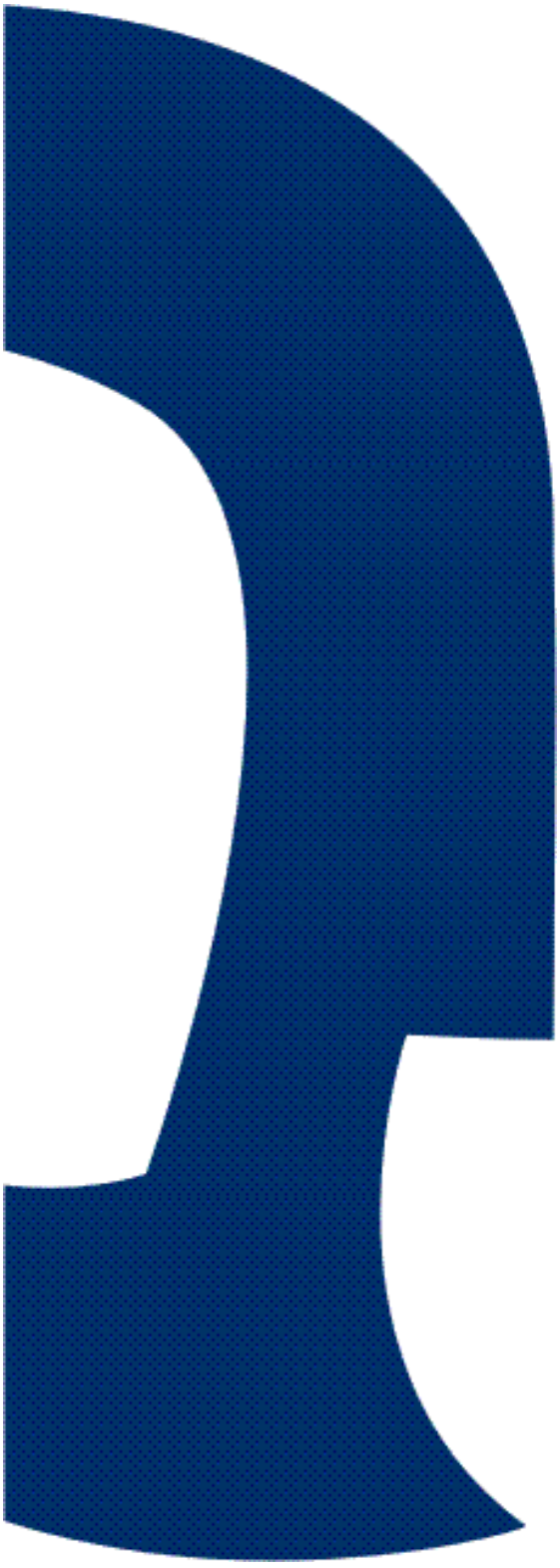


Titulo: The incidence of neonatal mortality and congenital malformations in the canine species, brachycephalic vs dolichocephalic, in bitches subject to programmed or emergency caesarean sections.

Estudiante:
Dácil Alemán Almeida.

Tutor:
Miguel Batista Arteaga.

Cotutor:
Carla Moreno Marrero

Curso Académico: 2022-2023

Content

Abstract

This study determined neonatal mortality and the frequency of congenital malformations in bitches subject to programmes or emergency Caesarean sections, distinguishing brachycephalic and dolichocephalic breeds. Data from 140 bitches subject to Caesarean sections were registered, as well as their neonates (n=689). The dolichocephalic breed bitches required a larger percentage of emergency Caesarean sections (44%; 29/66) compared to the brachycephalic breed bitches (9,5%; 7/74). Total neonatal mortality was 11,17% (77/689), and there were higher rates of neonatal deaths in emergency surgeries (13,7%; 20/146) compared to those in programmed surgeries (2,95%; 16/543). Bitches < 4 years of age subject to Caesarean sections registered more incidence of neonatal mortality, meanwhile, the lowest incidence was that of bitches > 4 years subject to programmed surgeries. The total rates of neonatal mortality did not vary between the different breed types, brachycephalic or dolichocephalic, however, it was slightly higher during the resuscitation stage or the first 48 hours after birth in brachycephalic breeds. The total incidence of congenital malformations was 4,5%; being higher in brachycephalic breeds; cleft palate and anasarca were the most frequent anomalies representing almost 65% of the total malformations. The breeding bitches that showed an insufficient state of health manifested a higher incidence of neonatal malformations (25%; 11/44) in comparison to those with a complete state of health (12%; 9/72). Likewise, litter coming from inbreeding showed more frequent malformations (12%; 6/50) than those from outbreeding (1,6%; 8/499). The results stated that bitches with larger litters (>6) registered a higher incidence of congenital malformations (21,6%; 11/51), in comparison to smaller litters (10,8%, litters < 2 neonates; 13,5%, litters 3-6 neonates). This study enables us to assure that the correct reproductive management is crucial to diminish neonatal mortality and the incidence of congenital malformations. The correct genetic selection of reproducers, an adequate sanitary state of health of the mother, as well as the age of the same, are decisive aspects when programming a successful gestation.

Introduction

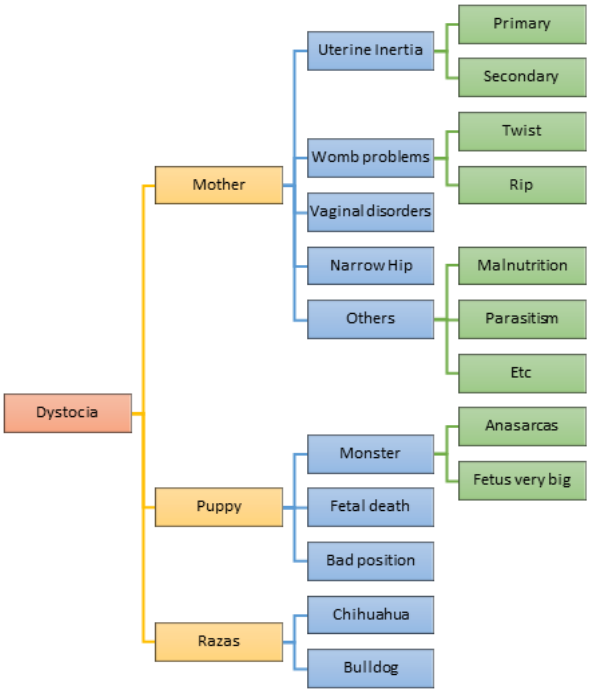
The domestic bitch (*Canis Familiaris*) is a monoestric breeder which normally ovulates spontaneously 48 hours after LH peak, oestrus will last an average of 15 to 18 days and gestation an average of 61 to 62 counting from ovulation (WSAVA 2002).

During this stage progesterone rises, which helps determine the exact moment of the bitches' reproductive cycle, starting with basal levels before LH peak, from 2ng/ml to 4 ng/ml during the LH peak, from 5 ng/ml to 12ng/ml at ovulation and later above 20ng/ml; this progesterone allows us to determine the best moments to cover the bitch. During gestation, progesterone is at maximum levels, starting to fall towards the parturition. Once progesterone lowers, another hormone rises, prolactin (WSAVA 2002).

When progesterone lowers below 2ng/ml without signs of imminent parturition, it is a sign that for some reason birth mechanisms are not developed and an emergency Caesarean section will be necessary.

Parturition begins with a series of events that start with fetal maturation; an increase of fetal cortisol secretion that in turn causes an increase of the mothers' cortisol secretion, the fetal stress caused by the limited supply of nutrients through the umbilical artery, stress caused by the limited space within the uterus. Moreover, maternal factors such as ruptured corpus lutheum provoking progesterone to lower meanwhile prostaglandin &2 and an increased sensibility of the uterus to oxytocin. When the fetus passes through the birth canal, distending the cervix and the vagina triggering Fergusson reflex – a neuroendocrine reflex that results in a liberation of oxytocin to the neurohypophysis. Oxytocin affects the ability of the uterus to contract, and relaxin helps relax pelvic ligaments and the birth canal. The three phases of birth are dilatation, fetus expulsion and placenta expulsion. (J Fusi y MC. Verones, 2022)

Whenever birth does not occur for whatever reason, it is called dystocia, meaning the incapacity to expulse fetus. Dystocia may occur due to causes related with the bitch or the fetus, what is more, there are canine breeds that are more predisposed to this condition (Prashant Kumar et al. 2018; Wyddooghe et al., 2013; Linde – Forsberg 2005; Johnston et al., 2001; Gilson, 2003).



Dystocia symptoms are:

- Failure to expulse the fetus 24 hours after the beginning of parturition.
- Persistent contractions during more than 30 minutes without delivery of the puppy.
- Fetal membranes discharge from the vulva more than 15 minutes without delivery of the puppy.
- Signs of the bitch being in pain.
- Haemorrhage.
- Uteroverdin without delivery of the puppy.

There are both medical and surgical treatments for dystocia. Medical treatment is based on oxytocin, glucose and calcium; however, this is not an option for every type of dystocia, if the condition is a result of an obstruction or uterus primary inertia, a surgical treatment will be necessary. Between 60 and 80% of bitches with dystocia require a Caesarean section (Linde-Forsberg, 2005; Gilson, 2003; Traas, 2008; Wykes y Olson, 2003; Fingland, 1996).

As above mentioned, the surgical treatment is a Caesarean section (hereafter C-section), this is the removal of the puppies directly from the bitch's uterus through an incision made on the middle line of the abdomen. The incision is protected with gaze pads and another incision is made on the cervix in order to be able to extract the puppies

one by one and pass them on to the resuscitation team. The uterus is stitched as is so the muscular and subcutaneous tissue and the skin of the abdomen.

Newborns will be given the APGAR test; 5 parameters will be checked, and this test will help assess the viability of each puppy and if any of them need more attention than the rest (Silva et al., 2009, Groppetti et al., 2010, Batista et al., 2014). Once the puppies are out of danger, a more exhaustive evaluation will be undertaken, in order to rule out any malformations or pathologies.

The objective of this study is to compare the neonatal mortality previous C-section, during resuscitation or 48 hours after the procedure, taking into consideration two distinguished types of breeds, dolichofacial and brachycephalic. Furthermore, neonates that present any type of malformation will be compared as well.

Material and methods

1. Animals

The study was conducted on 140 domestic bitches and 689 puppies that were treated in the Veterinary Clinic Cania during the period from 2015 to 2021. Some of the bitches received a reproductive control and later a programmed C-section meanwhile others underwent an emergency C-section. We understand emergency C-section as those that are performed once the parturition has been initiated (first stage of labour) and some kind of problem to fulfil normal birth occurs (dystocia). The bitches were identified according to breed, table 1, and age, table 2. Anamnesis determined vaccination state, the possibility of endogamy (inbreeding or outbreeding), the type of feeding and the administration of supplements during gestation.

Table 1. Number of females in the study according to breed

Nº of animals	Breed	Nº of animals	Breed
40	American Bully	3	Labrador
1	Beagle	3	Mix Breed
2	Boston Terrier	1	German Shepherd
4	Boxer	1	Malinois
1	German Pointer	1	Pointer
7	Bull Terrier	17	Presa Canario
14	French Bulldog	2	Schnauzer
2	English Bulldog	5	Scottish Terrier
2	Pug	1	Shar Pei
22	Chihuahua	2	Staffordshire Bull Terrier
1	Dogo of Burdeaux	1	Welsh terrier
1	Fox Terrier	3	West Highland White Terrier
1	Golden Retriever	1	Yorkshire Terrier
1	Great Dane		

Table 2. Nº of females distributed according to age

Age	Nº of specimens
< 2 years	11
2-4 years	75
4-6 years	38
>6 years	16

2. Monitoring the gestation

The monitoring of the bitch's gestation included 3 ultrasounds; these were performed by the same veterinarian using the GE Medical Systems Ultrasound scanner model MWA150019A. As a rule, the time used for each ultrasound is from 30 to 45 minutes, taking into consideration the size of the breeder, her character, and the number of

puppies. The ultrasounds were performed at days 30, 45 and 58, evaluating the following:

1. First scan: 25 to 30 days

The first control to determine if or not the bitch is expectant, if the embryos have a heartbeat and if there are any reabsorptions.

2. Second scan: 43 to 46 days

The second control will determine:

- Size and development according to the gestational age, based on formulas of biparietal and body diameter.
- Formation and development of the different organs according to gestational age.
- Absence of anomalies that might predict birth complications.
- Correct state of the placenta and the different fetal fluids.
- Clean amniotic liquid without alterations.
- Normal fetal cardiac frequency (>200 BPM)

3. Third scan: 57 to 59 days

The third scan will evaluate biparietal diameter (BPD)and heartbeat per minute (BPM). Various measurements will be taken of various puppies.

3. Prior the C-section

A complete evaluation of the bitch is performed before the surgical treatment (cardiac frequency, mucosa, temperature, arterial tension, etc.), a blood sample is taken to measure progesterone, coagulation and biochemical, using the analyser IDEXX Catalyst One, and a haemogram that is checked with the haematological analyser IDESS ProCyte Dx. In addition, another ultrasound scan is performed to evaluate vitality, heartbeat and possible puppy stress before entering surgery.

4. Caesarean Section

4.1 Anaesthesia Protocol

Before medicating, the bitch is prepared for surgery, shaved and intravenous catheter is inserted in the cephalic vein. Depending on the level of excitement of the bitch, a micro dosage of dexmedetomidine (Dexdomitor 0,5% 10ml Zoetis 1mcg/kg intravenous) will be administered followed by oxygen during to minutes with soft lighting and no noise to relax the bitch and allow correct oxygenation. From this moment on, the animal will be on intravenous fluid Gelaspan (40mg/ml Braun 500ml for a bitch of 30 kg). Propofol will be used to reach profound deep place (Propovet 10mg/ml Ecuphar a dosage of 0,2 mg/kg) and fentanyl (Fentanest 0,05mg/ml Kern a dosage of 2mcg/kg) IV, the animal is intubated (using the adequate size of tracheostomy tube) and the connection to the anaesthesia machine in order to sleep the bitch with Isoflurane (IsoFlo), inducing with 5% and later on maintaining it at 0,8%.

While preparing the animal, it is placed dorsal ventrally, slightly on the side, and the abdomen is cleaned. The animal will have been injected metoclopramide (Primperan 10mg/ml a dosage of 0,1 mg/kg) and amoxicillin (Veterinarian Clamoxyl LA 150 mg/ml a dosage of 15 mg/ml) subcutaneous. After the procedure, pain will be controlled with tramadol (Tranvetol 50mg/ml Virbac a 4 mg/kg) and the animal is sent home with antibiotic treatment (amoxicillin).

4.2 Surgical procedure

After all the material has been sterilized and the bitch is prepared, the surgeon infiltrates lidocaine (Lidor 20mg/ml) in the middle line. An incision is made, from 6 to 12cm depending on the size of the animal and the size of the uterus. The patient is opened layer by layer and the surgeon debrides until she reaches the middle line, once it is found, the muscular tissue is opened, and the uterus is carefully taken out of the abdomen.

Before taking out the puppies, the team will be sure 20 minutes have past since the animal was induced, assuring the puppies will not have rests of Propofol in blood. An incision of about 4 to 6cm long is made on the uterus, where the puppies are taken out and placed on the resucitation area; after each puppy the placentas are extracted. Once all the puppies are out, oxytocin is intravenously administered (1UI per bitch) and the uterus is closed using Cushing stitches with absorbable material 3/0 (Monosyn violet).

Viscera is cleaned with warm saline solution (33 to 35ºC) to eliminate any waste or maternal liquid that might have entered the abdominal cave. Layer by layer the bitch is closed using intradermic suture.

4.3 Monitoring

The animal is completely monitored from the beginning, using the IMECC8 Mindray VET monitor, we control heartbeat, respiratory frequency, saturation, the amount of carbon dioxide, the electrocardiogram, the temperature, and the blood pressure. If the animal shows any signs of instability or pain, the amount of isoflurane can be adjusted and it is possible to force breathing momentarily, if necessary, administer fentanyl or even repeat the dosage of dexmedetomidine.

The estimated time for this surgical procedure is 1,5 to 2 hours depending on the size of the bitch and the condition of the uterus, furthermore, if the owner desires to sterilize the bitch during the C-section or prefers to reconstruct the reproductive area.

5. Resuscitation and neonatal evaluation

5.1 Preparation of the resuscitation area

The resuscitation area is prepared considerably before the C-section begins, the area must have a heating pad, soaker pads, tissue paper to clean the puppies, identifying collars that must be placed first thing the puppies reach the area, and the necessary medicine: routine wise puppies are administered naloxone (O,4 mg/ml Kern Pharma a dosage of 0,008mg/puppy), atipamezole (Revazol 5mg/ml Dechra diluted 0,1:10 and injected 0,02ml SC), oxygen and glucose serum (Glocosavet Braun 40%; 1ml diluted 40%)to all the puppies. In addition, an incubator will be prepared at 37º. The resuscitation team will be prepared waiting, normally one person for every 2 to 3 puppies, and a person specifically to link the surgery and the resuscitation areas.

5.2 Resuscitation Manoeuvres

Once puppies are extracted from the uterus, they can come along with their placenta or not, this will depend on the work done in surgery and the status of the placenta. They will be identified with a coloured collar orderly, hence, this collar will help indicate whether a puppy needs more medication or more intense manoeuvres. Medication is administered (naloxone to revert the possible effects of fentanyl and atipamezole to

revert the effects of dexmedetomidine, both SC), mouth and nasal cavities are aspired from all fluids, puppies are oxygenated, some glucose serum is distilled sublingual, and the puppies are rubbed. Heartbeat, oxygenation, and breathing is checked.

Depending on the animal, it may be necessary to repeat some of the previous steps, such as aspiration of nasal cavity and the mouth. Once the puppies are breathing and show a normal colour, the umbilical cord can be clamped, and finally the puppies can be passed to the incubator until the rest arrive the same condition.

If any of the puppies does not stabilize with the normal resuscitation manoeuvres, adrenalin will be administered, thoracic compressions made, and oxygen inhalation will be forced. After 45 minutes without signs of life the puppy is considered dead.

5.3 Neonatal Evaluation

Puppies undergo the APGAR test both before and after the resuscitation manoeuvres, to appropriately check the viability of the puppy. This test includes 5 parameters, and the sum of theses classifies puppies in three categories:

- 0-3 points → low vitality
- 4-6 points → moderate vitality
- 7-10 points → normal vitality

Table 3. Parameters and points used in APGAR tests

SIGNS	0 POINTS	1 POINT	2 POINTS
Cardiac Frequency (lpm)	<180	180-220	>220
Respiratory Frequency	Absent	weak	strong
Reflexes	Absent	weak	strong
Muscular tone	Absent	weak	strong
Mucosae	cianotic	pale	pink

Puppies will also be weight and their coat colour and sex identified, all of which will be noted and registered if any other measurement was needed. Puppies will be completely checked, head (making sure no Fontanelle), eyes and nose, mouth (making sure there is no cleft palate or cleft lip), legs and paws to assure there are no incorrect positioning, the navel to make sure it has no hernia, the knot is correct and disinfected, spinal column to assure there is no deficit of closing, the annus, the vulva or pennis

(making sure there are no herma vertebras). If any non-viable malformation is found such as an anasarca or monster puppy, a humanitarian euthanise will be performed.

Results

Table 4 reflects the percentage of programmed C-section and emergency C-sections that were performed at the clinic. Almost 75% were programmed and when observing the breeds involved, almost 90% of the C-sections of brachycephalic bitches are programmed meanwhile those of dolichocephalic ones is slightly above 50%.

Table 4. Comparison between urgency and programmed C-sections depending on the breed.

	Emergency C-sections	**Programmed C-sections**	**Total**
Dolichocephals	43,93% (29/66)	56,06% (37/66)	47 % (66/140)
Brachycephals	9,45% (7/74)	90,54 % (67/74)	52% (74/140)
Total	25,71% (36/140)	74,28 (104/140)	

The neonatal mortality (Table 5A) shows a value slightly above 11%, without significant differences between the three periods taking into consideration (before, during and48 hours after the surgery). On another note, if we take a look at the age of the bitches, the younger they are, the more mortality there is, being that of the bitches less than 2 years old 16,07% and the bitches more than 6 years old the one with less percentage of neonatal mortality, furthermore, the percentage in programmed C-sections was 0%.

Table 5A. Neonatal mortality based on age of the mother and perinatal period

Age	Mortality prior surgery	Mortality during resuscitation	Mortality 48hours after birth	Total
< 2 years	14,28 % (8/56)	0% (0/56)	1,78% (1/56)	16,07% (9/56)
2-4 years	4,42% (17/ 385)	5,71 % (22/385)	4,67% (18/385)	12,73 % (49/385)
4-6 years	4,44% (8/180)	3,33% (6/180)	1,66% (3/180)	9,44% (17/180)
>6 years	4,41% (3/68)	1,47% (1/68)	1,47% (1/68)	7,35% (5/68)
Total	4,5% (31/689)	4,20% (29/689)	3,33% (23/689)	11,17% (77/689)

On the other hand, regardless the age of the mother (Table 5B), there was always a higher incidence of neonatal mortality in emergency C-sections (19,28% dead neonates), with considerably higher values of mortality prior the surgery (13,7% vs 2,95%; emergency C-sections vs programmed C-sections; $p < 0.01$), as well as during the resuscitation (12,3% vs 2,02%; emergency C-sections vs programmed C-sections; $p < 0.01$); there is hardly any difference between the mortality during the first 48 hours after birth. Based on the age, the highest incidence of mortality was detected in the bitches from 2 to 4 years of age under emergency C-sections (39,65%), while the lowest level (0,0%) was detected in bitches from 4 to 6 years of age under programmed C-sections.

Table 5B. Neonatal Mortality based on the age of the mother and the type of C-section (programmed/emergency)

Age and type of C-section	Mortality prior C-section	Mortality during resuscitation	Mortality after 48 hours	Total
< 2 years	50%	0 %	0%	50%
emergency	(6/12)	(0/12)	(0/12)	(6/12)
< 2 years	4,54%	0%	2,27%	6,82%
programmed	(2/44)	(0/44)	(1/44)	(3/44)
2-4 años	15,51%	19%	5,17%	39,65%
emergency	(9/58)	(11/58)	(3/58)	(23/58)
2-4 años	2,45%	3,36 %	3,36%	9,17%
programmed	(8/327)	(11/327)	(11/327)	(30/327)
4-6 años	4,34%	13,04%	6,52 %	23,91%
emergency	(2/46)	(6/46)	(3/46)	(11/46)
4-6 años	4,47%	0 %	0%	4,47%
programmed	(6/134)	(0/ 134)	(0/134)	(6/134)
>6 años	10%	3,33%	3,33%	16,67%
emergency	(3/30)	(1/30)	(1/30)	(5/30)
6>años	0%	0%	0%	0%
programmed	(0/38)	(0/38)	(0/38)	(0/38)
Total	13,7%	12,32 %	2,73%	19,18%
emergency	(20/146)	(18/146)	(4/146)	(28/146)
Total	2,95%	2,02 %	2,76 %	8,84
programmed	(16/543)	(11/543)	(15/543)	(48/543)

The percentage of bitches that showed neonatal mortality is expressed in the following table: almost 40% (55/140) of the bitches were affected. The period of time in which less puppies died (7,86%) was during the resuscitation without significant differences between the various ranges of age of the mother. Similar results were observed in the bitches under 2 years of age, these are the ones to suffer more neonatal

mortality, more than 50%. The older they are, mortality decreases under 9% in bitches above 6 years of age.

Table 6. Percentage of bitches that suffered neonatal mortality based on the age and the stages studied

Age	Mortality prior C-section	Mortality during resuscitation	Mortality after 48 hours	Total
< 2 years	27,27% (3/11)	9,09 % (1/11)	9,09% (1/11)	54,54% (6/11)
2-4 years	15,79% (12/76)	7,89% (6/76)	18,42% (14/76)	42,10 % (32/76)
4-6 years	18,92% (7/37)	8,11% (3/37)	8,11% (3/37)	35,13% (13/37)
>6 years	12,5% (2/16)	6,25% (1/16)	6,25% (1/16)	8,69% (4/16)
Total	17,14% (24/140)	7,86% (11/140)	13,57% (19/140)	39,28 % (55/140)

If we compare neonatal mortality taking the breed into consideration (table 7), we can observe that there are no significant differences when comparing brachycephalic or dolichocephalic breeds, the last presenting more than 30% mortality (33,7% *vs* 36,6%; brachycephalic vs dolichocephalic). In both breed types we can see the period before C-section is the one with more mortality; the number of dead neonates in dolichocephalic breeds practically doubled ($p<0.01$) that of the brachycephalic (12,16%, 9/74 vs 22,72%, 15/66; brachycephalic vs dolichocephalic). The stage that registered less amount of neonate mortality was immediately after the surgery (resuscitation period) regardless of the breed.

Table 7. Percentage of neonatal mortality in brachycephalic breeds versus non brachycephalic breeds. Percentage of affected bitches.

Type of Breed	Mortality prior C-section	Mortality during resuscitation	Mortality after 48 hours	Total
Brachycephalic	12,16% (9/74)	9,46% (7/74)	12,16% (9/74)	33,78% (25/74)
Dolichocephalic	22,72% (15/66)	6,06% (4/66)	7,57% (5/74)	36,36% (24/66)
Total	17,14 (24/140)	7,86% (11/140)	10% (14/140)	35% (49/140)

Table 8 describes the incidence of congenital malformations, total and based on the type of malformation. In this study, 4,48% of puppies show malformations, 61,29% of them belonging to brachycephalic breeds and 38,71% dolichocephalic breeds. The most frequent malformations, independently of the breed, are anasarca and mouth abnormalities, first cleft palate and second cleft lip, reaching 75%.

Table 8. Distribution of the different type of congenic malformations in neonates in brachycephalic and dolichocephalic breeds.

Type of malformation	Total	Brachycephalic Breeds	Dolichocephalic Breed
Anasarca	38,70% (12/31)	36,84% (7/19)	41,67% (5/12)
Cleft palate	25,80 % (8/31)	26,31% (5/19)	25% (3/12)
Lip Palate	12,9% (4/31)	15,79% (3/19)	8,3% (1/12)
Missing limbs or issues	6,45% (2/31)	5,2% (1/19)	8,3% (1/12)
Monster	3,22% (1/31)	0% (0/19)	8,3% (1/12)
Hernia	9,68 % (3/31)	5,2% (1/19)	16,66% (2/12)
Eviscerated	3,22% (1/31)	5,2% (1/19)	0% (0/12)
Default skin closure	3,22% (1/31)	5,2% (1/19)	0% (0/12)
Total	4,48% (31/691)	61,29% (19/31)	38,71% (12/31)

Table 10 reflects the incidence of congenital malformations based on the age of the bitches, we can observe the period from 2 to 4 years of age is when less percentage of puppies with malformations is born (12%; $p<0.05$), versus the rest of the periods of age, 18 to 21%. Meanwhile, table 11 reflects the influence of the size of the litter on the incidence of congenital malformations: the more numerous the litter, the more puppies with malformations, up to double times in litters of more than 6 compared to litters of 2 or less.

Table 10. Incidence of congenital malformations based on the age of the mother.

Age of the bitch	Nº of bitches with malformed puppies
<2 años	18,18% (2/11)
2-4 años	12% (9/75)
4-6 años	21,05% (8/38)
>6 años	18,75% (3/16)

Table 11. Incidence of congenital malformations based on the size of the litter

Size of the litter	Nº of litter with malformations
< 2 cachorros	10,81% (4/37)
3-6 cachorros	13,46% (7/52)
>6 cachorros	21,57% (11/51)

The percentage of congenital malformation based on the type of breeding is represented in table 12, in which we can observe significantly larger numbers of inbreeding neonate puppies. Hence, the number of bitches that have malformations in their litters is considerably larger in inbreeding in comparison with the bitches who are outbreed (23,97% vs 8,89% respectively; p>0.01)

Table 12. Impact of the size of the litter on the number of congenital malformations.

Type of breeding	Neonates with malformations/ nº of puppies	Bitches with neonates with malformations /nº of bitches
Outcrossing	1,60% (8/499)	8,89% (8/90)
Inbreeding	12% (6/50)	23,97% (3/13)
Total	2,55% (14/549)	10,68% (11/103)

Depending on the state of health of the breeding bitch (table 13) we can observe differences between the percentage of malformed puppies. On the one hand, when we have an incomplete state of health we can see a higher number of bitches with

malformation, 25%, in comparison with the 12,5%. On the other hand, this incomplete state of health also affects the number of puppies presenting malformations, 9,34% versus 3,13%.

Table 13. Incidence of congenital malformations in regard of the health state of the mother.

State of Health	Bitches with malformations	Percentage of neonates with malformations
Complete	12,5% (9/72)	3,13% (12/383)
Incomplete	25% (11/44)	9,34 % (17/182)
Unknown	8,33% (2/24)	1,58% (2/126)
Total	15,71% (22/140)	4,49% (31/691)

Finally, table 14, shows the distribution of the C-sections throughout the year, as well as the percentage of congenital malformations in each season. During the year, the season in which we find more C-sections is fall (32%), practically duplicating the number of C-sections performed in Spring. Likewise, fall is the season with more incidence of congenital malformations (6,54%), as well as a higher percentage of litters with malformations (20%).

Table 14. Percentage of C-section per season and congenital malformations dase don the moment of birth

Season	Neonates with malformations	C-sections with puppies with malformations	C-sections per season
Spring	4,5% (5/111)	17,39% (4/23)	16,42% (23/140)
Summer	3,3% (6/ 182)	11,43% (4/35)	25% (35/140)
Fall	6,54% (14/ 214)	20% (9/45)	32,14% (45/140)
Winter	3,26% (6/184)	13,51% (5/37)	26,43% (37/140)

Discussion

This retrospective study evaluated the neonatal mortality before the C-section, during the stage of resuscitation and the stage immediately after the surgery and the 48 hours following birth, valuing the influence of the breed and the age of the bitch. Moreover, this study has allowed us to define the frequency of congenital malformations in an elevated number of neonates, as well as the influence of age and state of health of the mother, the size of the litter, the type of breeding and the season of the year.

Our study reflected that brachycephalic breeds register a superior number of programmed C-sections. Said breeds are more predisposed to suffer dystocia during parturition caused by their anatomic conformation (Wydooghe et al., 2013, Batista et al., 2014). The owners are familiar with this condition and usually request a more intense veterinarian control during the gestation and more strictly during the period of time previous to parturition, scheduling serial ultrasounds or the measurement of plasmatic progesterone the days prior to the delivery, in order to determine with more certainty the day of the C-section (Nöthling et al., 2022).

By contrast, dolichocephalic breeds register a lower percentage of dystocia (Vilar et al., 2018), the owners, by ignorance or by their own decision, frequently choose a natural parturition over a programmed C-section. In our study, the percentage of emergency C-sections corresponds to bitches that did not initiate parturition or had trouble during it, registering numbers above 5%. Our study confirms dolichocephalic breeds register less mortality; this may be due to the fact that they are breeds that tolerate stress better and normally suffer from fewer pathologies. In the same way, brachycephalic neonate breeds show somewhat less vitality at birth, evidenced by the lower levels in APGAR tests (Batista et al., 2014). Dolicocephalic breeds show more mortality prior to the surgery because there are less predisposed to dystocia, meaning when a C-section is performed it is the result of a specific reason, the birth has been interrupted or there might be neonatal death (Johnston et al., 2001, Gilson, 2003; Linde-Forberg, 2005).

The total rates of neonatal mortality were situated above 11%, a rate that was more than doubled in emergency C-sections than in programmed ones. This lower rate of mortality in programmed C-sections may be caused by factors such as less fetal stress

manifested by the puppies in a programmed, as well as a better general state of the mother towards the surgical intervention.

As a matter of fact, in our study, the neonatal mortality in emergency C-sections focused on the stages prior to the surgery and the resuscitation stage, not registering differences regarding programmed C-sections in the percentage of neonatal death in the first 48 hours after birth. Unlike other studies that describe mortality between 13% and 14% in puppies born after a C-section (Moon et al., 2000, Moon y Erb 2002) Groppetti et al., 2010), our study reflects much lower mortality in programmed C-sections (4,78%), similar to those described by authors that perform programmed C-sections (Vilar et al., 2018; Veronesi et al., 2022). Employing anaesthetic standards that are safe for both mother and puppies (De Cramer et al., 2017, Batista et al., 2014), as well as a specialised resuscitation team and the performance of APGAR test, to evaluate the viability of the neonates are imperative elements in order to reduce neonate mortality in the first hours after the C-sections.

The age of the bitch is another factor to bear in mind due to its influence on neonatal mortality. Coherent with the previous information, neonatal mortality was higher in emergency C-sections, without regard towards age, although it was notorious in groups <2 and from 2 to 4 years. One of the causes that predispose dystocia is the age of the bitch (Bergström et al., 2006; Doebeli et al., 2013), being more frequent in very young or very old bitches. This higher incidence in young bitches may be because they have not reached full reproductive maturity, inexperience being it her first delivery, in addition to the inexperience of the owner and his ability to detect the signs of parturition or not having scheduled a complete gestation control. It is noteworthy that programmed C-sections register higher mortality in bitches from 4 to 6 years of age (4,47%) and bitches under 6 years (0,0%). This may be due to the reproductive life of the bitch being near its end, therefor owners schedule more gestation controls and take more care of the process to reduce mortality in the last litter.

Regarding the congenital malformations, the total percentage was near 4,5%, , the highest incidence was in brachycephalic breeds (> 60% of the total anomalies). Our results were higher than those found by Veronesi et al., (2009), with values of 1,6%, but at the same time under others (Moon y Erb, 2002; Wydooghe et al., 2013, Batista et al., 2014; Vilar et al., 2018), with values between 7 and 14%. In our study, anasarca and cleft palate were more frequent, representing about 65% of the total congenital

malformations, very similar to other studies (Wydoghe et al.; 2013; Batista et al., 2014; Vilar et al., 2018). Genetic factors and family predispositions have been described as factors owards development of anasarca in brachycephalic breeds (Johnston et al., 2001; Hopper et al., 2004), registering up to 30% more risk of cleft palate in brachycephalic breeds (Ingwersen, 2005).

It has been proven that inbreeding with high levels of consanguinity translate to more malformations, this might be due to incompatible genes (Wydooghe et al., 2013, Batista et al., 2014). Regarding the previous, our study confirmed that the incidence of anomalies was much higher in inbreeding, both with brachycephalic and dolichocephalic breeds. The causes of this endogamy may be the breeder's choice for certain reproducers or the geographic position of the islands, making it difficult to find other reproducers.

Other aspects analysed were the size of the litter, the state of health of the mother and the season of the year. Different authors have said there is a correlation between the size of the litter and the increase in neonatal mortality (Groppeti et al., 2010, Batista et al., 2014), which has been registered in our study with an incidence of over 20% of the litters showing malformations when the number of the litter was above 6. This higher incidence of anomalies in the development could be due to the fact that bigger litters can generate inadequate placentation and consequently fewer nutrients reach some of the neonates, which means abnormal development of the animal. Regarding the state condition of the mother, our study confirms bitches with a correct vaccination and de-worming show less congenital malformations. It is accepted that some pathogens predispose more abortions and the development of congenital malformations (Verstengen et al., 2008), much higher in bitches with poor or inexistent state of health compared to those who are correctly vaccinated, even if they are exposed to these agents.

Finally, our study confirmed there is more malformation during the fall, which is also when there are more C-sections. It may be due to the fact that neonates born in fall (September to December) come from gestation during July and September, meaning the bitches might be affected by the high temperatures. No other study has valued the influence of the season of the year on the congenital malformations in the canine species, but it has been proved that there is a reduction of fertility in the hottest months of the year (Sui et al., 2022). It would not be rare to discover something similar may

influence the make semen quality, the levels of ovulations of the bitch or the later development of the embryo.

The global analysis of the study manifests that the correct reproductive management is essential towards gestation and the safe birth of neonates. A correct reproducer genetic selection, an adequate sanitary health condition, as well as the age of the reproducers, are crucial factors when programming gestation. Moreover, owners must be advised to realize a strict control and monitoring during gestation in order to prevent situations that may lead to dystocia, that will cause emergency C-sections and higher neonatal mortality rates.

Conclussions

- Relying on the results of this retrospective study, we can elaborate on the following conclusions:
- Emergency C-sections were more frequent in dolichocephalic breeds than brachycephalic breeds.
- Total neonatal mortality was 11,17%, being higher in emergency C-sections than programmed C-sections.
- The bitches <2 years submitted to emergency C-sections registered more neonatal mortality, while the bitches that registered less were those >4 years of age and submitted to programmed C-sections.
- Mortality during the stage previous to the C-section was not different depending on the type of breed.
- The incidence of congenital malformations was near 4.5%, anasarca and cleft palate were the most common anomalies.
- The age of breeding does not seem to influence the development of congenital malformations.
- An excessive size of the litter, an incomplete sanitary state of the bitch or inbreeding favour the development of congenital anomalies.

Reference

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, vol. 146, issue 3-4, (2014), pp: 218-226

Bergström, A., Fransson, B., Lagerstedt, AS., Olsson K. Primary uterine inertia in 27 bitches: aetiology and treatment. Journal of small animal practice. Wiley Online Library. Volume 47, Issue 8 (2006), pp. 456-460

De Cramer K.G.M., Joubert K.E., Nöthling J.O. (2017) Puppy survival and vigor associated with the use of low dose medetomidine premedication, propofol induction and maintenance of anesthesia using sevoflurane gas-inhalation for section in the bitch. Theriogenology, volume 96, 1 July 2017, pp 10-15

Doebeli, A., Michel, E., Bettschart, R., Hartnack, S., & Reichler, I. M. (2013). Apgar score after induction of anesthesia for canine cesarean section with alfaxalone versus propofol. Theriogenology, 80(8), 850-854.

Fingland R.B. (1996). Cirugía de ovarios y útero. En: Birchar SJ, Sherding R. (Ed): Manual Clínico de Pequeñas Especies. México. McGraw_hill, Inter- Americana, Ediores. 1996, pp. 1081-1084

Fusi, J., Veronesi, MC. Canine parturition: what is known about the hormonal setting?. Domest Anim Endocrinol. January 2022; Volumen 78, 106687

Gilson, S.D. Cesarean section. D. Slatter (Ed.), Textbook of Small Animal Surgery, WB Saunders Co., Philadelphia, PA (2003), pp. 1517-1520

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 (2010), pp. 1187-1196

Hanna, M., Aurélien, G, Marine, D., Claire Maria, N., Alexandre, F., Sylvie, Chastant-Maillard. Monitoring of the newborn dog and prediction of neonatal mortality. Preventive Veterinary Medicine, Volume 143, 1 August 2017, pages 11-20

Ingwersen, W. Congenital and inherited anomalies of the digestive system. Kahn C.M. (Ed.). The Merck Veterinary Manual. 9th Edition, Merck and Co., Whitehouse Station NJ, (2005). p. 131-137

Johnston, S.D., Kustritz, M.V., Olson, P.N. . Disorders of the Canine Uterus and Uterine Tubes (Oviducts). Canine and Feline Theriogenology. WB Saunders Co., Philadelphia, PA (2001), pp. 206-224

Linde-Forsberg C. Adnormalities in Pregnancy, parturition and the periparturient period. Ettinger SJ, Felman EC. (Ed). Textbook of Veterinary Internal Medicine. 6ed. Piladelphia. Saunders. 2005. P1655-1667

Linde-Forsberg, C., Persson, G. A survey of dystocia in the Boxer breed Acta Vet. Scand., 49 (2007)

Moon P.F., Erb, H.N. Perioperative factors associated with puppy vigor after delivery by cesarean section. J. Am. Anim. Hosp. Assoc., 38 (2002), pp. 90-96

Moon, P.F., Erb, H.N., Ludders, J.W., Gleed, R.D., Pascoe, P.J. Perioperative risk factors for puppies delivered by cesarean section in the United States and Canada. J. Am. Anim. Hosp. Assoc., 36 (2000), pp. 359-368

Nöthling, J.O., Jonne, C. J., Hegarty, E., Schooley E.K., de Cramer K.G.M. Use of a point-of-care progesterone assay to predict onset of parturition in the bitch. Frontiers in Veterinary Science, (2022), volume 9, pp. 23

Prashantkumar, K. A., Walikar, A. (2018). Evaluation of treatment protocols for complete primary uterine inertia in female dogs. Pharm Innov J, 7, 661e4.

Silva, L.C., Lúcio, C.F., Veiga, G.A., Rodrigues, J.A., Vannuchi, C.I., 2009. Neonatal clinical evaluation, blood gas and radiographic assessment after normal birth, vaginal dystocia or caesarean section in dogs. Reprod. Domest. Anim. 44 (Suppl. 2), 160–163.

Traas, A.M. . Surgical management of canine and feline distocia. Theriogenology, 70 (2008), pp. 337-342

Veronesi, M.C., Panzani, S., Faustini, M., Rota, A. An Apgar scoring system for routine assessment of newborn puppy viability and short-term survival prognosis. Theriogenology, 72 (2009), pp. 401-407

Verstegen-Onclin K., Verstegen J. Endocrinology of pregnancy in the dog: a review. Theriogenology. 70(3), (2008), pp. 291-299

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. Animal Reproduction Science, vol 190, (2018), pp. 53-62

WSAVA 2002 Congress. Patrick W. Concannon, PhD, Dipl ACT (Hon). Department of Biomedical Sciences, Cornell University Ithaca, NY, USA

Wydooghe, E., Bergmans, E., Rijsselaere, T., Van Soom A. . International breeder inquiry into the reproduction of the English bulldog. Vlaams Diergen. Tijds., 82 (2013)

Wykes, P.M., Olson P.N. Normal and abnormal parturation. D. Slatter (Ed.), Textbook of Small Animal Surgery, WB Saunders Co., Philadelphia, PA (2003), pp. 1510-1517