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Association of Estradiol-17β, Progesterone, and Follicular Development with Standing Estrus and Ovulation After PGF₂α Treatment in Barbari Goats

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ABSTRACT

This study aimed to characterize ovarian follicular activity and circulating hormonal patterns in Barbari goats following a single, randomly timed injection of $PGF_2\alpha$ during the breeding season. Ovarian structures were monitored every 12 hours using B-mode ultrasonography, while blood samples for estradiol-17β and progesterone measurement were collected at the same interval. Detection of standing estrus was performed every 6 hours using apron-fitted bucks. After $PGF_2\alpha$ administration, the onset of standing estrus and the time of ovulation differed significantly ($p < 0.05$) among early- ($n = 7$), intermediate- ($n = 6$), and late-responding ($n = 6$) goats. Peak estradiol-17β concentrations occurred 12 hours before ovulation. No significant differences ($p > 0.05$) were noted among goats in ovulatory follicle diameter or duration of standing estrus. Regression of the corpus luteum was faster ($p < 0.05$) in early responders compared with intermediate and late responders. Dominant follicle size and estradiol-17β concentrations varied ($p < 0.05$) among the groups. Although overall progesterone levels showed no significant group differences ($p = 0.065$), temporal changes in progesterone concentrations varied significantly ($p < 0.05$) across goats. These findings indicate that the timing of estrus onset, estrus cessation, and ovulation after $PGF_2\alpha$ administration may differ among Barbari goats due to variations in follicular development and hormone dynamics during the luteal phase.

Keywords: PGF2α, Barbari goats, Ovarian Dynamics, Estrus, Hormonal Profile, Ovulation

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Introduction

The rapid growth of the human population across the Asian subcontinent continues to drive a strong demand for higher meat production, making the development of an efficient livestock system essential for ensuring regional food security. Nearly 46.9% of the global goat population is concentrated in China, India, and Pakistan [1]. Among the breeds commonly raised in SAARC nations—particularly Pakistan, India, Sri Lanka, and Afghanistan—the Barbari goat holds significant importance [2]. This medium-sized breed is valued for both milk and meat, and is recognized for its superior feed conversion ability, strong reproductive performance, high prolificacy, early sexual maturity, elevated kidding rates, and natural resistance to a range of bacterial and parasitic infections [3-5]. Barbari goats are also well-suited to the harsh conditions of arid and semi-arid regions in South Asia due to the expression of heat shock protein genes HSP60 and HSP70, which enhance thermal tolerance [6].

In small ruminant reproduction, prostaglandin F2α (PGF2α) and its analogs are widely applied to synchronize estrus during the breeding season [7]. These compounds are advantageous because they are easy to administer

intramuscularly, disperse quickly within the body, and are almost entirely metabolized in the lungs [8]. Estrus can be successfully induced in more than 70% of goats with a single PGF2α injection and in over 90% with two injections given 11–14 days apart during the breeding season [9]. The success of PGF2α treatment is influenced by multiple factors, including timing, dosage, route of injection, seasonal conditions, the presence of males, and ovarian follicular status, although an active corpus luteum is essential for the treatment to be effective [10-13].

In both ewes and goats, PGF2α fails to initiate luteolysis if administered before day 4 or on day 17 of the estrous cycle [14,15]. During the luteal phase, estrus induction rates in ewes using a single PGF2α dose range from 40% to 80%, largely because the corpus luteum can be at different developmental stages [16]. Likewise, dairy goats exhibit estrus responses between 67% and 85% when PGF2α is administered on day 6 or 12 of the cycle [7]. Beetal goats, for instance, show ovulation between 60 and 96 hours following a single PGF2α injection, with most ovulations occurring around 72 hours. Corresponding fluctuations have also been noted in estradiol-17β, luteinizing hormone (LH), progesterone decline, dominant follicle growth, and corpus luteum regression between early and late ovulators [17].

Other findings indicate that goats treated with PGF2α on day 5 of the cycle and again on days 11 and 16 exhibit earlier estrus expression. Collectively, published research demonstrates that both estrus response rate and the timing of estrus onset after PGF2α administration vary depending on when synchronization protocols begin during the luteal phase [18]. Variations in circulating levels of estradiol-17β, progesterone, and LH during the follicular phase have been associated with estrus onset and ovulation in goats, ewes, dairy cows, and buffalo [19-22].

Barbari goats are spontaneous ovulators known for high fertility, frequently producing twins or triplets, with an ovulation rate of 1.43; however, environmental conditions and feeding also influence reproductive success [23,24]. Studies have examined how diets such as rice dried distillers grain (rDDGS) affect physical and behavioral indicators of estrus in this breed [25]. Other work has evaluated genetic and environmental influences on growth, productivity, and reproductive traits in Barbari goats raised under semi-intensive management [2,4]. Additional studies have explored the relationship between caprine pregnancy-associated glycoproteins (caPAG) and gestational progress, litter size, and parity in this breed [26,27]. Despite these contributions, detailed information on follicular dynamics and changes in circulating estradiol-17β and progesterone levels in connection with estrus onset following PGF2α treatment remains lacking.

Therefore, the objective of this study was to characterize the timing of standing estrus, the interval to ovulation, and preovulatory follicular changes relative to plasma concentrations of estradiol-17β and progesterone after PGF2α administration in Barbari goats.

Materials and Methods

Animals and management

The study was carried out at the Small Ruminant Training and Research Centre (SRT&RC) in Pattoki, University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan (31°03′29.0″ N, 73°52′42.9″ E). All procedures were performed following the ethical guidelines approved by the UVAS Animal Care and Ethical Review Committee (protocol 6853) and Yangzhou University (SYXK [Su] 2017-0044). Nineteen multiparous, clinically sound goats with regular estrous cycles, body weights between 30–40 kg, and body condition scores ranging from 2.5 to 4 were used. The animals were maintained in open housing and were provided sorghum fodder (4–5 kg/head/day), a concentrate mix composed of corn gluten, corn grain, wheat bran, soybean meal, and canola meal (400–500 g/head/day), along with unrestricted access to water. The trial was conducted during the natural breeding period from September to December 2021.

Selection and treatment of animals

Ultrasonographic screening of a 30-goat herd was performed to locate animals carrying a functional corpus luteum, from which nineteen goats (n = 19) were selected. Each selected animal received a single intramuscular dose of PGF2α (Delmazine®, Fatro, Italy; 1 mL) immediately after corpus luteum identification. Ovarian assessments were conducted every 12 hours using a 7.5 MHz probe (Honda® 1600, Tokyo, Japan), starting at the time of treatment (0 h) and continuing until ovulation. Ovulation was confirmed when a dominant follicle that had been previously identified was no longer present at the next 12-hour evaluation [28]. Estrus behavior was checked every 6 hours using teaser bucks equipped with aprons, and a doe was classified as being in standing estrus only when she remained stationary and accepted a mount [29].

Experimental design and methodology

From an initial pool of fifty Barbari goats, only nineteen animals were ultimately eligible for experimentation. Twenty of the original animals (n = 20) were pregnant, while eleven others (n = 11) were either in very early (≤4 days) or very late (≥17 days) luteal stages or were already showing estrus signs; these animals were excluded. The goats included in the trial (n = 19) were healthy, multiparous, and approximately 3.5 ± 0.3 years old with no history of reproductive abnormalities.

PGF2α was administered once a mature corpus luteum was confirmed between days 4 and 16 of the estrous cycle. A baseline blood sample for hormone analysis was collected at treatment, another sample was taken 24 hours later, and subsequent samples were collected at 12-hour intervals until ovulation occurred. Concurrently, ultrasonography was performed every 12 hours to document follicular growth and luteal regression. Behavioral estrus detection took place every 6 hours using apron-wearing bucks.

Substantial variability was observed in the timing of estrus onset and ovulation among the animals. Therefore, the goats were classified into three groups: early-responding (n = 7), intermediate-responding (n = 6), and late-responding (n = 6), based on their ovarian condition and hormone status at the time of treatment.

Although studies focusing on reproductive physiology often involve larger sample sizes, only nineteen goats could be included here due to limitations related to funding, herd availability, seasonal breeding patterns of Barbari goats, and management constraints. Expanding the number of animals and reducing the interval between blood sampling and ultrasonographic monitoring in future work would allow for more detailed characterization of the interactions among hormone profiles, follicular development, estrus behavior, and ovulation.

Blood sampling for hormonal analyses

To monitor endocrine changes, blood was obtained from the jugular vein at eight scheduled times—0, 24, 36, 48, 60, 72, 84, and 96 hours following the PGF2α injection. Each sample was collected in 5 mL EDTA vacutainer tubes. The blood was spun immediately after withdrawal at 3000× g for 15 minutes, allowing the plasma fraction to be collected and stored at −20 °C until hormone testing. Plasma estradiol-17β and progesterone concentrations were quantified using a double-antibody RIA system (Immuno Tech®, Beckman Coulter, Prague, Czech Republic), performed according to the earlier outlined procedure [30]. The assays were validated for goats. For progesterone and estradiol-17β, the intra-assay variation was 7.6% and 13.8%, respectively, while the inter-assay variation was 8.4% and 14.4%. Sensitivity limits were 0.03 ng/mL for progesterone and 0.02 pg/mL for estradiol-17β.

Statistical analyses

All numerical data are presented as mean ± SEM, with significance set at $p \leq 0.05$. Before conducting statistical comparisons, values were transformed to meet normality requirements: corpus luteum size and progesterone concentrations were adjusted via a 1/x transformation, while estradiol-17β values were processed using a Log10 transformation. Correlation analyses (Pearson) assessed the associations between estradiol-17β and preovulatory follicle diameter, and between progesterone levels and corpus luteum measurements. Differences in the timing of standing estrus among goats categorized as early, intermediate, or late responders were evaluated with one-way ANOVA. In addition, pooled data for corpus luteum regression, progesterone patterns, and preovulatory follicle size relative to PGF2α administration were modeled using a generalized linear approach (SPSS v16.0, IBM, Chicago, IL, USA).

Results

Response to estrus management

Every treated doe (n = 19) eventually entered standing estrus, with the average onset occurring 50.6 ± 4.8 hours after PGF2α administration. Across all animals, standing estrus persisted for an average of 22.3 ± 2.8 hours. From the moment estrus began, ovulation followed roughly 32.5 ± 4.4 hours later, and ovulation took place approximately 9.4 ± 1.5 hours after the end of standing estrus **(Table 1)**. Considerable variation ($p < 0.05$) occurred between animals regarding when estrus began and when ovulation occurred relative to treatment **(Table 1)**. The earliest responders (n = 7) expressed estrus at 44 ± 2.0 hours post-PGF2α, those classified as intermediate responders (n = 6) at 51 ± 3.0 hours, and late-responding animals (n = 6) at 60 ± 0.0 hours. However, the length of standing estrus, the delay between estrus cessation and ovulation, and the diameter of the preovulatory follicle

measured 12 hours before ovulation showed no statistical differences ($p > 0.05$) among these three response groups **(Table 1)**.

Table 1.

Parameter	Overall (n = 19)	Early group (n = 7)	Intermediate group (n = 6)	Late group (n = 6)
Time from PGF2α to onset of standing estrus (hours)	50.6 ± 4.8	44.0 ± 2.0[a]	51.0 ± 3.0[b]	60.0 ± 0.0[c]
Duration of standing estrus (hours)	22.3 ± 2.8	18.0 ± 3.5	24.0 ± 0.0	27.0 ± 3.0
Time from end of standing estrus to ovulation (hours)	9.4 ± 1.5	10.0 ± 0.2	9.0 ± 3.0	9.0 ± 3.0
Time from PGF2α to ovulation (hours)	82.3 ± 4.0	72.0 ± 0.0[a]	84.0 ± 0.0[b]	96.0 ± 0.0[c]
Interval from onset of estrus to ovulation (hours)	32.5 ± 4.4	28.0 ± 1.0	33.0 ± 1.5	36.0 ± 0.0
Diameter of preovulatory follicle 12 h before ovulation (mm)	7.1 ± 0.3	7.5 ± 0.4	6.5 ± 0.3	7.1 ± 0.1
Corpus luteum diameter 12 h after PGF2α (mm)	8.9 ± 0.4	8.4 ± 0.4	8.9 ± 0.5	10.0 ± 0.5

[a, b, c] denote difference at $p < 0.05$ among groups.

Association between dominant follicle, estradiol 17β, and standing estrus

Analysis of periovulatory follicular activity revealed that most goats (13 out of 19; 68%) carried two dominant follicles following PGF2α treatment. The primary dominant follicle consistently exhibited a greater diameter than the secondary one (7.3 ± 0.3 mm vs. 6.6 ± 0.3 mm; $p = 0.00$; **Figure 1A**). Despite this size difference, both follicles expanded at a comparable rate as ovulation approached **(Figure 1B)**. Plasma estradiol-17β concentrations rose in a linear relationship with the average diameter of preovulatory follicles ($r = 0.98$, $p = 0.003$). Peak estradiol-17β levels appeared 12 hours before ovulation, and standing estrus concluded roughly three hours afterward **(Figure 2)**. Early-responding goats displayed larger dominant follicles than those in the intermediate or late groups ($p = 0.005$) after PGF2α administration **(Figure 3)**. The pattern of estradiol-17β secretion across early-, intermediate-, and late-responding goats followed the same general trend **(Figure 4)**.

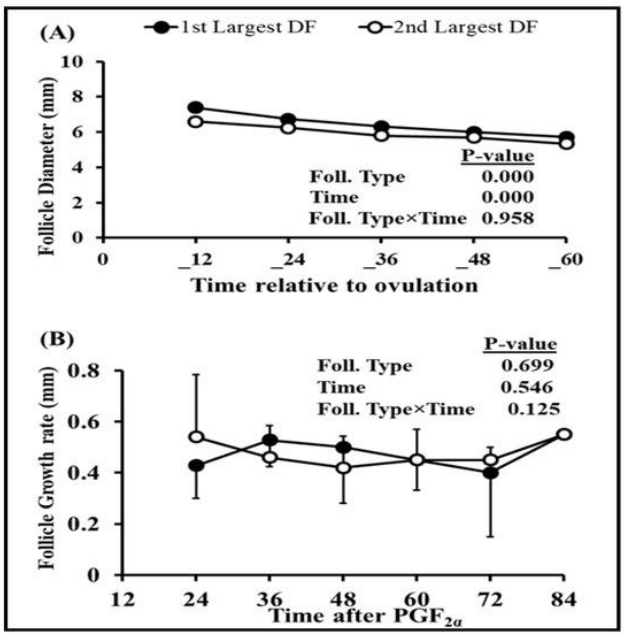

Figure 1. When follicle measurements were aligned with the moment of ovulation (0 h), the primary dominant follicle (n = 19) consistently exhibited a greater mean diameter than the secondary dominant follicle (n = 13) ($p = 0.00$) (A). Despite this size difference, both follicles followed a comparable pattern of enlargement over time following PGF2α administration, showing no significant divergence in growth rate (B)

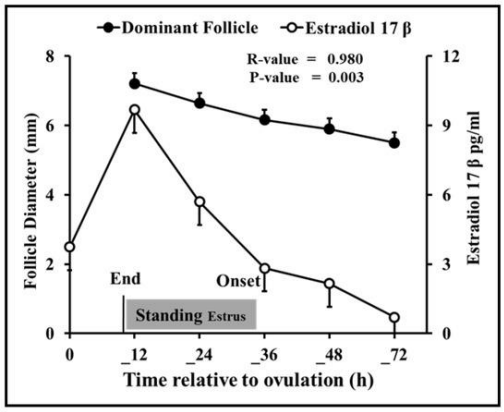

Figure 2. Across the periovulatory period, rising plasma estradiol 17β levels (n = 19) closely mirrored the progressive increase in dominant follicular diameter (mean ± SEM; n = 19). The hormone reached its maximum concentration 12 h before ovulation, coinciding with the largest follicular size. Standing estrus in the Barbari goats appeared roughly 19.3 h prior to this hormonal peak

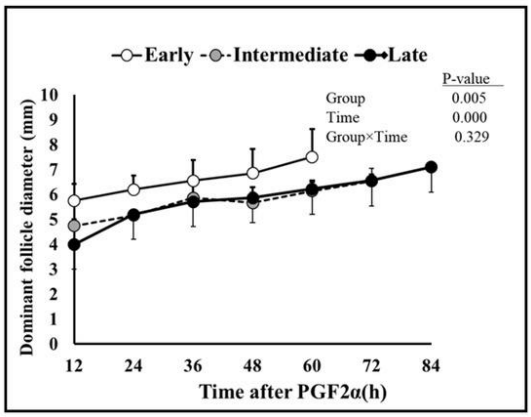

Figure 3. When dominant follicle size (mean ± SEM) was evaluated among early- (n = 7), intermediate- (n = 6), and late-responding (n = 6) groups, goats entering estrus earlier displayed noticeably larger dominant follicles following PGF2α exposure

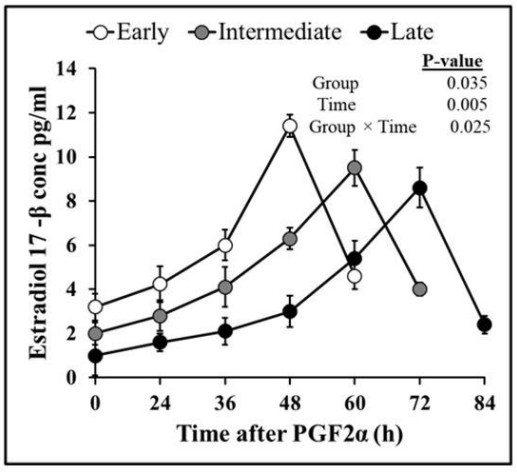

Figure 4. A similar temporal shift was observed for plasma estradiol 17β, with early-responding goats (n = 7) attaining their maximal hormone concentration sooner than goats in the intermediate (n = 6) or late (n = 6) response categories

Relationship between corpus luteum, progesterone, and ovulation

Following PGF2α treatment, most goats (68%) were found to possess two corpora lutea. Measurements taken 12 hours post-injection showed the first corpus luteum averaging 9.6 ± 0.2 mm and the second 9.2 ± 0.6 mm in diameter. Over time, both structures underwent significant regression ($r = -0.98$, $p = 0.00$), with their sizes

remaining statistically similar until ovulation (p = 0.42; **Figure 5**). Concurrently, plasma progesterone levels decreased proportionally to the combined size reduction of the two corpora lutea (r = 0.92, p = 0.00; **Figure 6**). The pace of luteal regression varied markedly among goats categorized as early-, intermediate-, or late-responders (p = 0.000; **Figure 7**). However, despite these differences in luteal regression, the absolute plasma progesterone concentrations did not differ significantly across the three groups (p = 0.065; **Figure 8**).

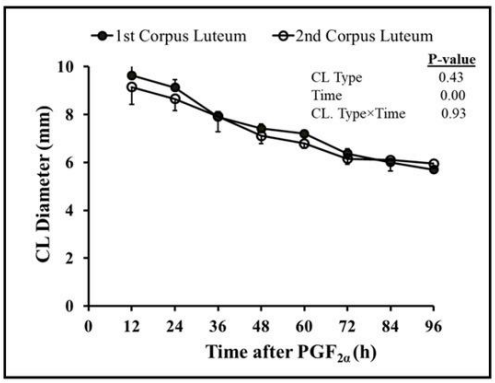

Figure 5. Size (mean ± SEM) of the primary corpus luteum (present in all 19 goats) versus the accessory corpus luteum (present in 13 goats) following PGF2α injection (time 0 h) in Barbari does. The two types of corpora lutea showed comparable diameters throughout the observation period (p = 0.43)

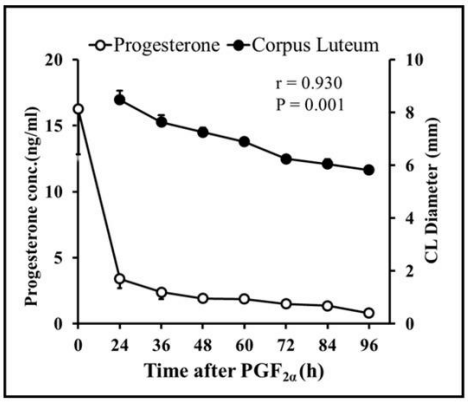

Figure 6. Close association between circulating progesterone levels and the average diameter of all corpora lutea (n = 19) after administration of PGF2α in Barbari goats. Progesterone concentration in plasma fell markedly within the first 24 hours

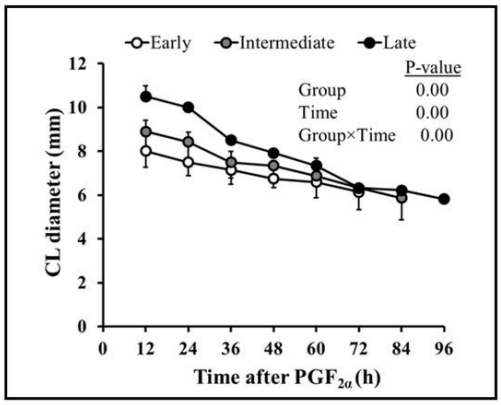

Figure 7. Time-course of corpus luteum diameter (mean ± SEM) after PGF2α (0 h) in goats classified as early responders (n = 7), intermediate responders (n = 6), and late responders (n = 6). The largest corpora lutea, mainly found in the late-responder group, displayed noticeably slower structural regression than those in the early- and intermediate-responder groups

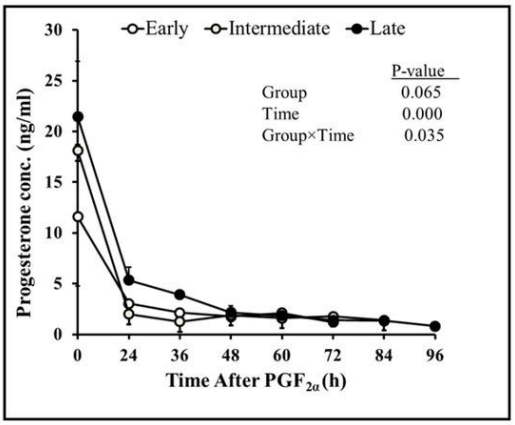

Figure 8. Plasma progesterone profiles (mean ± SEM) after PGF2α treatment (0 h) across early- (n = 7), intermediate- (n = 6), and late-responder (n = 6) Barbari goats. In every group, progesterone dropped sharply within 24 hours. While the overall magnitude of the decline was similar between groups (p = 0.065), the temporal pattern of the fall in progesterone differed significantly among the three response categories (p = 0.03)

Discussion

This investigation explored ovarian activity and associated hormonal fluctuations in Barbari goats following a single PGF2α injection administered indiscriminately during the luteal phase. The treatment elicited a full estrus response, with all goats exhibiting standing estrus, which is consistent with earlier observations in Beetal goats treated with two PGF2α injections [31]. The timing of estrus onset and ovulation following a single dose in Barbari goats was also similar to what has been reported in Shiba goats receiving a single injection [19]. In contrast, compared with Beetal goats receiving two injections [32], both the interval to estrus and ovulation were extended in the present study (estrus onset: 50.6 ± 4.8 vs. 36.0 ± 1.2 h; ovulation interval: 82.3 ± 4.0 vs. 66.0 ± 2.7 h), suggesting a more variable response when a single PGF2α dose is applied at different stages of the luteal phase. Nevertheless, our findings for estrus response, estrus onset, and ovulation timing are in close agreement with Beetal goats that received PGF2α after ultrasonographic confirmation of a mature corpus luteum [17].

Variability in follicular development during the luteal phase appeared to influence the duration to estrus and ovulation. Previous work indicates that follicular growth in the early or late luteal stage can significantly affect the outcome of PGF2α treatment [18,33]. In this study, goats that exhibited early estrus had larger dominant follicles at the time of injection compared with those with delayed estrus **(Figure 3)**. This was accompanied by an earlier peak in plasma estradiol-17β among early responders relative to intermediate and late responders **(Figure 4)**, underscoring the link between follicular status and hormonal dynamics.

The timing of PGF2α administration in this study was based on the presence of corpora lutea rather than the exact estrous cycle day, meaning goats likely ranged from the 5th to 17th day of the luteal phase. Earlier reports suggest that luteolysis is faster when PGF2α is administered during the early (day 5) or late (day 16) luteal stages compared with the mid-luteal phase (day 11) [18, 34]. Progesterone levels are typically higher in the mid-luteal phase than in early or late stages [18], which corresponds with our observation that early responders experienced a more rapid decline in plasma progesterone after treatment than intermediate and late responders **(Figures 7 and 8)**. It has been suggested that a larger luteal mass and higher progesterone concentration at treatment can delay luteolysis, whereas the presence of a dominant follicle can accelerate estrus induction [35,36]. In line with this, early-responding goats in our study were more responsive due to smaller corpora lutea, lower progesterone levels, and larger dominant follicles **(Figures 3, 7, and 8)**.

Key reproductive parameters in Barbari goats—such as the interval from estrus onset to ovulation (32.5 ± 4.4 vs. 28.6 ± 3.8 h), number of ovulations (1.7 ± 0.4 vs. 1.95 ± 0.2), and preovulatory follicle diameter (7.1 ± 0.3 vs. 7.15 ± 0.3 mm)—were comparable to Beetal goats under similar treatment conditions [17]. Collectively, the results highlight that estrus onset and ovulation after PGF2α in Barbari goats can vary depending on the luteal stage at the time of injection. Future research could benefit from administering PGF2α on precisely defined estrous cycle days, using larger sample sizes, and incorporating more frequent hormonal sampling and ultrasonographic monitoring to better characterize the relationship between ovarian dynamics and hormonal profiles.

In the present study, the pattern of estradiol-17β changes relative to the onset of standing estrus in Barbari goats resembled that reported for Shiba goats [19]. However, the decline in progesterone occurred later in Barbari goats (24 h) compared to Shiba goats (12 h), likely due to the more frequent plasma sampling performed in the latter. Generally, the duration of estrus behavior in goats varies widely—from 20 to 58 hours—depending on factors such as breed, age, season, and the presence of males [37]. In this research, Barbari goats exhibited a relatively short standing estrus of approximately 22 hours, comparable to the 22-hour estrus observed in Angora [38] and Shiba goats [19], but shorter than that reported in Boer goats (37 h) [39] and Beetal goats (30.8 ± 3.9 h) [17] after PGF2α administration during the breeding season.

Conclusions

This study demonstrates that variations in ovarian follicular dynamics and plasma hormonal profiles—specifically estradiol-17β and progesterone—at the time of PGF2α administration strongly influence reproductive outcomes, including estrus expression and ovulation, in Barbari goats. The differences appear largely dependent on the size of preovulatory follicles and the corpus luteum on the day of treatment. At the time of injection, most goats (68%) had two dominant follicles, which matured significantly over time ($p = 0.00$), although their growth rates remained similar ($p = 0.124$) until ovulation. Likewise, 68% of the goats had two corpora lutea, which regressed significantly over the observation period ($p = 0.00$), with no significant difference in regression rates between them ($p = 0.93$). A strong positive correlation ($r = 0.98$) was observed between the combined diameter of preovulatory follicles and plasma estradiol-17β levels measured during each ultrasonographic scan. Similarly, luteal regression and progesterone decline were closely associated ($r = 0.93$) until ovulation.

The attainment of maximum ovulatory follicle size occurred earlier in early-responding goats (7.5 ± 0.4 mm) than in late responders (6.5 ± 0.3 mm). Initial estradiol-17β levels also varied among goats, producing a staggered peak of estradiol-17β at 48, 60, or 72 hours post-treatment. correspondingly, the corpus luteum diameter 12 hours after PGF2α was smaller in early-responding goats (8.4 ± 0.4 mm) compared to intermediate (8.9 ± 0.5 mm) and late responders (10 ± 0.5 mm). Additionally, goats with lower initial progesterone concentrations experienced a more rapid hormone decline within 24 hours (11.2 ± 0.3 vs. 19.1 ± 0.1 vs. 21.5 ± 0.5 ng/mL), resulting in significant variability in estrus onset, estradiol peak, progesterone drop, and ovulation timing across individuals.

This research represents the first comprehensive profiling of follicular dynamics and ovarian steroid changes following induced luteolysis during the preovulatory period in Barbari goats. Future studies could enhance these findings by administering PGF2α on precisely defined estrous cycle days, utilizing larger cohorts, and employing more frequent hormonal sampling and ultrasonography to achieve a more accurate determination of estrus onset and ovulation in this breed.

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Conflict of Interest: None

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Ethics Statement: None

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