Comparative analysis of the birth process and maternal-offspring interactions in bitches raised under different environmental conditions

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ABSTRACT

Pregnancy, parturition, and the postpartum period are critical stages in dog breeding that are significantly influenced by environmental factors. This study aimed to investigate how different birthing environments (home and farm) affect gestation duration, birth dynamics, and interactions between mothers and their offspring in small and medium breed dogs. A total of 68 pregnant bitches were included in the study, with 50 giving birth in a home environment (Group 1, G1) and 18 in a farm environment (Group 2, G2). Parameters such as birth weight, gestation length, neonatal mortality within the first 48 hours, duration of labor, time to first maternal licking, and time to first nursing were evaluated. The results indicated that bitches in G1 had a significantly shorter gestation period (P=0.021) and a higher incidence of dystocia (28%), whereas no cases of dystocia were observed in G2. Moreover, significant differences were observed between the groups regarding the time to first maternal licking and first nursing (P<0.05). The neonatal mortality rate was higher among G2 puppies (16.36%) compared to G1 puppies (3.60%). These findings highlight the impact of environmental conditions and human intervention on parturition dynamics and maternal-neonatal interactions. Long-term studies may provide further insights into optimizing birth management strategies for improved reproductive outcomes in dogs.

Introduction

In canine reproduction, the gestational and periparturient periods represent critical windows that necessitate both physiological readiness and behavioral competence from the dam. However, in the absence of such readiness, adverse outcomes such as insufficient maternal bonding, delayed onset of nursing, and neglect of neonatal care may arise, compromising early postnatal development (18, 40). Perinatal mortality, encompassing stillbirths and early neonatal deaths, affects 8-24.6% of puppies, with variations across breeds (39). Risk factors for neonatal mortality include fetal, maternal, and environmental factors (28). Contributing factors include low birth weight, stillbirths, inadequate milk production, maternal neglect, and neonatal anatomical immaturity (34). Despite these biological contributors, inadequate postpartum management remains the predominant underlying cause (35).

Maternal-neonatal interaction plays a central role in mitigating these risks and ensuring neonatal survival and vitality. Timely initiation of maternal behaviors plays a crucial role in stimulating vital physiological functions such as thermoregulation, defecation, and passive transfer of immunity (27, 41). Colostrum quality and intake during

the initial 48 hours postpartum are particularly decisive. Colostrum is rich in immunoglobulins and bioactive compounds critical for the establishment of neonatal immune protection. Failure to ingest sufficient colostrum within this narrow window may result in immunodeficiency and increased neonatal mortality (17, 26, 35).

Maternal behavioral deficiencies often arise from disruptions in endocrine signaling pathways (e.g., suppression of oxytocin) and are exacerbated by environmental stressors that interfere with instinctive caregiving behaviors (11). Elevated stress levels in the dam may diminish responsiveness to neonatal cues, delaying or suppressing behaviors such as licking, pup retrieval, and nursing. These behavioral impairments may significantly compromise thermoregulation, feeding initiation, and immune system priming during the early neonatal period (9, 10, 11, 15).

Importantly, as parturition approaches, bitches instinctively seek secluded, calm, and secure environments conducive to whelping. In the absence of such conditions, the progression of labor may be either abnormally prolonged or prematurely accelerated. Stress-induced restlessness in the dam can negatively impact her capacity to initiate maternal care, particularly early licking behaviors, which are essential for establishing mother-pup bonding (29). When such disruptions occur, they may delay meconium passage, prolong the interval to first nursing, and elevate the risk of obstetric complications, including dystocia, stillbirth, and neonatal death (2, 38).

Emerging evidence also highlights that the implications of peripartum stress extend beyond the immediate postpartum period. Interactions between maternal microbiota, stress-related hormonal profiles, and maternal care behaviors appear to influence not only early immune development and neuroendocrine functioning but also long-term behavioral and stress-regulation outcomes in the progeny (10, 19).

Dystocia, in particular, represents a significant obstetric emergency that can exhaust the dam, reducing or eliminating the brief yet critical window for maternal-infant bonding. In such scenarios, the delay or failure of timely colostrum ingestion can significantly compromise passive immune acquisition and neonatal viability (17, 26). To mitigate these risks, it is imperative to establish a stress-minimized birthing environment, ensure continuous monitoring, and optimize postnatal management during the initial hours after delivery (37).

A growing body of research on environmental enrichment demonstrates that supportive periparturient conditions reduce maternal stress, enhance lactational performance, increase maternal care frequency, and improve neonatal behavioral outcomes (9). Thus, the birthing environment is not merely a passive physical setting but an active modulator of maternal physiology and behavioral expression. Although genetic selection remains central to improving reproductive performance in dog breeding, suboptimal environmental conditions may negate genetic advantages by disrupting maternal-infant synchrony and elevating early-life mortality risks (10, 11). Therefore, both intrinsic and extrinsic factors must be considered in designing optimal breeding and neonatal care protocols.

Building on this conceptual framework, the present study was designed to comparatively evaluate the influence of two distinct birthing environments—home and farm—on reproductive and maternal-neonatal parameters across various small and medium-sized dog breeds. Specifically, the study examines gestation length, prepartum body temperature fluctuations, number of live births and stillbirths, incidence of neonatal mortality within the first 48 hours, birth weights, duration of parturition, time to first maternal licking, and time to first nursing.

Materials and Methods

Animal Material and Groups: The animal material for the study consisted of 68 pregnant dogs of various breeds and ages, all healthy and having been pregnant at least once before, whose births were monitored in home and farm environments. The dogs that gave birth in a home environment (Group 1, n=50) included the following breeds: Yorkshire (n=10), Maltese (n=10), Cocker (n=10), Poodle (n=10), and King Charles Spaniel (n=10). The breeds of dogs that gave birth in a farm environment (Group 2, n=18) were Beagle (n=6), Fox Terrier (n=6), and Cocker (n=6). The ages of the dogs in both groups ranged from 2 to 5 years.

Housing Conditions and Data Collection: During the prepartum period (from the 28th day of pregnancy until delivery), the dogs were provided with a balanced commercial dog food diet in two meals per day. All dogs included in the study were subjected to similar care and feeding programs. Feeding protocols were standardized across groups, including identical feed brands, feeding times, and portion sizes adjusted for weight and gestational stage.

For the dogs housed in the home environment, data were collected based on anamnesis information provided by the owners. Owners received written observation cards containing instructions and timing guidelines for recording specific parameters. These cards served as a standardized protocol that the owners were required to follow. Dog owners in Group 1 were not required to provide continuous video recordings; instead, all observations were based on the structured cards and daily

communication. They were trained through illustrated instruction materials and video tutorials prepared by the research team. These were supplemented with scheduled remote consultations by a single supervising veterinarian during the study period to ensure correct data collection procedures. All maternal behaviors in Group 1 were evaluated based on owner-recorded cards, with daily confirmation and clarification by the supervising veterinarian via video call.

For the dogs housed in the farm environment (Group 2), data collection began on the 30th day of pregnancy and continued until 48 hours postpartum. Dog owners were trained using illustrated instructional materials and video tutorials prepared by the research team. This system allowed uninterrupted observation and recording of their behavior and birthing process. The reason for using a camera system on the farm was that the dogs were not always with their owners. Video data from Group 2 formed the primary observational dataset for coded maternal behaviors such as first licking and first nursing. The camera recordings also provided detailed data during the birth and the subsequent 48 hours post-birth.

Farm Environment Detail: The farm was a private, non-commercial, family-run facility, not used for large-scale breeding but maintained under controlled and enriched conditions to allow natural observation of maternal behaviors in a low-stress setting. The farm had a dedicated kennel area for the dogs, and external animal entry into the farm was entirely prohibited to ensure the safety and health of the dogs. Dogs housed in the farm environment were kept in an isolated area with access to free movement in a controlled environment. This area was designed to allow dogs to exhibit natural social behaviors and was free of dominant individuals that could cause conflicts or aggressive interactions. Each dog was kept individually during the peripartum period. Aggressive behavior was limited to protective reactions triggered by external auditory stimuli, such as the sound or passage of another dog near the facility, and did not involve direct interaction. The dogs spent a quarter of the day roaming freely around the farm. Environmental enrichment consisted of preparing a soft-surfaced birthing area, providing visual contact with conspecifics, ensuring human caretaker interaction, and placing the dam's preferred toys near the whelping box to create a familiar and comforting environment.

Data Collection Process: Data related to the birthing process of the dogs were collected based on the following parameters: gestation duration determined from the reported mating dates, changes in prepartum body temperature, the number of puppies born alive or stillborn, the number of puppies that died within the first 48 hours, birth weights of the puppies measured within the first 24 hours using a precision digital scale (Voit SC20 Precision Digital Scale), duration of labor, and the time until first nursing and first licking. The onset of the birthing process was identified by observing uterine contractions, straining, licking of the vulva area, and restlessness. The observed behaviors "first licking" and "first nursing" were defined operationally as follows: "first licking" refers to the average time it took for the dam to start licking her puppies, indicating acceptance behavior; "first nursing" refers to the average time it took for the puppies to successfully attach to a teat and begin suckling. The health status of the newborn puppies was monitored and recorded for 48 hours postpartum.

Pregnancy Examination and Prepartum Body Temperature Measurement: Pregnancy in the dogs used in the study was confirmed 25-30 days after mating through ultrasonographic examination. The presence of embryonic sacs, fetal development status, and viability were observed using ultrasonography (Mindray Z5Vet, 5-8 MHz linear probe) (1). Ultrasonographic examinations were performed following the same standardized protocol by a veterinarian for both groups of dogs. In addition, the rectal body temperatures of the dogs in both groups were measured during the late pregnancy period using a digital thermometer (Braun PRT1000 Digital Thermometer). Prepartum temperature changes were recorded and correlated with the birthing process. Rectal temperature measurements were taken daily at the same time to ensure consistency.

Determination of Delivery Method: The delivery method (normal delivery or cesarean section) for the dogs included in the study was determined through continuous monitoring of the birthing process, with interventions made as necessary. As the expected delivery time approached, dogs in both the home and farm environments were observed 24 hours a day to identify any complications during labor. The decision-making process for determining the delivery method was based on specific criteria. For normal delivery, the evaluation focused on observing labor signs, ensuring that uterine contractions were regular, the puppy progressed through the birth canal, and the overall process proceeded without requiring intervention. In cases of normal labor progression, minimal intervention was provided to support the natural process.

Criteria for Deciding on a Cesarean Section Due to Dystocia Included: The criteria included the failure of the puppy to enter the birth canal within 2 hours after the onset of labor signs, a delay of more than 30 minutes in the birth process after the puppy had entered the birth canal, signs of extreme maternal fatigue, and inadequate uterine contractions. In cases where the first two criteria were met,

oxytocin administration was attempted. If this intervention did not yield results, a cesarean section was performed. Oxytocin was administered only in cases of confirmed dystocia with proper fetal positioning and presentation. Considering the pharmacokinetics and short half-life of oxytocin, as well as its targeted use before delivery, the administration was not expected to influence postpartum maternal behavior or subsequent data collection. Oxytocin was administered to 5 dogs in Group 1 who met the defined dystocia criteria. While oxytocin can influence maternal behavior in some contexts, its limited use (1–2 doses within 90 minutes before C-section) was deemed unlikely to affect the subsequent behavioral observations in this study.

Oxytocin Administration: Oxytocin was administered at intervals of 30-40 minutes (Vetas Oksitosin, Türkiye, 1-5 IU IV) and repeated up to a maximum of two times. If no response was observed after the second administration, a cesarean section was performed (21). The cesarean sections were conducted using a gas anesthesia protocol with propofol and sevoflurane. Postoperative analgesia was provided with meloxicam (Bavet Meloxicam, Türkiye, 0.04 ml/kg/day). Newborn puppies were dried with clean towels, and their umbilical cords were treated with a local antiseptic solution (10% povidone-iodine solution, Adeka, Türkiye).

Statistical Analysis: The data collected were compared between the two groups based on the identified parameters. Normality was tested using the Shapiro-Wilk test. For data meeting the normality assumption, the independent samples t-test was applied, while the Mann-Whitney U test was used for non-normally distributed data. Results were presented as mean ± standard error, and the threshold for statistical significance was set at $P<0.05$. Statistical analyses were conducted using SPSS software (SPSS Inc., Chicago, IL, USA).

Results

The average ages of dogs giving birth in Group 1 and Group 2 were 3.80 years and 3.44 years, respectively ($P>0.05$). Both groups experienced a decrease of approximately 1°C in prepartum body temperatures. Specifically, just before delivery, the body temperatures recorded were 37.4°C for Group 1 and 37.25°C for Group 2 ($P=0.067$). Regarding gestation duration, dogs delivering in a home setting had a significantly shorter gestation period ($P=0.021$). While birth duration and puppy weight were higher in Group 1, these differences did not achieve statistical significance ($P>0.05$). Notably, significant differences were observed between the groups in the time taken for the puppies to nurse for the first time and for the mother to lick her offspring ($P<0.05$). The differences between the groups are summarized in Table 1.

The incidence of dystocia was observed to be 28% in Group 1, whereas no cases of dystocia were reported in Group 2. In Group 1.12% of all births ultimately required a cesarean section due to dystocia. In Group 1, analysis revealed that the three last-born puppies were stillborn, and two puppies from different mothers died within the first 48 hours after birth. In Group 2, four puppies from four mothers (including two firstborn and two lastborn) also did not survive. Additionally, five puppies from five different mothers died within the first 48 hours. Notably, no anomalies or clinical signs were detected in the deceased puppies. The total number of puppies born was 139 in Group 1 and 55 in Group 2. A total of 5 neonatal deaths (3 stillbirths and 2 early neonatal deaths) were recorded in Group 1, while Group 2 experienced 9 neonatal deaths (4 stillbirths and 5 early neonatal deaths). Accordingly, the neonatal mortality rate was calculated as 3.60% in Group 1 and 16.36% in Group 2. This difference was found to be statistically significant ($P=0.042$).

Table 1. Some prepartum and postpartum parameters in dogs housed in home and farm environments

Parameters	G1	G2	P
Age (years)	3.80±0.18	3.44±0.29	0.304
Gestation duration (days)	63.58±0.51^{a}	66.27±1.28^{b}	0.021*
Prepartum body temperature (°C)	37.4±0.43	37.25±0.56	0.067
The average number of puppies born	2.78±0.14	3.05±0.2	0.314
The average number of puppies survived	2.68±0.13	2.55±0.16	0.712
Duration of labor (hour)	3.52±0.28	3.28±0.32	0.956
Puppy weight (gram)	144.85±4.66	142.09±7.55	0.746
Time for the mother to first lick (hour)	2±0.17^{a}	1.75±0.23^{b}	0.035
Time for the puppy to first nurse (hour)	2.53±0.17^{a}	2.12±0.22^{b}	0.019

The data are presented as mean ± standard error. Different superscripts (^{a}, ^{b}) indicate significant differences between groups ($P<0.05$).

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Table 2. Dystocia and normal birth outcomes in animals giving birth in a home environment

Parameters	Dystocia	Normal birth	P
Age (years)	4.07±0.43	3.67±0.19	0.314
Gestation duration (days)	63.61±1.03	63.75±0.58	0.907
Prepartum body temperature (°C)	37.42±0.10	37.41±0.50	0.945
The average number of puppies born	2.74±0.18	3±0.21	0.374
Duration of labor (hours)	4.38±0.67	3.27±0.30	0.147
Puppy weight (gram)	139.51±10.2	146.62±5.45	0.305
Time for the mother to first lick (hour)	2.52±0.42[a]	1.84±0.20[b]	0.024
Time for the puppy to first nurse (hour)	2.90±0.37[a]	2.38±0.19[b]	0.006

The data are presented as mean ± standard error. Different superscripts ([a], [b]) indicate significant differences between groups (P<0.05).

Table 3. Analysis of dogs giving birth by gender within groups

Group 1	Gender	Results	P
Puppy weight (gram)	Female	123.02±6.37[a]	0.001
	Male	156.68±6.71[b]	
Time for the mother to first lick (hour)	Female	1.93±0.27	0.916
	Male	2.11±0.26	
Time for the mother to first nurse (hour)	Female	2.48±0.26	0.807
	Male	2.64±0.26	
Group 2	**Gender**	**Results**	**P**
Puppy weight (gram)	Female	125.24±6.21[a]	0.001
	Male	162.36±6.55[b]	
Time for the mother to lick (hour)	Female	1.95±0.26	0.792
	Male	2.11±0.25	
Time for the mother to nurse (hour)	Female	2.46±0.25	0.907
	Male	2.63±0.26	

The data are presented as mean ± standard error. Different superscripts ([a], [b]) indicate significant differences between groups (P<0.05).

Among Group 1, those with normal deliveries had significantly shorter times to first licking (P=0.024) and first nursing (P=0.006) compared to those that experienced dystocia (Table 2). Analyses conducted within the gender groups showed no statistical differences in the time to first licking or first nursing in either Group 1 or Group 2. However, a significant difference was observed in birth weights between the genders (Table 3).

Discussion and Conclusion

Neonatal mortality rates have been reported to range from 9.23% to 26% (12, 20, 26, 33, 38). These losses are commonly associated with management factors, inadequate nutrition, congenital abnormalities, and infectious diseases (8). This substantial difference suggested that environmental factors might have exerted a strong influence on early neonatal survival, even when maternal parity and average litter size were comparable between groups. Further analysis examined variables like litter size, maternal age, gestation duration, and birth weight. Although not statistically significant, Group 2 had a numerically higher average litter size than Group 1, which may have imposed greater physiological demands on the dam. Although litter size and age were similar between groups, Group 2 had a slightly lower average birth weight compared to Group 1. Delayed maternal behaviors and the increased incidence of dystocia in Group 1 were expected to affect neonatal outcomes negatively; however, in our data, early neonatal mortality was actually higher in Group 2 despite stronger maternal care behaviors there. This suggests that increased litter size may have imposed greater physiological demands on the dams, potentially leading to reduced care efficiency or increased neonatal vulnerability.

In dogs, the stillbirth rate in normal deliveries varies between 5.5% and 33%, while the average stillbirth rate for cesarean sections is approximately 8.5%. Enhancing care and feeding programs during the prenatal, delivery, and postnatal periods significantly increases the live birth and survival rates of puppies (20, 37). Reyes et al. (35) highlighted that maternal size and birth order can lead to physiological and metabolic changes in neonatal dogs, especially affecting first- and last-born puppies. Cornelius et al. (7) similarly reported that last-born puppies are at higher risk of stillbirth, with a stillbirth rate of 18.7% versus 4.6% in earlier-born pups. In our study, the

stillbirths observed in the last three-born puppies of Group 1 support these findings. In contrast, Group 2 had two stillbirths among firstborn puppies and two among last-born puppies, suggesting that other variables—such as environment and maternal condition—may influence this association. In addition, higher average litter size in Group 2 might have created additional postnatal care demands on the dam, potentially affecting neonatal outcomes regardless of maternal behavior quality.

Breed size and maternal age significantly affect litter characteristics and birth outcomes. Borge et al. (5) noted that toy breeds average 3.5 puppies, whereas giant breeds average 7.1. Martins et al. (24) reported that increasing maternal age is associated with fewer pups but a higher male pup ratio. Groppetti et al. (14) found that birth weight is influenced by maternal age and size, with lighter pups seen in toy breeds and very young/old dams. Tønnessen et al. (39) highlighted increased neonatal mortality in older and primiparous bitches. Although Group 2 included generally larger breeds, the average birth weight did not differ significantly between the two groups in our study. Yet, Group 2 had significantly higher neonatal mortality, suggesting that birth weight alone does not fully predict outcomes and that environmental or postnatal care differences may also contribute. A significant gender difference in birth weight was also observed in both groups, with male puppies being heavier. This supports findings from other studies indicating gender-related physiological differences at birth (43).

Although the farm group exhibited stronger and earlier maternal behaviors, this did not directly correlate with lower neonatal mortality. This paradox may relate to the timing and underlying causes of death. In Group 1, most losses occurred in the last-born puppies, possibly due to prolonged labor or placental insufficiency, which are not necessarily linked to postnatal care quality. Cornelius et al. (7) emphasized that last-born puppies face a higher risk due to extended inter-pup intervals and fetal stress. In contrast, no dystocia cases were recorded in Group 2, indicating that the observed neonatal deaths in this group may have resulted from intrinsic neonatal vulnerabilities rather than complications during labor. Therefore, while maternal behavior was more robust in the farm setting, intrapartum factors such as dystocia and labor duration were likely more critical contributors to mortality.

Münnich and Küchenmeister (25) identified hypoxia, hypoglycemia, and hypothermia as leading causes of neonatal deaths. Their study found that 63 of 183 hypoxic puppies died, with 92.7% dying within 48 hours. In our study, although specific diagnostic tests were not performed, clinical observations suggest that maternal rejection (refusal to nurse or lick) may have contributed to deaths via hypoxia or hypothermia. These interpretations remain speculative, as no pathological or microbiological evaluations were conducted, but align with common clinical patterns seen in canine neonatology.

Bawaskar et al. (3) also reported a higher incidence of dystocia in first-time mothers, while Guardini et al. (15) emphasized the role of environmental conditions on maternal behavior. In our study, only multiparous bitches were included, eliminating parity-related risks. However, the high dystocia rate and delayed maternal behaviors in home births aligned with literature findings that highlight the role of environment and stress on birth outcomes. It is also possible that frequent human intervention in the home setting, particularly by inexperienced owners, may have unintentionally contributed to birth complications or delays in maternal behavior expression.

Baqueiro-Espinosa et al. (2) reported that dystocia alters maternal behavior, often increasing contact with pups as a compensatory mechanism. Lúcio et al. (22, 23) linked dystocia to adverse neonatal physiology, including low Apgar scores, acidosis, and cortisol elevation. In our study, dogs with dystocia (Group 1) showed delayed licking and nursing, reinforcing the concept that parturition stress can disrupt normal maternal behavior. This observation further supports the hypothesis that birth complications can impair the timely onset of maternal care. While we did not measure biochemical stress markers, behavioral delays support this theory. Importantly, no cases of dystocia were recorded in Group 2, indicating that other, more intrinsic neonatal factors (e.g., immaturity, respiratory failure) may have been responsible for the higher neonatal mortality in that group.

The average gestation duration in dogs is around 63±3 days; however, this varies with breed, litter size, and maternal age. Okkens et al. (30, 31) and Vinaykumar et al. (42) reported that smaller breeds and larger litters tend to shorten gestation, while maternal age shows less consistent effects. In our study, Group 2 had significantly longer gestation periods, possibly due to breed composition (larger breeds). Contrary to initial assumptions, Group 2 did not have smaller litter sizes; in fact, the average number of puppies per litter was higher than in Group 1. These findings align with the current literature, though the potential influence of increased fetal load on maternal stress and care distribution should be further investigated.

Maternal licking stimulates respiration, thermoregulation, and excretion (4, 13, 36). Early onset of licking and nursing has been linked to lower mortality and is typically seen in less-intervened, farm-based environments (15, 32). Guardini et al. (16) emphasized consistent observer definitions in maternal behavior research. In our study, maternal behaviors were delayed in home-raised dogs, especially in dystocia cases, which may be linked to stress and owner interventions. This methodological discrepancy may have influenced the

reliability of behavioral metrics. While farm births were continuously monitored via video, the home group relied on structured observation cards and owner reports. Although owners were trained via scheduled video calls and provided written protocols, subjectivity in their observations may have affected precision. We recommend that future studies employ synchronized video tracking across all environments to reduce bias (6).

This study was conducted as an observational field study and included several methodological limitations. Firstly, data collection methods differed between the groups: while births in the farm environment were continuously monitored using a camera system, video recording was not feasible in the home environment due to ethical, technical, and privacy concerns. Instead, structured owner anamnesis forms and observation cards were utilized. Secondly, hormonal or biochemical parameters such as oxytocin or cortisol levels, which could provide insight into the physiological basis of maternal behaviors, were not measured. This limited the ability to support certain findings with biological mechanisms. Also, all animals were multiparous and clinically healthy; the difference in breed composition between the groups is a potential limitation. Finally, causes of mortality were assessed solely based on clinical observations; no pathological or microbiological analyses were conducted. This study highlights that environmental conditions and human intervention can significantly influence parturition outcomes and maternal-offspring interactions in dogs. Although farm-raised bitches exhibited earlier and more robust maternal behaviors, neonatal mortality was higher in this group, suggesting that intrapartum factors such as dystocia and prolonged labor may outweigh the benefits of strong postnatal care. Conversely, home births were associated with higher dystocia rates and delayed maternal behaviors, likely due to increased stress and owner interventions. Despite similar litter sizes and birth weights, differences in gestation length, maternal response timing, and neonatal survival between the groups emphasize the multifactorial nature of reproductive outcomes. Future studies with larger sample sizes and the inclusion of hormonal and physiological mechanisms are recommended to enhance the validity and generalizability of these findings.

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Ethical Statement

The study was approved by Selcuk University Faculty of Veterinary Medicine Experimental Animal Production and Research Center Ethics Committee (Decision no: 2024/190, Date: 26.12.2024).

Conflict of Interest

The authors declare that they have no conflict of interests.

Author Contributions

Conceptualization: YP. Writing – original draft preparation: YP, DA, GB, ÖA. Data analysis: GB, YP. Writing – review and editing: YP, DA, GB, ÖA, İİK. All authors reviewed and approved the final version of the manuscript.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

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