The effect of hCG administration on reproductive performance in undernourished lactating hair goats synchronized during non-breeding season

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Abstract

This study aimed to assess the effects of short and long synchronization protocols, combined with post-synchronization human chorionic gonadotropin (hCG) administration, on the reproductive performance of lactating hair goats during the non-breeding season, considering their inadequate pasture conditions. A total of 60 goats were randomly divided into four groups (G1, G2, G3, G4). Progesterone (flugeston acetate)-impregnated intravaginal sponges with were used for 5 days in G1 and G2 and 12 days in G3 and G4. All received a 500 IU pregnant mare's serum gonadotropin (PMSG) injection 48 h before sponge removal and were exposed to bucks 12 h later. Groups G2 and G4 received a 500 IU hCG injection on the eighth day after mating. Groups G1 and G3 did not receive any application after mating. Blood samples were collected on the 8th, 15th, and 22nd days for post-mating progesterone analysis, and pregnancy examinations were performed on the 35th day. The study showed a 90% total oestrus rate (54/60). However, there were no significant differences in conception, pregnancy, and kidding rates among the groups. Serum progesterone concentrations significantly increased on the 15th day in G2 and G4, where hCG was administered. In summary, hCG raised progesterone levels but did not significantly affect the reproductive performance of undernourished, lactating goats in a non-breeding season, suggesting that environmental factors and animal nutrition play a crucial role in synchronization outcomes.

Caprine, lactation, pregnancy, reproduction, synchronization

The seasonal nature of reproduction in small ruminants limits annual production. Reproductive management of small ruminants is essential for increasing production. Synchronization processes are utilized to control the reproductive activities of small ruminants, improving reproductive management and increasing productivity (Dogan et al. 2023). For this purpose, intravaginal devices containing progesterone or synthetic analogues, such as medroxyprogesterone acetate or fluorogestone acetate, are generally the most commonly used exogenous hormones for synchronizing oestrus and ovulation in goats (Gonzalez-Bulnes et al. 2020). In small ruminants, these intravaginal devices containing progesterone are used for either short periods (5–7 days) or long periods (12–14 days) to stimulate or synchronize oestrus (Martinez-Ros et al. 2019; Turgut and Koca 2024). Additionally, equine chorionic gonadotropin (eCG) is often applied in conjunction with intravaginal devices containing progesterone to enhance both the oestrus response and the ovulation rate outside the breeding season (Kuru et al. 2018).

To ensure a successful pregnancy, it is essential that a harmonious relationship exists between the embryo, the ovaries, the fallopian tubes, and the uterus. If this relationship is problematic, it can lead to embryonic losses (Montes-Quiroza et al. 2018). Progesterone hormone is absolutely necessary for the formation and maintenance

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of pregnancy (Rodrigues et al. 2022). Elevated progesterone concentrations following fertilization have a positive impact on both embryo development and the secretion of interferon-τ (IFN-τ), ultimately enhancing embryonic survival due to the favourable interaction between the embryo and the uterus (Spencer 2013; Arosh et al. 2016). One of the primary causes of embryonic loss is insufficient luteal function (Mann et al. 2006; Montes-Quiroza et al. 2018). To ensure a successful pregnancy, continuous progesterone secretion by the corpus luteum (CL) is essential (Diskin and Morris 2008). Using luteotropic hormones like human chorionic gonadotropin (hCG) and gonadotropin-releasing hormone (GnRH) has been reported to enhance pregnancy rates by supporting the corpus luteum and elevating P4 concentrations (Fonseca et al. 2018; Côrtes et al. 2021). Research studies have demonstrated the efficacy of hCG application in promoting CL formation and enhancing P4 (progesterone) concentrations (Fernandez et al. 2018; Fonseca et al. 2018).

Nutrition plays a crucial role in both the synthesis and release of gonadotropic hormones, and nutritional issues can lead to disruptions in hormone metabolism (Koşal et al. 2021; Fernández-Foren et al. 2023). Malnutrition can lead to reduced pulsatile luteinizing hormone (LH) release, resulting in decreased progesterone concentrations (Ali et al. 2019). Decrease in progesterone levels may also lead to increased embryonic mortality (Rodrigues et al. 2022). This study investigated the impact of hCG administration on the fertility of lactating hair goats under different synchronization protocols during the non-breeding season, while they were exclusively fed on pasture.

Materials and Methods

Ethical statement

This study was carried out with the approval of the Van Yuzuncu Yil University Animal Experiments Local Ethics Committee, as indicated by Decision Number 2021/07-05 on 29/07/2021.

Location, animals and feeds

The study included 60 hair goats which were bred in a private enterprise located in the rural regions of Van, a city located in the eastern part of Türkiye, on the border with Iran. These goats were selected during the non-breeding season, were aged between 2 and 5 years, had previously given birth, were in good health, and were currently in a lactating state, being milked once a day. The study utilized a total of eight buck goats, all of which were confirmed to be in good health and known to be fertile. The animals received two daily feedings, in the morning and evening, through grazing on the pasture. They had *ad libitum* access to water while they were in the pen.

Study protocols

The animals used in the study were divided into four groups. An intravaginal sponge (20 mg of flugestone acetate, Chronogest®, France) was simultaneously inserted into the animals in all the groups (Plate XI, Fig. 1). On the third day, animals in both group 1 (G1, n = 15) and group 2 (G2, n = 15) received an intramuscular injection of 500 IU of pregnant mare's serum gonadotropin (PMSG) (Chrono-Gest/PMSG®, MSD Animal Health, Unterschleissheim, Germany). The intravaginal sponges were removed from the animals in both groups (G1 and G2) on the 5th day, and they were subsequently exposed to the buck 12 h later. In group 3 (G3, n = 15) and group 4 (G4, n = 15), animals received a 500 IU intramuscular PMSG injection on the 10th day. The intravaginal sponges were then removed on the 12th day, followed by exposure to the buck 12 h later. The mating of animals in all groups was identified and recorded, and goats in oestrus were housed with bucks for up to 24 h. On the 8th day after mating, mated animals in both G2 and G4 groups were administered 500 IU hCG (Chorulon®, MSD Animal Health). Mating animals in G1 and G3 groups did not receive any additional applications. Blood samples were collected from all mated animals on the 8th, 15th, and 22nd days for post-mating blood progesterone analysis. The blood samples collected during the procedure were centrifuged at 1,500 *g* for 10 min, and the resulting serums were stored at −20 °C until measurements were conducted. Serum P4 concentrations were determined using the automated Elecsys® Immunoanalyser method (Roche Diagnostics, Mannheim, Germany) in combination with commercial kits. Pregnancy examinations of the animals were conducted through transrectal ultrasonography (utilizing a 7.5 MHz Linear Probe, Honda HS 1500, Japan) on the 35th day after mating. This approach was employed to ascertain the pregnancy status of the goats within the entire study group.

Throughout the study, the nutrition of animals that were grazing under natural conditions was closely monitored. It is important to note that the study took place during a year when the Van region experienced a severe drought

(Demir and Şen 2021). Unfortunately, due to the scarcity of supplementary rations beyond pasture feeding and milking, it was impossible to provide intervention for animals found to be undernourished. Consequently, the study was designed to consider nutritional deficiencies, and the results were evaluated accordingly.

The oestrus rate, conception rate, pregnancy rate and kidding rate in the groups were calculated using the following formulas:

Oestrus rate (%) = (number of goats showing oestrus / number of synchronized goats) × 100

Conception rate (%) = (number of goats getting pregnant / number of goats showing oestrus and mating) × 100

Pregnancy rate (%) = (number of goats pregnant / number of goats synchronized) × 100

Kidding rate (%) = (number of goats giving birth / number of goats getting pregnant) × 100

Statistical analysis

When calculating the sample size, we aimed in our study for a minimum power (power of the test) of 80% for each variable, while maintaining a Type 1 error rate of 5%. The Shapiro-Wilk (n < 50) and Skewness-Kurtosis tests were employed to assess the normality of the distribution of continuous measurements in the study. Since the measurements were found to be normally distributed, parametric tests were utilized. Descriptive statistics for the continuous variables in the study included the mean and standard deviation. Categorical variables were presented as the number (n) and percentage (%). One-Way Analysis of Variance (ANOVA) was conducted to compare measurements based on categorical groupings. Subsequent to ANOVA, Duncan *post hoc* multiple comparison test was employed to ascertain the differences between the groups. Repeated Measures ANOVA was used to compare the indices according to time, followed by Bonferroni *post hoc* multiple comparison test to determine the times that created the difference. One-sample Chi-square test was employed to compare the incidence rates among the groups. In the calculations, the statistical significance level (α) was set at 5%, and the analysis was performed using the SPSS (IBM SPSS for Windows, version 26) statistical software package.

Results

It was observed that none of the intravaginally placed sponges were expelled in any of the goats. No instances of abortion, premature birth, or stillbirth were noted throughout the study. The oestrus rate, conception rate, pregnancy rate, and kidding rate for the synchronization protocol conducted in the non-breeding season are provided in Table 1.

In our study, the overall oestrus rate was established at 90% (54/60). According to the results obtained through transrectal ultrasonography examination on the 35th day after mating, no significant differences ($P > 0.05$) were observed with regard to conception rate, pregnancy rate, and kidding rate among the G1, G2, G3, and G4 groups (Table 1). Conception/pregnancy/kidding rates were markedly higher in G2 and G4 groups compared to G1 and G3 but differences were non-significant probably due to low numbers of animals in the groups.

Table 1. The oestrus rate, conception rate, pregnancy rate, and kidding rate in the study groups G1, G2, G3, and G4.

Indicator	G1	G2	G3	G4	Total	*P* value
Oestrus rate % (n)	100 (15/15)	80 (12/15)	86.7 (13/15)	93.3 (14/15)	90 (54/60)	> 0.05
Conception rate % (n)	6.7 (1/15)	25 (3/12)	7.7 (1/13)	14.3 (2/14)	13 (7/54)	
Pregnancy rate % (n)	6.7 (1/15)	20 (3/15)	6.7 (1/15)	13.3 (2/15)	11.7 (7/60)	
Kidding rate % (n)	0 (0/1)	66.67 (2/3)	0 (0/1)	50 (1/2)	42.9 (3/7)	

G1: 5-d progesterone-impregnated sponge + pregnant mare's serum gonadotropin (n = 15); G2: 5-d progesterone-impregnated sponge + pregnant mare's serum gonadotropin + human chorionic gonadotropin (n = 15); G3: 12-d progesterone-impregnated sponge + pregnant mare's serum gonadotropin (n = 15); G4: 12-d progesterone-impregnated sponge + pregnant mare's serum gonadotropin + human chorionic gonadotropin (n = 15)

For the analysis of progesterone concentrations, oestrous detected and mated animals were included. As a result, Table 2 displays serum progesterone concentrations on the 8th, 15th, and 22nd days following mating.

Serum progesterone concentrations exhibited a significant increase on the 15th day in the G2 and G4 groups where hCG was administered ($P < 0.05$). Conversely, for all groups, serum progesterone values on the 22nd day were significantly lower compared to other time points ($P < 0.05$) (Table 2).

Table 2. Serum progesterone concentrations (mean ± standard deviation) in the study groups G1, G2, G3, and G4.

Days after mating	G1 (n = 15)	G2 (n = 12)	G3 (n = 13)	G4 (n = 14)
8 days	7.54 ± 3.79[a]	8.44 ± 4.19[b]	7.92 ± 4.30[a]	5.54 ± 3.10[b]
15 days	8.35 ± 3.53[a]	12.57 ± 6.74[a]	8.72 ± 3.49[a]	12.14 ± 5.81[a]
22 days	4.34 ± 4.88[b]	5.19 ± 4.32[c]	2.02 ± 1.72[b]	5.13 ± 4.94[b]

Values with different superscripts in columns within the same group are significantly different ($P < 0.05$).

G1: 5-d progesterone-impregnated sponge + pregnant mare's serum gonadotropin; G2: 5-d progesterone-impregnated sponge + pregnant mare's serum gonadotropin + human chorionic gonadotropin; G3: 12-d progesterone-impregnated sponge + pregnant mare's serum gonadotropin; G4: 12-d progesterone-impregnated sponge + pregnant mare's serum gonadotropin + human chorionic gonadotropin

Discussion

In this study, we explored the impact of hCG injection on short- and long-term synchronization in hair goats during the non-breeding season. As the animals used in this research were owned by a private enterprise and were not subject to our intervention, it is important to note that the results obtained during the study period were influenced by malnutrition. In this regard, after the synchronization process, no significant differences were observed between the groups in terms of the oestrus rate, conception rate, pregnancy rate, and kidding rate ($P > 0.05$). Furthermore, a significant increase in blood progesterone concentrations was observed following the hCG injection in both the short and long-term groups ($P < 0.05$). Nevertheless, it is noteworthy that this increase in blood progesterone did not confer any advantages in terms of conception rate, pregnancy rate, or kidding rate.

Variations in oestrus rates were observed in oestrus synchronization procedures conducted in the non-breeding season among small ruminants. In their study, İbiş and Ağaoğlu (2022) observed that 100% of the Saanen goats displayed oestrus during the synchronization procedure, which involved intravaginal progesterone application for 11 days and was conducted in the non-breeding season. Similarly, in the studies conducted by Kılboz and Karaca (2010) involving oestrus synchronization with intravaginal sponge application in young goats in the non-breeding season, they achieved a 100% oestrus rate. In their research, Baril et al. (1992) reported an 80.7% oestrus rate. According to Wildeus et al. (2003), when intravaginal sponges were used for oestrus synchronization in older goats over an 8-day period, only 50% of the goats displayed oestrus. In our study, the overall oestrus rate obtained was 90% (54/60). This result was found to be consistent with findings in the existing literature.

Sarıbay et al. (2008) achieved a conception rate of 33.3% following synchronization with intravaginal progestagen in lactating hair goats conducted in the non-breeding season. In our study, the overall conception rate was calculated to be 13% (7/54). The 13% rate we observed was lower than that reported by Sarıbay et al. (2008). Kılboz and Karaca (2010) reported a 50% pregnancy rate in the short-term trial group and a 5% pregnancy rate in the long-term trial group in their oestrus synchronization studies involving young goats and intravaginal sponge application conducted the non-breeding season. They hypothesized that the low pregnancy rate in the long-term sponge-applied group might be attributed to the detrimental effects of extended exposure to progesterone on follicular development in the animals. In a previous study of long-term (12 days) intravaginal progestagen applications in sheep by Viñoles et al. (2001), a deceleration in follicular development in the later stages of the application was observed, leading to an extended process of ovulatory follicle development. According to Viñoles et al. (1999), this condition results in an increased frequency of LH waves but leads to the formation of a consistently large follicle, as the LH peak fails to occur. In our study, the overall pregnancy rate was determined to be 11.7% (7/60). In our short-term application group, the pregnancy rate, including the hCG applied group, was observed to be 13.4% (4/30), whereas in our long-term application group it was 10% (3/30), also including the hCG applied group.

In order to mitigate early pregnancy losses in small ruminants, the administration of luteotropic hormones during either the early or late luteal phase represents a strategy to elevate progesterone concentrations (Rodrigues et al. 2022). Diverse outcomes have been observed in studies investigating the effects of administering GnRH or hCG, aimed at supporting the luteal structure and increasing progesterone concentrations, following mating or artificial insemination, with the goal of enhancing pregnancy outcomes. There is variability in the findings of different studies. Some studies, such as those by Fukui et al. (2001), Fernandez et al. (2019), and Ozmen et al. (2022) report that hCG application after mating has no significant effect on the pregnancy rate. On the other hand, some studies including Moeini et al. (2009), Mirzaei et al. (2014), and Rodrigues et al. (2022), have reported a 10–20% increase in the pregnancy rate when hCG or GnRH treatment is administered. According to Catalano et al. (2015), the application of hCG following mating results in an increase in plasma progesterone concentration. However, their study did not find a significant change in the pregnancy rate. Similarly, Ibiş and Ağaoğlu (2022) observed that progesterone concentrations increased after buserelin injection on the 12th day following mating, yet no significant difference was noted in the pregnancy rate. In our study, a substantial increase ($P < 0.05$) in blood progesterone concentrations was noted on the 7th day (15th day after mating) following hCG injection administered on the 8th day after mating. Nevertheless, it was observed that this difference had dissipated in the measurement results taken on the 22nd day.

When considering all of these results collectively, the disparities in outcomes could be attributed to variations in the protocols employed, administration methods, nutritional status, or physiological conditions arising from distinct experimental circumstances. We believe that the outcomes we observed were influenced by factors including the documented drought in our study area (Demir and Sen 2021), the concurrent lactation status of the animals, and the absence of supplementary feed beyond pasture. When a balanced and adequate diet is not available to meet the daily needs of animals, negative energy balance (NEB) occurs. Plasma insulin-like growth factor-1 (IGF-I) levels have been shown to decrease due to NEB. IGF-I exerts hormonal and autocoidal effects on metabolic activity and ovarian functions (Taylor et al. 2004; Velazquez et al. 2008). It is primarily produced by the liver in response to growth hormone (GH) (Lucy 2001; Taylor et al. 2004; Velazquez et al. 2008). Additionally, it is produced by the granulosa cells of the follicle (Pushpakumara et al. 2002) and the CL (Perks et al. 1999; Wathes et al 2003). IGF-1 stimulates glucose uptake and utilization in fat and muscle tissues, leading to reduced blood glucose concentration systemically (Perez-Martin et al. 2003). Following birth, IGF-1 production rapidly decreases due to GH receptor inhibition in the liver (Radcliff et al. 2003). The reduced blood IGF-1 levels disrupt the negative feedback on GH secretion, resulting in elevated blood GH concentrations (Veldhuis et al. 2001). Elevated GH concentration stimulates gluconeogenesis in the liver and accelerates the lipolysis of stored fats in the body. This, in turn, leads to an increased release of non-esterified fatty acids (NEFA). Elevated GH and NEFA concentrations lead to insulin resistance in animals. Reduced circulating insulin, IGF-1, and glucose concentrations inhibit the pulsatile release of LH by restricting active primary follicle production (Lucy 2007). Positive correlations have been observed between energy balance, IGF-1 concentration, and progesterone concentrations, indicating inadequate luteinization and premature luteolysis (Spicer et al. 1990). This effect has been linked to reduced fertilization rates and embryonic losses (Santos et al. 2016). When nutritional factors lead to low IGF-1 concentrations, animals tend to lose condition, directly affecting ovarian function. Malnutrition and deconditioning during lactation can reduce embryonic survival by impacting luteal activity (Snijders et al. 2000; Lonergan 2011). In our study, the trajectory of blood progesterone concentrations following synchronization and the findings from our pregnancy examinations were linked to the NEB we suspect developed in the animals.

In conclusion, oestrus in goats in the non-breeding season can be effectively induced with short and long-term intravaginal sponge applications. Furthermore, post-mating hCG application raises blood progesterone concentrations. However, the effect of increasing progesterone concentration on reproduction needs to be studied on a larger number of experimental animals. Generally, the low pregnancy rate in this study shows the dependence of reproductive performance on environmental conditions and nutritional status.

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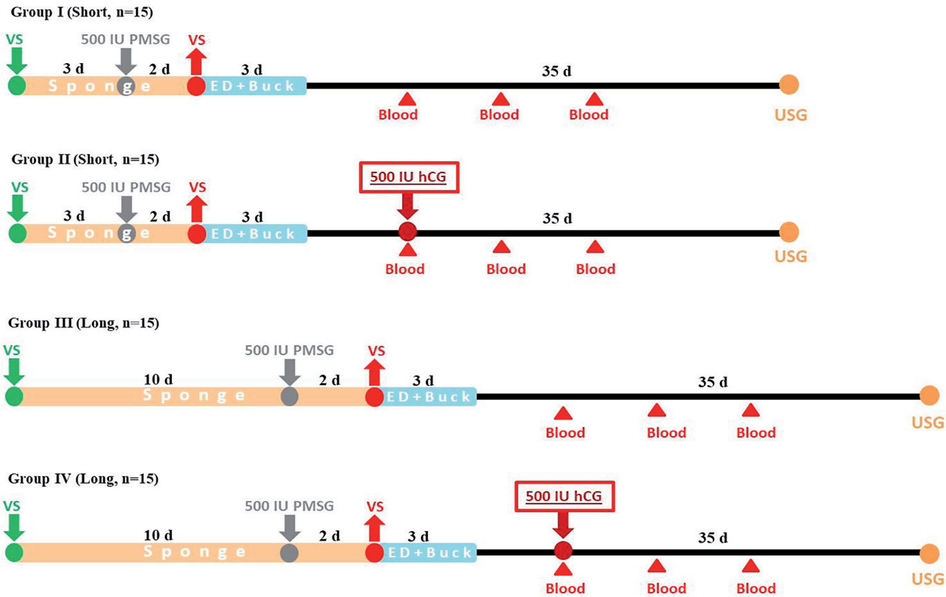

Fig 1. Experimental design of the groups

VS: vaginal sponge; PMSG: pregnant mare's serum gonadotropin; ED: oestrus detection; hCG: human chorionic gonadotropin; USG: ultrasonography