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Original Research Article

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

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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

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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.

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.

**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 |

### 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.


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

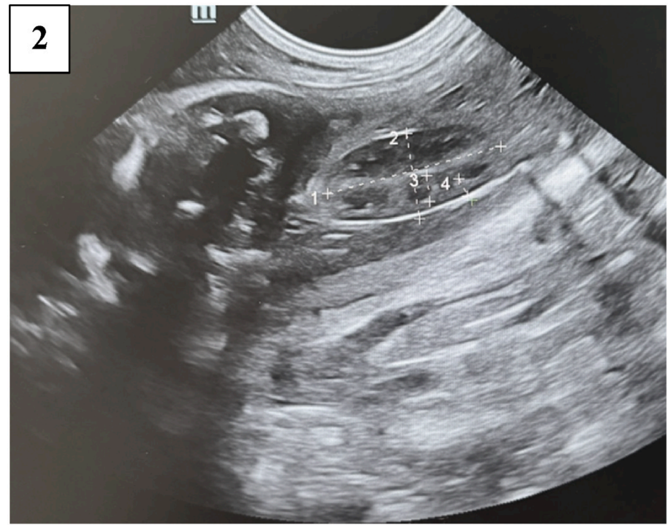


**Fig. 2.** Ultrasonographic images of fetal kidneys illustrating biometric measurements: (1) longitudinal length (cranial to caudal pole), (2) vertical height (maximum perpendicular width), (3) medullary thickness, and (4) cortical thickness.

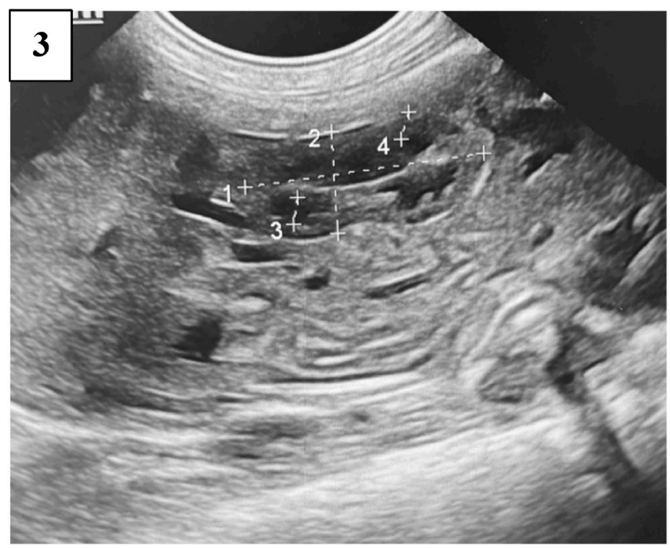


**Fig. 3.** Ultrasonographic images of fetal kidney illustrating: (1) longitudinal length, (2) vertical height, (3) medullary thickness, and (4) cortical thickness.

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.

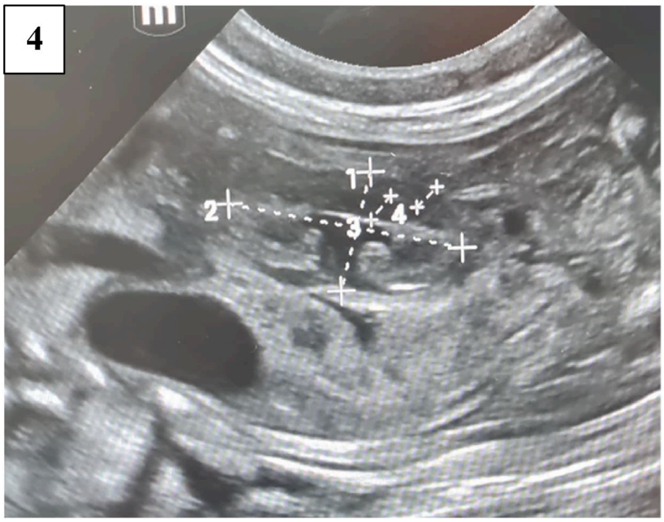


**Fig. 4.** Additional ultrasonographic view of a fetal kidney: (1) vertical height, (2) longitudinal length, (3) medullary thickness, and (4) cortical thickness.

### 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

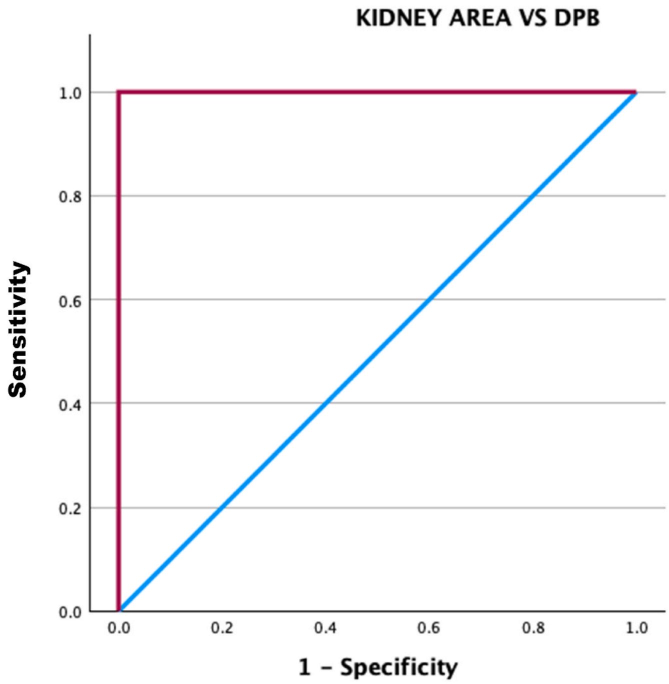


**Fig. 5.** Receiver operating characteristic (ROC) curve illustrating the diagnostic performance of fetal kidney area in predicting fetal biparietal diameter (BPD) thresholds.

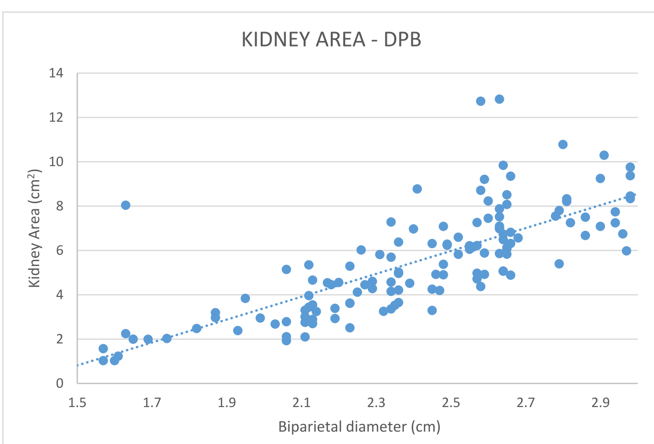


**Fig. 6.** Scatter plot showing the linear relationship between fetal biparietal diameter (BPD) and fetal kidney area. The dotted line represents the fitted linear regression model.

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

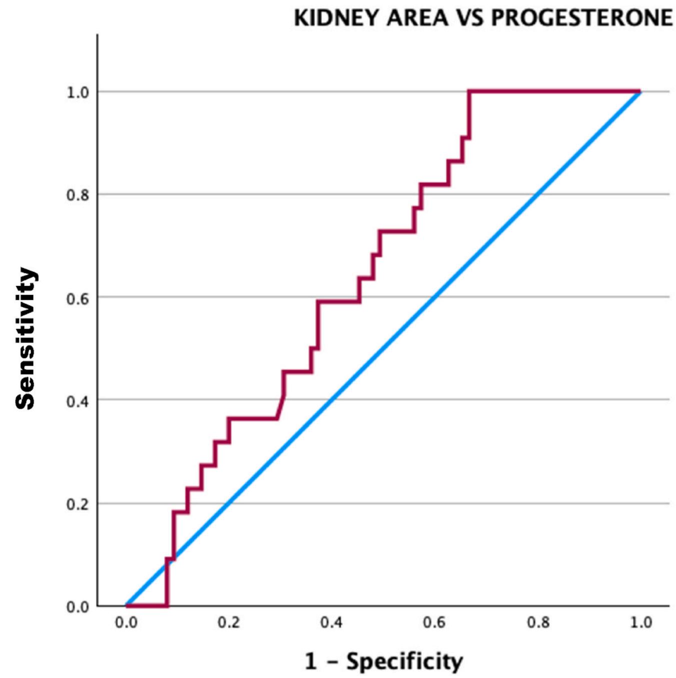


**Fig. 7.** Receiver operating characteristic (ROC) curve showing the diagnostic performance of fetal kidney area in predicting maternal serum progesterone concentration.

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.

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