batista Miguel:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Iusupova Ksenia:** Writing – review & editing, Methodology, Formal analysis. **Alamo Desirée:** Writing – review & editing, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Veronesi MC, Fusi J. Neonatal severity indicators in dogs. Rev Bras Reprod Anim 2021;45:525–32. https://doi.org/10.21451/1809-3000.rbra2021.071.
[2] Groppetti D, Pecile A, Del Carro AP, Copley K, Minero M, Cremonesi F. Evaluation of newborn canine viability by means of umbilical vein lactate measurement, Apgar score and uterine tocodynamometry. Theriogenology 2010;74:1187–96. https://doi.org/10.1016/j.theriogenology.2010.05.020.
[3] Cornelius AJ, Moxon R, Russenberger J, Havlena B, Cheong SH. Identifying risk factors for canine dystocia and stillbirths. Theriogenology 2019;128:201–6. https://doi.org/10.1016/j.theriogenology.2019.02.009.
[4] Rodríguez-Trujillo R, Batista-Arteaga M, Iusupova K, Rosario-Medina I, Alamo-Santana D. Comparison of propofol and alfaxalone as anesthetic drugs in bitches undergoing ovariohysterectomies (healthy bitches and with pyometra) and cesarean sections. Animals 2024;14:1343. https://doi.org/10.3390/ani14091343.
[5] Plavec T, Knific T, Slapšak A, Raspor S, Lukanc B, Pipan MZ. Canine neonatal assessment by vitality score, amniotic fluid, urine, and umbilical cord blood analysis of glucose, lactate, and cortisol: possible influence of parturition type? Animals 2022;12:1247. https://doi.org/10.3390/ani12101247.
[6] Teifke JP, Peuckert C, Rudnick JC, Büttner K, Bostedt H. Computed tomographic and histopathologic studies of lung function immediately post natum in canine neonates. Animals 2023;13(11):1741. https://doi.org/10.3390/ani13111741.
[7] Axnér E, Axelsson R, Hermansson U. Evaluation of canine neonatal health by breeders: a prospective questionnaire study on the association between neonatal scores (modified APGAR), parturition, birth weight, growth, and puppy mortality. Animals 2023;13(23):3605. https://doi.org/10.3390/ani13233605.
[8] Abreu RA, Almeida LL, Rosa Filho RRD, Angrimani DSR, Brito MM, Flores RB, Vannucchi CI. Canine pulmonary clearance during feto-neonatal transition according to the type of delivery. Theriogenology 2024;224:156–62. https://doi.org/10.1016/j.theriogenology.2024.05.022.
[9] Alonge S, Melandri M. Effect of delivery management on first-week neonatal outcome: how to improve it in Great Danes. Theriogenology 2019;125:310–6. https://doi.org/10.1016/j.theriogenology.2018.11.006.
[10] Batista M, Moreno C, Vilar J, Golding M, Brito C, Santana M, Alamo D. Neonatal Viability evaluation by Apgar score in puppies delivered by cesarean section in two brachycephalic breeds (English and French bulldog). Anim Reprod Sci 2014;146: 218–26. https://doi.org/10.1016/j.anireprosci.2014.03.003.
[11] Veronesi MC, Faustini M, Probo M, Rota A, Fusi J. Refining the APGAR score cutoff values and viability classes according to breed body size in newborn dogs. Animals 2022;12(13):1664. https://doi.org/10.3390/ani12131664.
[12] Veronesi MC. Assessment of canine neonatal viability—the Apgar score. Reprod Domest Anim 2016;51:46–50. https://doi.org/10.1111/rda.12787.
[13] Wu TW, Tamrazi B, Hsu KH, Ho E, Reitman AJ, Borzage M, Wisnowski JL. Cerebral lactate concentration in neonatal hypoxic-ischemic encephalopathy: in relation to time, characteristic of injury, and serum lactate concentration. Front Neurol 2018; 9:293. https://doi.org/10.3389/fneur.2018.00293.
[14] Deshpande SA, Platt MW. Association between blood lactate and acid-base status and mortality in ventilated babies. Arch Dis Child Fetal Neonatal Ed 1997;76(1): F15–20. https://doi.org/10.1136/fn.76.1.f15.
[15] Marconi AM, Cetin I, Ferrazzi E, Ferrari MM, Pardi G, Battaglia FC. Lactate metabolism in normal and growth-retarded human fetuses. Pediatr Res 1990;28(6): 652–6. https://doi.org/10.1203/00006450-199012000-00022.
[16] Tennent-Brown B. Blood lactate measurement and interpretation in critically ill equine adults and neonates. Vet Clin Equine Pract 2014;30(2):399–413. https://doi.org/10.1016/j.cveq.2014.04.006.
[17] Castagnetti C, Pirrone A, Mariella J, Mari G. Venous blood lactate evaluation in equine neonatal intensive care. Theriogenology 2010;73(3):343–57. https://doi.org/10.1016/j.theriogenology.2009.09.018.
[18] Vassalo FG, Simões CRB, Sudano MJ, Prestes NC, Lopes MD, Chiacchio SB, Lourenço MLG. Topics in the routine assessment of newborn puppy viability. Top Companion Anim Med 2015;30:16–21. https://doi.org/10.1053/j.tcam.2015.02.003.
[19] Mila H, Grellet A, Feugier A, Chastant-Maillard S. Differential impact of birth weight and early growth on neonatal mortality in puppies. J Anim Sci 2015;93: 4436–42. https://doi.org/10.2527/jas.2015-8971.
[20] Johnson CA. Glucose homeostasis during canine pregnancy: insulin resistance, ketosis, and hypoglycemia. Theriogenology 2008;70(9):1418–23. https://doi.org/10.1016/j.theriogenology.2008.09.008.
[21] Henrique FV, Silva A O da, Silva A V A da, Carneiro R dos S, Araújo NL de S. Maternal dystocia by primary uterine inertia associated with hypoglycemic shock in dog: case report. Arq Ciênc Vet Zool 2015;18(3):179–83.
[22] Oliva VNLS, Queiroz MCVG, Albuquerque VB, Carreira JT, Souza TFB, Ferreira GTNM, Abimussi CJX, Vides JP. Vitality evaluation methods for newborn puppies after cesarean section performed under general inhalation anesthesia. Pesq Vet Bras 2018;38(6):1172–7. https://doi.org/10.1590/1678-5150-PVB-5350.
[23] Srithunyarat T, Jitpean S, Wipoosak P, Nonthakotr C, Boonbal N, Kunkitti P, Seesupa S. Apgar scores in puppies following the induction of etomidate compared with alfaxalone or propofol for cesarean section. Vet World 2024;17:527–34. https://doi.org/10.14202/vetworld.2024.527-534.
[24] Doebeli A, Michel E, Bettschart R, Hartnack S, Reichler IM. Apgar score after induction of anesthesia for canine cesarean section with alfaxalone versus propofol. Theriogenology 2013;80:850–4. https://doi.org/10.1016/j.theriogenology.2013.07.006.
[25] Sofyan LM, Martinez-Taboada F. Comparison of alfaxalone versus propofol as anaesthetic induction agents in increasing the rate of survival and vigour of neonates. Veterinary Evidence 2021;6:e002. https://doi.org/10.18849/ve.v6i2.344.
[26] Bolis B, Scarpa P, Rota A, Vitiello T, Veronesi MC. Association of amniotic uric acid, glucose, lactate and creatinine concentrations and lactate/creatinine ratio with newborn survival in small-sized dogs – preliminary results. Acta Vet Hung 2018;66:125–36. https://doi.org/10.1556/004.2018.012.
[27] Bienboire-Frosini C, Muns R, Marcet-Rius M, Gazzano A, Villanueva-García D, Martínez-Burnes J, Mota-Rojas D. Vitality in newborn farm animals: adverse factors, physiological responses, pharmacological therapies, and physical methods to increase neonate vigor. Animals 2023;13(9):1542. https://doi.org/10.3390/ani13091542.
[28] Castagnetti C, Pirrone A, Mariella J, Mari G. Venous blood lactate evaluation in equine neonatal intensive care. Theriogenology 2010;73(3):343–57. https://doi.org/10.1016/j.theriogenology.2009.09.018.
[29] Di Mauro FM, Schoeffler GL. Point of care measurement of lactate. Top Companion Anim Med 2016;31:35–43. https://doi.org/10.1053/j.tcam.2016.05.004.
[30] Mamão LD, Malm C, Maia MZ, Reis FG, Mendes ALS, Santos DFTD, Silveira CFD. Comparison of Apgar score, serum lactate, and blood gas analysis in neonates born by elective cesarean section or cesarean section after dystocia. Ciência Rural 2022; 53(4). https://doi.org/10.1590/0103-8478cr20201009.
[31] Fusi J, Faustini M, Bolis B, Veronesi MC. Apgar score or birthweight in Chihuahua dogs born by elective Caesarean section: which is the best predictor of the survival at 24 h after birth? Acta Vet Scand 2020;62:1–8. https://doi.org/10.1186/s13028-020-00538-y.
[32] Mila H, Grellet A, Delebarre M, Mariani C, Feugier A, Chastant-Maillard S. Monitoring of the newborn dog and prediction of neonatal mortality. Prev Vet Med 2017;143:11–20. https://doi.org/10.1016/j.prevetmed.2017.05.005.
[33] Münnich A. Fading kitten syndrome: factors predisposing to 'faders' and treatment options. J Feline Med Surg 2022;24:243–56. https://doi.org/10.1177/1098612X221079710.
[34] Pereira KHNP, Fuchs KdM, Corrêa JV, Chiacchio SB, Lourenço MLG. Neonatology: topics on puppies and kittens neonatal management to improve neonatal outcome. Animals 2022;12:3426. https://doi.org/10.3390/ani12233426.
[35] Castagnetti C, Cunto M, Bini C, Mariella J, Capolongo S, Zambelli D. Time-dependent changes and prognostic value of lactatemia during the first 24 h of life in brachycephalic newborn dogs. Theriogenology 2017;94:100–4. https://doi.org/10.1016/j.theriogenology.2017.01.040.
[36] Vanspranghels R, Houfflin-Debarge V, Deken V, Rakza T, Maboudou P, Storme L, Ghesquiere L, Garabedian C. Umbilical cord arterial and venous gases, ionogram, and glucose level for predicting neonatal morbidity at term. Eur J Obstet Gynecol Reprod Biol 2020;252:181–6. https://doi.org/10.1016/j.ejogrb.2020.06.022.
[37] Lúcio CF, Silva LCG, Vannucchi CI. Perinatal cortisol and blood glucose concentrations in bitches and neonatal puppies: effects of mode of whelping. Domest Anim Endocrinol 2021;74:106–483. https://doi.org/10.1016/j.domaniend.2020.106483.
[38] Bienboire-Frosini C, Muns R, Marcet-Rius M, Gazzano A, Villanueva-García D, Martínez-Burnes J, Mota-Rojas D. Vitality in newborn farm animals: adverse factors, physiological responses, pharmacological therapies, and physical methods to increase neonate vigor. Animals 2023;13(9):1542. https://doi.org/10.3390/ani13091542.

[39] Münnich A, Küchenmeister U. Causes, diagnosis and therapy of common diseases in neonatal puppies in the first days of life: cornerstones of practical approach. Reprod Domest Anim 2014;49:64–74. https://doi.org/10.1111/rda.12329.
[40] Reyes-Sotelo B, Ogi A, Mora-Medina P, Mariti C, Olmos-Hernández A, Hernández-Ávalos I, Domínguez-Oliva A, Rosas ME, Verduzco-Mendoza A, Gazzano A. Early blood analysis and gas exchange monitoring in the canine neonate: effect of dam's size and birth order. Animals 2022;12(12). https://doi.org/10.3390/ani12121508.
[41] Gill MA. Perinatal and late neonatal mortality in the dog. Sydney, Australia: University of Sydney; 2001. Ph.D. Thesis, http://hdl.handle.net/2123/4137.
[42] Mila H, Grellet A, Chastant-Maillard S. Prognostic value of birth weight and early weight gain on neonatal and pediatric mortality: a longitudinal study on 984 puppies. In: Proceedings of the 7th international symposium on canine and Feline reproduction—ISCFR. Canada: Whistler, BC; 26–29 July 2012.
[43] Grundy SA. Clinically relevant physiology of the neonate. Vet Clin North Am Small Anim Pract 2006;36:443–59. https://doi.org/10.1016/j.cvsm.2005.12.002.
[44] Kuttan KV, Joseph M, Simon S, Ghosh KNA, Rajan A. Effect of intrapartum fetal stress associated with obstetrical interventions on viability and survivability of canine neonates. Vet World 2016;9:1485–8. https://doi.org/10.14202/vetworld.2016.1485-1488.
[45] de Almeida LL, Abreu RAD, Brito MM, Gardés TP, Flores RB, Rosa Filho RRD, Vannucchi CI. Both spontaneous vaginal delivery and elective caesarean section influence neonatal redox status in dogs. Vet Rec 2022;190(5):e1082. https://doi.org/10.1002/vetr.1082.
[46] Kredatusova G, Hajurka J, Szakallova I, Valencakova A, Vojtek B. Physiological events during parturition and possibilities for improving puppy survival: a review. Vet Med 2011;56:589–94. https://doi.org/10.17221/4436-VETMED.
[47] Ruiz CC, Del Carro AP, Rosset E, Guyot E, Maroiller L, Buff S, Portier K. Alfaxalone for total intravenous anaesthesia in bitches undergoing elective caesarean section and its effects on puppies: a randomized clinical trial. Vet Anaesth Analg 2016;43:281–90. https://doi.org/10.1111/vaa.12298.
[48] Haythornthwaite B. Comparison of the safety of alfaxalone and propofol as anaesthetic induction agents in bitches undergoing C-section. Vet Evid 2020;5:e3. https://doi.org/10.18849/ve.v5i3.313.

8. CONCLUSIONES

Artículo 1.
Based on the findings of this study, propofol and alfaxalone showed similar efficacy for healthy bitches and bitches with pyometra undergoing an ovariohysterectomy, with no significant differences in hemodynamic stability during surgery. Both anesthetic protocols proved to be effective for cesarean sections. Alfaxalone was associated with a higher neonatal vitality as indicated by immediate post-birth Apgar scores. However, it is important to note that this did not translate into a statistically significant difference in neonatal survival rates between the two protocols.

Según los hallazgos de este estudio, el propofol y la alfaxalona mostraron una eficacia similar en perras sanas y perras con piómetra sometidas a ovariohisterectomía, sin diferencias significativas en la estabilidad hemodinámica durante la cirugía. Ambos protocolos anestésicos demostraron ser eficaces en cesáreas. La alfaxalona se asoció con una mayor vitalidad neonatal, según lo indicado por las puntuaciones de Apgar inmediatamente después del parto. Sin embargo, es importante destacar que esto no se tradujo en una diferencia estadísticamente significativa en las tasas de supervivencia neonatal entre ambos protocolos.

Artículo 2.
The present study confirmed the influence of maternal factors over the type of cesarean sections and the incidence of neonatal mortality. Scheduled cesareans were more frequent in bitches with a complete health status, multiparous and a litter size between 4 and 6 puppies, while the higher incidence in emergency C-sections was recorded in bitches without previous birth, with incomplete health status and a big litter size (>6 puppies). Neonatal mortality was notable higher in emergency C-sections, in bitches without previous births and with a not proper sanitary management. Finally, inbreeding and incomplete sanitary health conditions were factors that could favor a higher incidence of congenital malformations. The comprehensive analysis of the study indicates that proper reproductive management is crucial for gestation and the safe delivery of neonates. Appropriate genetic selection of breeders, maintaining good sanitary health conditions, and considering the age of the reproducers, are pivotal factors in planning for gestation.

El presente estudio confirmó la influencia de los factores maternos sobre el tipo de cesáreas y la incidencia de mortalidad neonatal. Las cesáreas programadas fueron más frecuentes en perras con un estado de salud completo, multíparas y un tamaño de camada entre 4 y 6 cachorros, mientras que la mayor incidencia en cesáreas de emergencia se registró en perras sin partos previos, con un estado de salud incompleto y un tamaño de camada grande (>6 cachorros). La mortalidad neonatal fue notablemente mayor en cesáreas de emergencia, en perras sin partos previos y con un manejo sanitario inadecuado. Finalmente, la endogamia y las condiciones sanitarias incompletas fueron factores que podrían favorecer una mayor incidencia de malformaciones congénitas. El análisis exhaustivo del estudio indica que el manejo reproductivo adecuado es crucial para la gestación y el parto seguro de los neonatos. La selección genética apropiada de los reproductores, el mantenimiento de buenas condiciones sanitarias y la consideración de la edad de los reproductores son factores fundamentales en la planificación de la gestación.

Artículo 3.

Fetal kidney area, in particular, may serve as an indirect marker of fetal maturity and could be clinically relevant in the context of elective cesarean section planning. Assessing whether fetal organ development is sufficient prior to delivery is essential to prevent iatrogenic prematurity, especially in high-risk pregnancies and brachycephalic breeds predisposed to dystocia. If further validated, fetal renal biometry could complement current protocols by providing additional criteria for the optimal timing of surgical parturition. While ultrasonography is widely established in veterinary obstetrics, additional research focusing on fetal organ development—particularly renal growth patterns—in pregnant bitches is warranted. The development of robust, breed-adjusted predictive models based on kidney biometry holds promise for improving clinical decision-making, minimizing perinatal morbidity, and enhancing the safety of canine deliveries.

El área renal fetal, en particular, puede servir como marcador indirecto de la madurez fetal y podría ser clínicamente relevante en el contexto de la planificación de cesáreas electivas. Evaluar si el desarrollo de los órganos fetales es suficiente antes del parto es esencial para prevenir la prematuridad iatrogénica, especialmente en gestaciones de alto riesgo y razas braquicefálicas con predisposición a la distocia. Si se valida aún más, la biometría renal fetal podría complementar los protocolos actuales al proporcionar criterios adicionales para el momento óptimo del parto quirúrgico. Si bien la ecografía está ampliamente establecida en obstetricia veterinaria, se justifica la investigación adicional centrada en el desarrollo de los órganos fetales, en particular los patrones de crecimiento renal, en perras gestantes. El desarrollo de modelos predictivos robustos y ajustados a la raza basados en la biometría renal es prometedor para mejorar la toma de decisiones clínicas, minimizar la morbilidad perinatal y aumentar la seguridad de los partos caninos.

Artículo 4.

Plasmatic neonatal lactate may serve as a valuable parameter for assessing metabolic stress and hypoxic conditions in neonates, particularly in high-risk populations such as those delivered via emergency cesarean section. However, its use as a standalone biomarker should be approached with caution, as lactate levels can be influenced by multiple perinatal factors, including delivery conditions, neonatal thermoregulation, and maternal metabolic status. Considering these complexities, lactate assessment should be incorporated into a broader diagnostic framework that includes additional biochemical and clinical parameters. A multimodal approach would allow for a more comprehensive evaluation of neonatal viability, facilitating more accurate clinical decision-making during the perinatal period.

El lactato plasmático neonatal puede ser un parámetro valioso para evaluar el estrés metabólico y las condiciones hipóxicas en neonatos, especialmente en poblaciones de alto riesgo, como los nacidos por cesárea de emergencia. Sin embargo, su uso como biomarcador independiente debe considerarse con cautela, ya que los niveles de lactato pueden verse influenciados por múltiples factores perinatales, como las condiciones del parto, la termorregulación neonatal y el estado metabólico materno. Considerando estas complejidades, la evaluación del lactato debe incorporarse a un marco diagnóstico más amplio que incluya parámetros bioquímicos y clínicos adicionales. Un enfoque multimodal permitiría una evaluación más completa de la viabilidad neonatal, facilitando una toma de decisiones clínicas más precisa durante el período perinatal.

Artículo 5.

This study highlights the critical role of glucose levels in neonatal viability, particularly in small animals undergoing cesarean sections due to dystocia. The findings reveal that neonatal hypoglycemia is a significant contributor to mortality within the first hours and days of life, underscoring the necessity for vigilant monitoring of glucose levels in neonates. The established cutoff values for glucose, based on Apgar scores, demonstrate that lower glucose concentrations correlate with poorer neonatal outcomes, thereby emphasizing the importance of timely interventions in cases of hypoglycemia. Specifically, a glucose level below 79.50 mg/dL is associated with a marked decrease in viability, which can severely impair a neonate's ability to suckle and respond to environmental stimuli. Furthermore, this study indicates that maternal factors, such as weight and glucosa levels, influence neonatal glucose status, though a direct correlation between maternal and neonatal glucose levels was not established. This suggests that while maternal health is pivotal, neonatal metabolic maturity and response to stressors also play a crucial role in determining glucose levels at birth.

Este estudio destaca el papel crucial de los niveles de glucosa en la viabilidad neonatal, en particular en animales pequeños sometidos a cesáreas por distocia. Los hallazgos revelan que la hipoglucemia neonatal contribuye significativamente a la mortalidad durante las primeras horas y días de vida, lo que subraya la necesidad de un monitoreo riguroso de los niveles de glucosa en neonatos. Los valores de corte establecidos para la glucosa, basados en las puntuaciones de Apgar, demuestran que las concentraciones más bajas de glucosa se correlacionan con peores resultados neonatales, lo que enfatiza la importancia de las intervenciones oportunas en casos de hipoglucemia. Específicamente, un nivel de glucosa inferior a 79,50 mg/dL se asocia con una marcada disminución de la viabilidad, lo que puede afectar gravemente la capacidad del neonato para mamar y responder a los estímulos ambientales. Además, este estudio indica que factores maternos, como el peso y los niveles de glucosa, influyen en el estado de la glucosa neonatal, aunque no se estableció una correlación directa entre los niveles de glucosa maternos y neonatales. Esto sugiere que, si bien la salud materna es fundamental, la madurez metabólica neonatal y la respuesta a los factores estresantes también juegan un papel crucial en la determinación de los niveles de glucosa al nacer.

9. CONTRIBUCIONES CIENTÍFICAS

PARTICIPACIÓN EN CONGRESOS NACIONALES E INTERNACIONALES.

- "Pyometra in bitches: a retrospective study over the clinical, histopathological and microbiological findings". **25th International EVSSAR Congress**. Poster comunication.

- The quality of dog semen on thawed samples: the effect of storage temperature and the use of post-thawed médium. **25th International EVSSAR Congress**. Poster comunication.

- Use of ultrasonography and plasma levels of canine prostatic specific esterase to value the prostate in dogs. **25th International EVSSAR Congress**. Oral presentation.

- Impact of umbilical lactate levels on puppy viability: correlations with neonatal and maternal parameters. **25th International EVSSAR Congress**. Oral presentation.

- Apgar test and plasmatic levels of glucose and lactate as viability indicators in puppies after C-section. **Joint ISCFR + EVSSAR Congress.** Poster comunication.

- Comparison of alfaxolone and Propofol administered as anesthettic induction agent for ovariohysterectomy in bitches. **Joint ISCFR + EVSSAR Congress.** Oral presentation.

- Valoración ecográfica y determinación de la esterasa prostática específica canina (CPSE) para el diagnóstico de la hiperplasia prostática en perros. **XVIII Congreso Andaluz de Veterinarios Especialistas en Animales de Compañía.** Póster.

- Neumotórax en neonatos recién nacidos por cesáreas distócicas. **XVIII Congreso Andaluz de Veterinarios Especialistas en Animales de Compañía.** Póster.

PUBLICACIONES CIENTÍFICAS (Q1)

- Rodríguez-Trujillo, R., Batista-Arteaga, M., & Iusupova, K. (2025). Glucose Levels as a Key Indicator of Neonatal Viability in Small Animals: Insights from Dystocia Cases. *Animals*, *15*(7), 956.

- Raquel, R., Miguel, B., Kseniia, I., & Desirée, A. (2025). Neonatal blood lactate as a predictor of early survival in puppies delivered by emergency cesarean section. *Theriogenology*, *241*, 117437.

- Raquel, R., Batista, M., Sara, A., & Kseniia, I. (2025). Fetal renal ultrasonography in canine pregnancy: relationship with maternal and fetal metrics for assessing fetal maturity. *Theriogenology*, 117612.

- Rodríguez-Trujillo, R., Batista-Arteaga, M., Iusupova, K., Rosario-Medina, I., & Alamo-Santana, D. (2024). Comparison of Propofol and Alfaxalone as Anesthesic Drugs in Bitches Undergoing Ovariohysterectomies (Healthy Bitches and with Pyometra) and Cesarean Sections. *Animals*, *14*(9), 1343.

- Rodríguez, R., Alemán, D., Batista, M., Moreno, C., Santana, M., Iusupova, K., & Alamo, D. (2024). Maternal and fetal factors for determining the cesarean section type (scheduled/emergency) in bitches. *Theriogenology*, *227*, 144-150.

CAPÍTULOS DE LIBROS

- Alamo Santana, D., Rodríguez Trujillo, R. D. C., Iusupova, K., & Batista Arteaga, M. (2023). Procedimientos para la recogida de semen en el perro y el gato.

- Iusupova, K., Santana, D. A., Trujillo, R. R., & Arteaga, M. B. (2023). Protocolos de refrigeración y congelación de semen en pequeños rumiantes. In *Técnicas reproductivas en las especies animales* (pp. 114-122). Elsevier.

- Santana, D. A., Iusupova, K., Trujillo, R. R., & Arteaga, M. B. (2023). Superovulación y transferencia de embriones en pequeños rumiantes. In *Técnicas reproductivas en las especies animales* (pp. 205-213). Elsevier.