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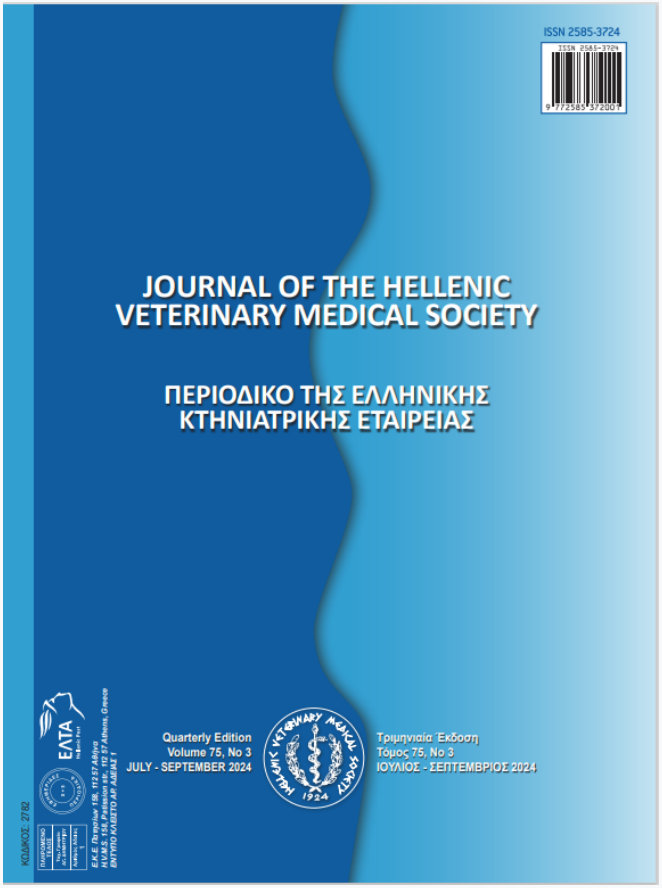

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Research article
Ερευνητικό άρθρο

Effects of exogenous progesterone on the ovine serum progesterone profile during the non-breeding season

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ABSTRACT: Exogenous progesterone (PRG) sources are used to induce ovine estrus during the non-breeding season. Intramuscular (IM) injection of PRG is an uncommon route for this purpose. This study investigated serum progesterone (P4) levels after IM administration of 50 mg of two different PRG products. Ten ewes were selected and received two consecutive $PGF_{2\alpha}$ injections at 24-h intervals. Serum samples were collected at Time 0, followed by the injection of the single doses of Fertigest® or Vetagesterone®. Serum P_4 levels were assayed at times (T) 0, 30 min, 3, 6, 12, 24, 48, 72, 96, and 120 h after injection. A large variation in the serum P_4 levels at T0 was detected. The serum P_4 level at T0 was 4.3 ng/mL, which is significant for anestrus ewes, following $PGF_{2\alpha}$ administration. The mean time to reach the maximum serum P_4 concentration was 5.8 h, with a mean concentration of 22.5 ng/mL. Serum P_4 levels returned to basal values (T0) after 48 h. An increased P_4 production rate (PR) was detected following PRG administration, which continued for 24 h. A single injection of PRG reduced the variability of serum P_4 levels from 24 h onward. The two different PRG sources in our test produced varying serum P_4 concentrations. This study also indicates an unusually elevated serum P_4 levels in anestrus ewes following $PGF_{2\alpha}$ administration. 48 h after a 50 mg PRG injection, the plasma levels of P_4 reached basal levels in anestrus ewes.

Keywords: exogenous progesterone; intramuscular; serum progesterone; non-breeding season; ewe

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INTRODUCTION

During the short-day period of the year, the reproductive activity of ewes is quiescent. Different aspects of photoperiod in the central nervous system (CNS) were investigated. Changes in cerebrospinal fluid proteomes and the steroid-modulating effects of estradiol on gonadotropin-releasing hormone (GnRH) neurons (Thiery et al., 2006), as well as differential expression of certain peptides such as kisspeptin and the steroid progesterone (P_4) in the anterior hypothalamus, are recognized effects of photoperiod on reproductive function (Goodman et al., 2012). Pinealectomy in ewes extended the breeding season, counteracting the inhibitory effects of melatonin on their reproductive function (Manca et al., 2014). Melatonin (Berean et al., 2021) and its combination with intravaginal progestin devices (Mansoor et al., 2020) are considered to activate estrus behavior during the non-breeding season in ewes. Trials to activate the reproductive cycle during anestrus commonly rely on exogenous progesterone (PRG) administered through intravaginal devices or subcutaneous implants (Awel et al., 2009; Yu et al., 2018). Several studies have evaluated the efficacy of the intramuscular (IM) route of PRG treatment for estrus induction in sheep. Hashemi et al. (2006) administered 20 mg of PRG for 12 days every other day, while Niasari-Naslaji et al. (2022) employed subcutaneous (SC) injections of 50 mg of PRG to induce estrus in Iranian breeds. To enhance the effectiveness of injected PRG in estrus induction, Dehkordi et al. (2022) administered a single injection of human chorionic gonadotropin (hCG) at the end of the PRG treatment. However, estrus responses differed between the two doses (20 or 50 mg) and routes of injection (SC or IM). The most important factor that may contribute to the efficacy of the IM route is the interval between injections. This study evaluated the serum levels of P_4 following a single injection of two different formulations of PRG in ewes during seasonal anestrus.

MATERIALS AND METHODS

This study was conducted at the Shuli station for Bakhtiari sheep breeding located in Chaharmahal and Bakhtiari province, Iran (32° 18' 45.1" N and 51° 3' 16.4" E) from May 22 to June 5, 2022. Ten ewes with a body weight of 55-65 kg (average 59.4±1.25 kg), 3 to 4 parities (average 3.4±0.16; mode: 3; median: 3), and 3-4 months postpartum (average 3.7±0.15; mode: 4; median: 4) were selected. Animals were fed on natural pasture with daily mineral supplementation. Water was provided ad libitum throughout the study. The animals were healthy and had received routine antiparasitic treatment. All procedures were approved by the Shahrekord University Ethics Committee Guidelines for Animals in Research. A GMP full-digital ultrasound device (V9, Shenzhen Empower Electronic Technology Co., Ltd, China) was used for transabdominal (with a convex probe of 5 MHz) and transrectal (with a rectal probe of 7.5 MHz) examinations of the uterus and ovaries before the start of the experiment. Furthermore, two doses of $PGF_{2\alpha}$ (5 mg Dinoprost tromethamine/mL, Vetalyse, Aburaihan, Iran) at a 24-hour interval were administered to ensure the absence of a corpus luteum on the ovary.

The ewes were equally assigned into two groups to receive one of the formulated progesterone products (PRG) 24 hours after $PGF_{2\alpha}$ administration: Group 1, the ewes (n=5; weight: 60±1.08 kg) received an IM injection of 1 mL of a human use product (Fertigest®; 50 mg P4/mL; Aburaihan, Iran) containing 50 mg of P_4. In group 2, ewes (n=5; weight: 58.1±1.51 kg) received an IM injection of 2 mL of an animal use product(Vetagesterone®; 25 mg P4/mL; Aburaihan, Iran) containing 50 mg of P_4. The blood samples were collected using the 2 mL Vacutainer® with no additives from the jugular vein at 0 (immediately before PRG injection), 30 min, and 3, 6, 12, 24, 48, 72, 96, and 120 h after PRG administration. The coagulated blood was centrifuged at 5000 rpm for 10 min, and the serum was separated and stored at -21°C until the assay.

Serum P_4 levels were assessed at various time points using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Accubind ELISA Microwells, Monobind, USA) that operates based on a competitive principle with a streptavidin-coated plate. The intra- and inter-assay coefficients of variation for the 20 samples were 3.8% and 7.5%, respectively. The sensitivity of P_4 detection was 0.105 ng/mL. The maximum cross-reactivities were 0.375% and 0.347% with 17OH-progesterone and corticosterone, respectively.

To estimate the mean release rate of P_4 in the blood after injection of the two types of PRG, we utilized the equation from Bedford et al. (1972). This equation estimates the metabolic clearance rate (MCR) of P_4 in anestrus ewes by infusing radiolabeled P_4 at a rate of 3.675 L/min.They estimated theproduction rate (μg/mL) of P_4(PR)in anestrus ewes based on MCR and endogenous P_4 as follows:

PR (μg/mL) = MCR (L/min) × endogenous P_4 (ng/mL).

A univariate normal plot with the Shapiro-Wilk test was used to assess the normality and homogeneity of the data.The serum P_4 values were analyzed by considering the group and time of sampling as fixed effects using the mixed procedure in SAS 9.1.3. Ewe was considered a random effect.Data is presented as the mean and standard error of the means. P values less than 0.05 are considered significant.

RESULTS

Figures 1 and 2 show that at time 0, the serum P_4 values were not normally distributed (Range: 1.4-12.3 ng/mL; P = 0.0002). However, IM administration of PRG statistically normalized the serum P_4 values up to 120 h.The variations in serum P_4 were stabilized from 48 h onward after PRG administration (Fig 1 and Fig 2).

The mean serum P_4 levels (ng/mL) at time 0, i.e., 24 h after the 2nd $PGF_{2\alpha}$ and before PRG administration, were 4.3±1.51 with a minimum of 1.4 and a maximum of 12.3, and 95% confidence limits of 0.83-7.79. Figure 3 shows that IM administration of the two types of PRG increased the serum levels of P_4, with a peak at 3 h, followed by a decline to reach a basal plateau at 48 h onward.

The mean time to reach maximum serum concentration did not differ between the two PRG. Therefore, the data were pooled, and the mean time to reach the maximum concentration was 4.7±1.01 h, with a mean maximum serum P_4 concentration of 22 ± 5.89 ng/mL. The data showed that 64.1% of the released P_4 from the injected PRG was detected in the serum during the first 24 h after injection.

The mean PR (μg/mL) was estimated at 7.4 ± 9.15 at time 0, with the highest values observed at 6 h (76.1±9.64) followed by a descending trend to reach basal levels at 24 h (9±9.15). Figure 4 displays the estimated PR following the injection of a single dose of 50 mg PRG.

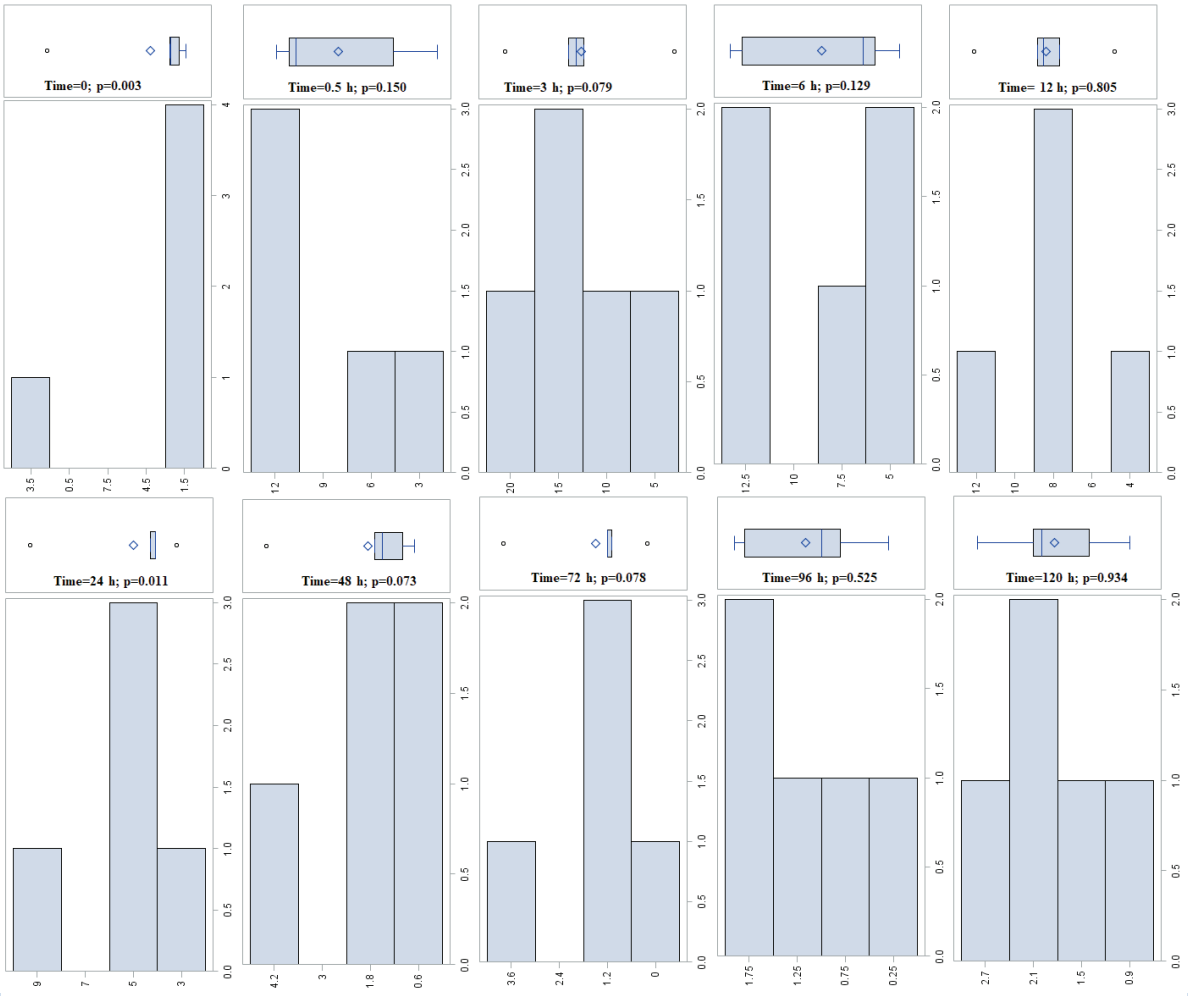

Fig 1. The histogram and box-whisker plots display the distribution of serum progesterone levels following the administration of PRG for animal use at various sampling times.

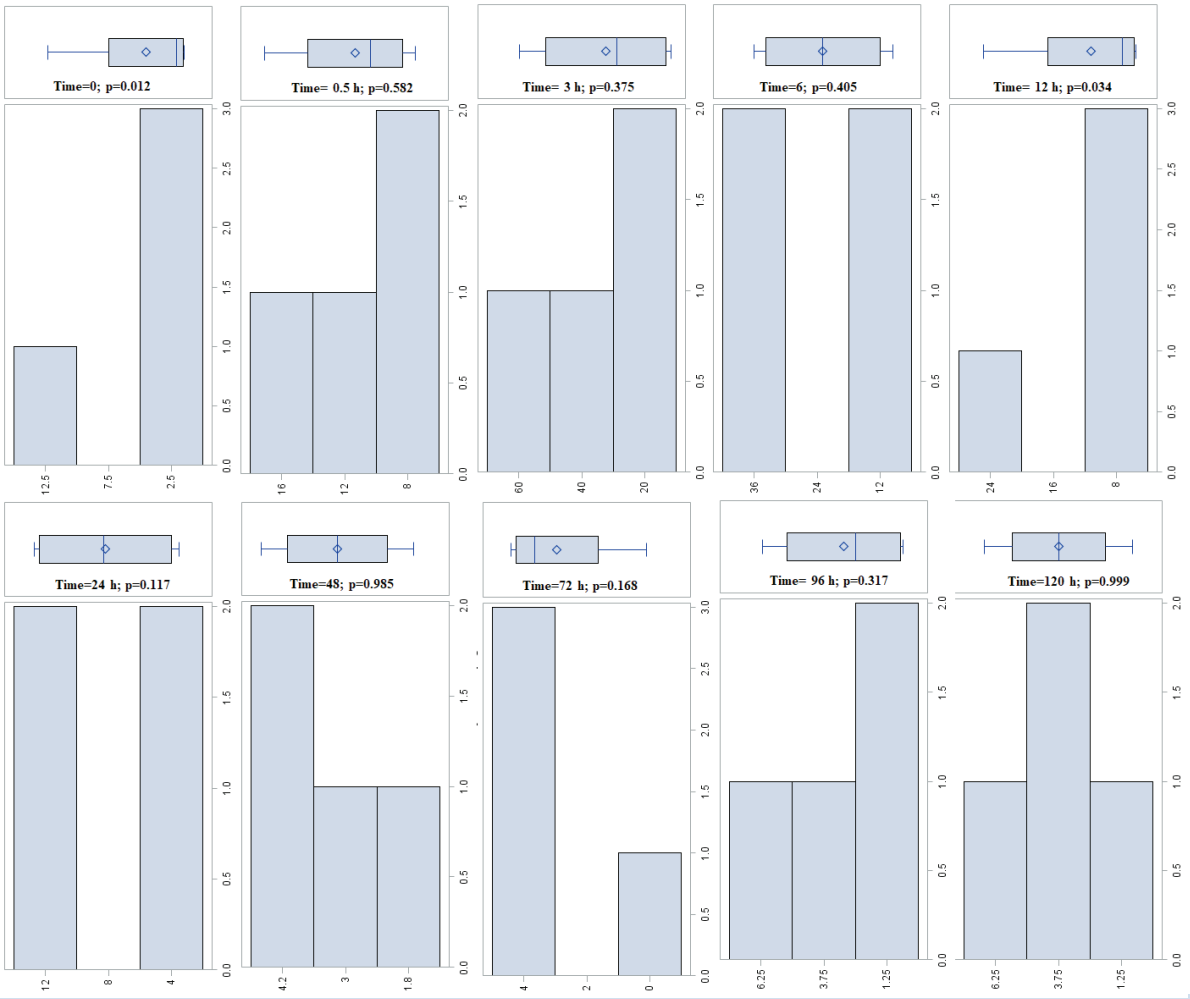

Fig 2. The histogram and box-whisker plots display the distribution of serum progesterone levels following the administration of PRG for human use at various sampling times.

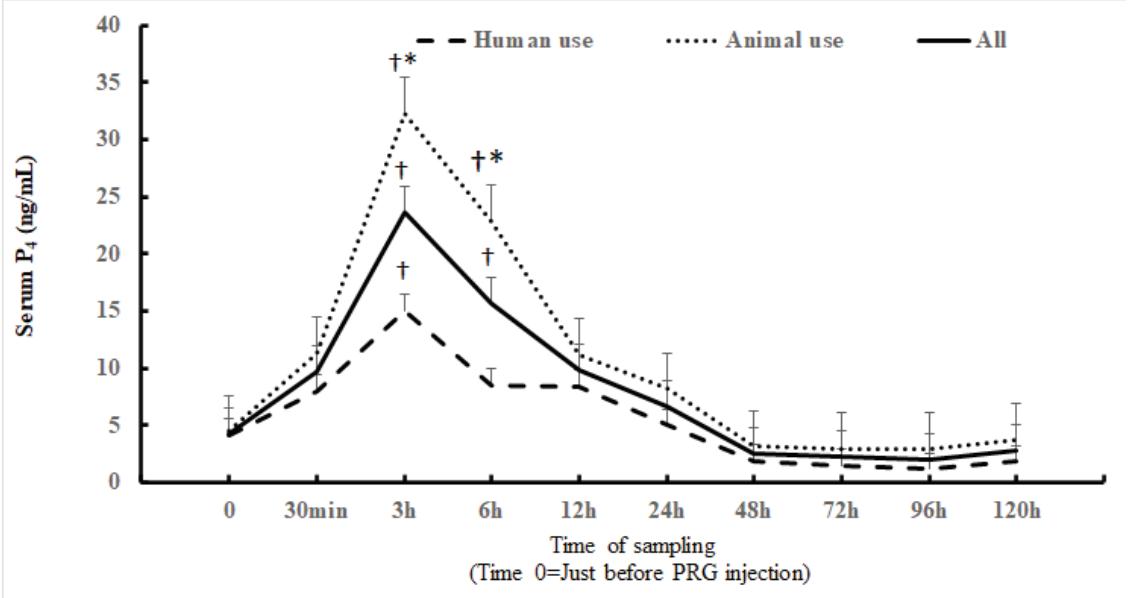

Fig 3. The mean serum progesterone profile following injection of two different progestins (for animal or human use) in non-breeding season Bakhtiari ewes. *indicates a significant difference between the two groups at each time point. †indicates a significant difference at each time point compared to time zero within the group.

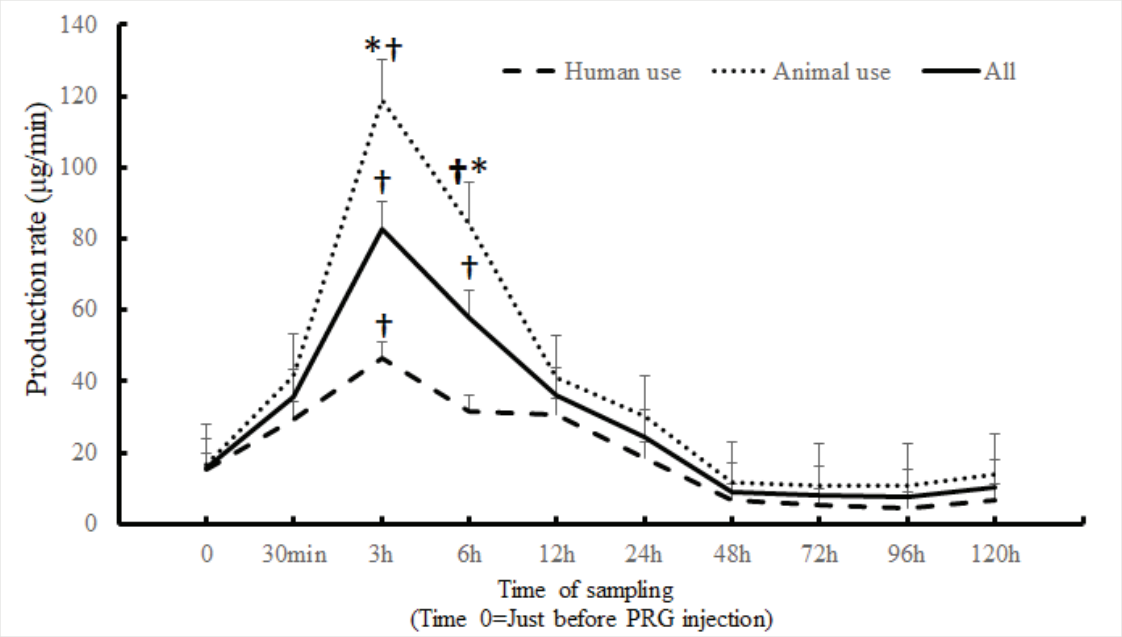

Fig 4. The estimated mean progesterone release rate following injection of two different progestogens (Human use and Animal use) in non-breeding season Bakhtiari ewes. *indicates a significant difference between the two groups at each time point. †indicates a significant difference at each time point compared to time zero within the group.

DISCUSSION

The results of this study showed that the mean serum P_4 levels in the non-breeding season in Bakhtiari ewes after two consecutive administrations of $PGF_{2\alpha}$ were more than 4 ng/mL, while two ewes had 12.2 and 12.3 ng/mL, indicating a large variation in serum P_4 in ewes. The two PRG products elevated the serum P_4 for 3-6 h, reaching basal concentrations after 24 h; whereas the estimated PR continued to increase up to 48 h. The injection of PRG, statistically homogenized the serum P_4 values and returned it to the basal level after 24 h. The two PRG products lead to different serum P_4 levels.

The mechanism by which the photoperiod influences reproductive function has been investigated in various studies. Higher concentrations of P_4 in the anterior hypothalamus and cerebrospinal fluid in long-day-exposed ewes than in short-day-exposed ewes indicate a greater affinity of P_4 to the CNS (Barker-Gibb and Clarke, 2000; Thiéry et al., 2003). The high levels of serum P_4 at time 0 in this study may be related to the pattern of luteinizing hormone (LH) secretion during the non-breeding season. Fluctuations in kisspeptin (Goodman et al., 2012) and neuropeptide Y (Barker-Gibb and Clarke, 2000), or the modulatory effects of estradiol on GnRH neurons (Thiery et al., 2006), occur during the anestrus season, potentially contributing to LH secretion.

In line with the results of the current study, Ayaseh et al. (2021) reported high levels of basal plasma P_4 in ewes after $PGF_{2\alpha}$ injection in non-breeding ewes. The mechanism behind higher levels of serum P4 during the non-breeding season may be attributed to increased levels of pituitary LH content in ewes (Chamley et al., 1976) and a greater amplitude of LH pulses in ovariectomized ewes during the non-breeding season (Barker-Gibb and Clarke, 2000). PRG induced a short-lived peak in serum P_4 for 3-12 h after IM injection, which subsequently returned to baseline levels of T0 after 24 h. It was assumed that the PRG normalized the values of serum P_4 concentrations at 48, 72, 96, and 120 h compared to time 0. This finding shows that the intervention of PRG in the serum P_4 profile and the establishment of its concentrations can be considered in estrus synchronization protocols during the non-breeding season.

CONCLUSION

In conclusion, significant variations and high concentrations were detected in the basal levels of serum

P_4 during the non-breeding season in ewes. A single injection of 50 mg of PRG elevated serum P_4 levels for 12 h and returned to basal levels within 24 hours. The product intended for human use released lower levels of P4 than the product intended for animal use during the experiment.

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