https://doi.org/10.52973/rcfcv-e33275 Revista Científica, FCV-LUZ / Vol. XXXIII, rcfcv-e33275, 1 – 7

Determination of the efficacy of human Chorionic Gonadotropin (hCG) administrations on reproductive performance, placentation, parturition, and neonatal parameters on different post–mating days in Kangal ewes sexually induced during anestrus

Determinación de la eficacia de la administración de Gonadotropina Corionica humana (hCG) sobre el desempeño reproductivo, la placentación, el parto y los parámetros neonatales en diferentes días posteriores al apareamiento en ovejas Kangal inducidas sexualmente durante el anestro

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ABSTRACT

This study aimed to determine the efficacy of post mating human Chorionic Gonadotropin (hCG) during anestrus on the formation of the accessory corpus luteum and some reproductive parameters. For this purpose. after synchronization of all the animal were divided into group 1 (n=100), group 2 (n=100), and group 3 (n=100) by applying 600 IU of hCG 6 d after sponge removal, 600 IU of hCG 8 d after sponge removal, and no hCG application (Control), respectively. The difference between groups in terms of reproductive parameters such as estrus, pregnancy, multiple pregnancy, litter size, and productivity was not statistically significant. The live birth weight of lambs was evaluated for singletons, twins, and triplets. The difference between group 1 and the control group was statistically significant in singleton lambs (P=0.04). The difference between group 1 and control (P<0.001) and between group 2 and control (P<0.001) was statistically significant for twins. In triplets, group 1 was different from both groups (P<0.001) and group 2 was different from the control group (P<0.001). In addition, when the placenta weight and the daily body weight gain of singleton lamb in the neonatal stage were examined, the values of both groups that were administered with post mating hCG were higher than the control group (P<0.001). The Progesterone (P_4) level in blood samples taken on the 21st d of pregnancy was found to be different between all groups. Furthermore, P_4 levels were found to be higher in group 1 compared to the other two groups (P<0.001). In the light of these findings, it was determined that hCG administration after mating contributed to placenta and offspring development by elevating P_4 levels. It was concluded that hCG should be administered 6 d after the sponge will be removed (on d 5 postmating) for optimal efficacy.

Key words: Anestrus; postmating; Kangal; sheep; progesterone; reproduction

RESUMEN

Este estudio tuvo como objetivo determinar la eficacia de los tratamientos con gonadotropina coriónica humana (hCG) posemparejamiento durante el anestro sobre la formación del cuerpo lúteo accesorio y algunos parámetros reproductivos. Para ello, después de la sincronización de todos los animales se dividieron en grupo 1 (n=100), grupo 2 (n=100) y grupo 3 (n=100) mediante la aplicación de 600 UI de hCG 6 d después de la eliminación de la esponja, 600 UI de hCG 8 d después de retirar la esponja y sin aplicación de hCG (Control), respectivamente. La diferencia entre grupos en términos de parámetros reproductivos como celo, preñez, preñez múltiple, tamaño de la camada y productividad no fue estadísticamente significativa. Se evaluó el peso vivo al nacer de los corderos para los únicos, mellizos y trillizos. La diferencia entre el grupo 1 y el grupo control fue estadísticamente significativa en los corderos únicos (P=0,04). La diferencia entre el grupo 1 y el control (P<0,001) y entre el grupo 2 y el control (P<0,001) fue estadísticamente significativa para los gemelos. En los trillizos, el grupo 1 fue diferente de ambos grupos (P<0,001) y el grupo 2 fue diferente del grupo control (P<0,001). Además, cuando se examinaron el peso de la placenta y la ganancia diaria de peso corporal de corderos únicos en la etapa neonatal, los valores de ambos grupos que recibieron hCG posemparejamiento fueron más altos que el grupo control (P<0.001). Se encontró que el nivel de progesterona (P_4) en las muestras de sangre tomadas el d 21 the preñez era diferente entre todos los grupos. Además, se encontró que los niveles de P_4 eran más altos en el grupo 1 en comparación con los otros dos grupos (P<0,001). A la luz de estos hallazgos, se determinó que la administración de hCG después del apareamiento contribuía al desarrollo de la placenta y la descendencia al elevar los niveles de progesterona. Se concluyó que la hCG debe administrarse 6 d después de retirar la esponja (el d 5 después del apareamiento) para una eficacia óptima.

Palabras clave: Anestro; apareamientos; Kangal; ovejas; progesterona; reproducción

Received: 30/05/2023 Accepted: 26/07/2023 Published: 08/08/2023

INTRODUCTION

Nowadays, embryonic loss is a major obstacle to livestock reproductive efficiency. In the 3-week period following fertile matings, sheep (*Ovis aries*) and goats (*Capra hircus*) experience 30–40% embryonic loss, and 70–80% of these preimplantation losses occur between 8–16 d following mating [1]. The primary cause of this condition is insufficient luteal function, which requires Progesterone (P_4) supplements early in pregnancy to prevent it. The increase in P_4 levels improves pregnancy rates and contributes to fetal development [1, 2, 3, 4, 5]. In addition to these factors, the seasonal dependence of sheep reproduction limits reproductive effectiveness. The investigation of the reproductive physiology of sheep has revealed that follicular dynamics persist during anestrus. Accordingly, interventions on ovulation can induce fertile estrus and ovulation at any time of the year, resulting in pregnancies [6]. However, during anestrus stimulation, luteal P_4 secretion is suppressed because ovulation and total luteal volume are lower compared to the breeding season. This reduction is further compounded by a decline in Gonadotropin support. Therefore, there is a greater need for direct P_4 supplements or interventions to increase Progesterone secretion during sexual stimulation in anestrus [6].

The exogenous administration of Gonadotropin-releasing hormone (GnRH) and human Chorionic Gonadotrophin (hCG) to sheep and cattle (*Bos taurus*) have the same goal. The purpose of using GnRH or hCG is to induce ovulation in the dominant follicle [7, 8, 9]. In sheep synchronizations, hCG applications are usually performed after the termination of P_4 applications. It is known that GnRH and hCG administered during this period do not cause a significant increase in reproductive efficiency [10, 11]. However, when this hormonal treatment is delayed until after mating, it can stimulate the formation of the accessory corpus luteum (CL) by inducing ovulation in the dominant follicle of the first wave of the following cycle. Therefore, this favorable situation results in increased plasma P_4 concentration. Increased P_4 levels during the maternal acceptance phase of pregnancy improve reproductive efficiency [5, 8, 12]. Post mating hCG administration increases P_4 levels, which significantly strengthens placentation during embryonic and fetal stages [13].

In the light of this information, the aim of the present study was to improve the function of the CL in the current pregnancy by increasing the accessory CL or luteinizing effect by ovulating the dominant follicle of the first follicle wave that develops after mating. On different postmating d, the efficacy of hCG administration on reproductive performance, placentation, and various parturition and neonatal stage parameters was determined.

MATERIAL AND METHODS

Location

The study was carried out at a sheep farm in Ortaklar Village, Yıldızeli District, Sivas Province, Turkey with coordinates 39°50'1" | 36°20'49", and an altitude of 1,290 meter above sea level. Its pasture is located in a geography dominated by steppe between high mountains.

Animals and treatment schedule

Before starting the study, 400 ewes and 40 rams that met the inclusion criteria were screened for general health. From these animals, 300 ewes and 30 rams of similar age and condition were selected for the study.

This study was carried out with 300 ewes (3–5 years old) that were conceived in the fall, gave birth in the spring, and nursed their lambs for approximately 50–70 d during the early anestrus period (April). At the beginning of the application, the average body weight of the animals was 44 ± 5 kg and the body condition score (BCS) were in the range of 2.5–3.25. Simultaneously, since the study was carried out during anestrus, 30 Kangal rams aged 4–6 years with proven fertility, weighing (Pinar, PR-110, Türkiye) 102 ± 8 kg and having a BCS of 3–5 were used to perform mating during sexual stimulation.

When the records of the establishment were examined, it was determined that although the sheep were housed with the rams during the anestrus period in previous years, no pregnancy occurred during this period. Therefore, P_4 levels were measured in blood samples collected prior to application. Examining the P_4 levels revealed that neither the study nor the control groups found any sheep with P_4 values at luteal and above (≥1 ng·mL^{-1}), and the P_4 values of animals in all groups were at sub-basal levels (<1 ng·mL^{-1}).

Animals were divided into 3 groups, each containing 100 sheep. On d 0, a vaginal sponge containing P_4 hormone (a white, 40 × 30 mm cylindrical polyurethane sponge containing 20 mg Chronolone flugestone acetate; Chronogest® CR, MSD, Turkey) was inserted in all groups. Seven d after this application (d 7), the inserted vaginal sponges were removed and $PGF_2\alpha$ hormone (263 µg Cloprostenol sodium equivalent to 250 µg Cloprostenol per ml; PGS®, Alke, Turkey) was applied. While the vaginal sponge was removed and $PGF_2\alpha$ hormone was administered, 480 IU of equine chorionic gonadotrophin (each mL of solution for injection contains 240 IU of Gonadotropin hormone; Chronogest/PMSG®, MSD, Turkey) was injected simultaneously. Rams were introduced one d later (d 8), and ewes were kept with rams for 5 d (until d 13). Mating animals were considered to be in estrus. Matings were done by natural insemination. Mated animals were considered estrus positive. 600 IU of hCG hormone (containing hCG at a concentration of 300 IU per mL when lyophilized solution powder was mixed with solvent; Chorulon® CR, MSD, Turkey) was administered to animals in group 1 on d 13 (6 d after removal of vaginal sponges) and to animals in group 2 on d 15 (8 d after removal of vaginal sponges), as for group 3 (control) sheep received no treatment. In all groups, animals showing estrus mated at 31 ± 3.2 h after $PGF_2\alpha$ hormone. There was no statistical difference between the groups.

Pregnancy examination

Two pregnancy examinations were performed: the first using the transrectal ultrasonographic method (Mindray DP50/Vet/US, Türkiye) 22 d after the introduction of rams (d 30) and the second by using the transabdominal ultrasonographic (Mindray DP50/Vet/US, Türkiye) method on d 68. All Pregnancy examinations were performed rectally in the supine position with a B-mode, linear array 5.0–7.5 MHz rectal probe ultrasonography device (Mindray DP50/Vet/US, Türkiye) to determine early pregnancies and litter counts, or transabdominally to determine embryonic and fetal losses that may occur in the following d of pregnancy. For transabdominal examination, the hairless area just above the breast, ventral to the right fasting pit, was preferred for probe insertion. The dorso-caudal aspect of the breast was scanned completely according to whether pregnancy-related findings could be obtained in this area. According to the stage of pregnancy detected in the ultrasonic examination, after the detection of the gestational

sac, it was decided that the animal was pregnant with the detection of the embryo or fetus, offspring membranes, fluids, heartbeat, and placentomes. The establishment was visited at certain periods (monthly) to follow up on the pregnancies. One week before the probable birth date, daily visits were made, and birth records were kept.

Housing and nutrition

Approximately two months before the start of the study, the rams were separated from the ewes in wooden pens in the same pen. However, this separation was not sufficient to block the pheromone. From the beginning of the application until the first pregnancy examination, the animals in all groups spent 6 h·d^{-1} in the newly awakened pasture and spent the rest of the time in the facility with a ration of 750 g meadow grass (*Poa annula* L.), 750 g wheat straw (*Triticum aestivum* L.), 500 g alfalfa grass (*Medicago sativa* L), and 250 g barley grits (*Hordeum vulgare* L.) per animal per d. After the first pregnancy examination, the animals spent the entire d grazing in the pasture.

After birth, the lambs were kept together with the mother in the paddocks, where only the pups could pass through and be fed *ad libitum* for a period of 1 week to 1 month. Water, dry alfalfa (*Medicago sativa* L), and commercial lamb starter feed were kept *ad libitum* in the section where only the lambs could enter and exit.

Collection and evaluation of blood samples

Samples for the first blood examination were taken from animals in all groups 1 week before the start of the study. The second blood samples were taken during the first pregnancy examination, 22 d after ram introduction (d 30). In order to determine the P_4 level, 10 mL of blood samples taken from the vena jugularis were kept at room temperature for half an h. The blood samples were then centrifuged in a refrigerated centrifuge (Nüve NF 800, Nüve Laboratory & Sterilization Technology, Türkiye) at 4,000 g/5min, and two 1 mL samples were taken into microcentrifuge tubes (Eppendorf, Hamburg, Germany) and stored at -80°C(Haier, DW-86L828S, China) until the time of measurement. P_4 measurement was made with chemiluminescence microparticle immunoassay [9] using the ARCHITECT Progesterone Chemiluminescence (7K77) Abbott test kit and a fully automated ARCHITECT -i2000SR instrument (Abbott Diagnostics, AbbottPark, USA) with an analytical sensitivity of ≤ 0.1 ng·mL^{-1} and a measuring range of 0.1–36.0 ng·mL^{-1}. The intra-assay coefficient of variation ranged between 3.4–5.5% and 1.6–2.2% for low- and high-level P_4 concentrations respectively. Analyses were validated for serum (in serum and blood collected in serum separator tubes) and plasma (with Na heparin, Li heparin, and K^{++}-Ethylene diamine tetra acetic acid (EDTA) anticoagulants) samples. Validation was not performed with anticoagulants other than those mentioned.

Estrus rate

Number of animals showing estrus in the group/total number of animals in the group

Pregnancy rate

Number of pregnant animals in the group/total number of animals in the group

Multiple pregnancy rate

Number of animals with multiple pregnancies in the group/total number of pregnant animals in the group

Embryonic–fetal mortality

Number of embryonic–fetal deaths in the group /total number of pregnant animals in the group

Number of births

Number of pregnant animals that completed pregnancy and gave birth

Number of offspring

Number of offspring born from the number of pregnant animals

Productivity

Total number of offspring/total number of pregnant animals

Lamb weight

Lamb weight was measured (Pinar, PR-110, Türkiye) half an h (30 in) after birth to allow for their mothers to dry them. Measurements were made at the end of this period.

Live weight at birth

Weight 30 min after birth (after complete drying)

Daily live weight gain in the neonatal stage

(Weight on d 30 – live weight at birth) / 30

Placenta weight

Weight of the placenta after cleansing of the fetal juices

Neonatal mortality

Number of lambs that died in the group between postnatal d 0–30/ total number of lambs born in the group

Difficult birth rate

Number of difficult births in the group /total number of births in the group

Statistical analysis

Data were analysed using SPSS version 26 (IBM Corp., Armonk, NY, USA). Reproductive parameters were analysed using the chi-square test. Lamb parameters and hormonal measurements were analyzed by one-way ANOVA and post-hoc Duncan's test, and the results were expressed as mean ± standard deviation (SD). Progesterone level determined all the sheep in the groups. But in statistic evaluation progesterone measurement belongs to non-pregnant sheep have been excluded. Statistical significance was set at $P<0.05$.

RESULTS AND DISCUSSION

After health screening, 300 healthy ewes with sub-basal P_4 levels were subjected to synchronization. P_4-impregnated sponges were kept in the vagina for 7 d and removed completely after 7 d. After synchronization, estrus was achieved in 79, 81, and 82 sheep in groups 1, 2, and 3, respectively. Two consecutive ultrasonographic examinations were performed to confirm pregnancy, the first on d 22 and the second on d 60 after mating. In the first pregnancy examination 73, 69, and 70

ewes were pregnant in groups 1, 2, 3, espectively. However, pregnancies detected at the first examination but not detected at the second examination were considered embryonic–fetal losses. There were 2 embryonic–fetal deaths in group 1, 2 in group 2, and 4 in group 3. There were 8, 5, and 4 triplet pregnancies and 32, 28, and 29 twin pregnancies in groups 1, 2, and 3, respectively. In order to record the births (litter size, mode of delivery, live birth weight, placenta weight, among others), we stayed in the facility during the birth period, and all births took place within 6 d. A total of 119, 104, and 100 lambs were born in 69, 67, and 69 births in groups 1, 2, and 3, respectively. Of these, 5 in group 1, 2 in group 2, and 3 in group 3 were considered difficult deliveries. At the 30–d neonatal stage, there were 5 neonatal losses in group 1, 4 in group 2, and 3 in group 3 (TABLE I).

TABLE I
Reproductive parameters, birth, and neonatal period data of the study

Parameters	Groups (n=100)		
	Group 1	Group 2	Group 3
Number of Animals in Estrus	79	81	82
Number of Pregnancy	73	69	70
Number of Twin Pregnancies	32	28	29
Number of Triplet Pregnancy	8	5	4
Number of Multiple Pregnancies	40	33	33
Number of Births	69	67	66
Embryonic–Fetal Mortality	2	2	4
Number of Lambs	119	104	101
Reproductivity	119/71	104/67	101/66
Difficult Birth Rate	5/71	2/67	3/66
Neonatal Mortality	5/119	4/104	3/101

Since the synchronization method was the same in all groups, estrus rates were similar in all groups (*P*=0.58) (TABLE II). The embryonic–fetal mortality rate was not significantly different between the groups (*P*=0.34), and there was no difference in neonatal mortality rates between the groups (*P*=0.62). Total pregnancy rates (*P*=0.8) and multiple pregnancy rates (*P*=0.24) were not different between the groups (TABLE III and TABLE IV respectively).

When P_4 levels were analyzed in blood samples taken during the first pregnancy examination on the 22nd d of pregnancy, a significant difference was found between all groups (*P*<0.001). As shown in FIG. 1, group 1 had higher P_4 levels compared to both group 2 and group 3, and group 2 had higher P_4 levels compared to group 3.

TABLE II
Estrus rates and statistical evaluation of the study groups

Estrus	Group 1	Group 2	Group 3	Total
Positive[1]	79[a] (79%)	81[a] (81%)	82[a] (82%)	242 (80.7%)
Negative[1]	21[a] (21%)	19[a] (19%)	18[a] (18%)	58 (19.3%)
Total	100	100	100	300

[1]: number of animals and (percentage), [a,b]: varied characters in the same row are statistically significantly different (*P*<0.05)

TABLE III
Pregnancy rates in the study groups and statistical evaluation of the results

Pregnancy	Group 1	Group 2	Group 3	Total
Positive[1]	73[a] (73%)	69[a] (69%)	70[a] (70%)	212 (70.7%)
Negative[1]	27[a] (27%)	31[a] (31%)	30[a] (30%)	58 (29.3%)
Total	100	100	100	300

[1]: number of animals and (percentage), [a,b]: varied characters in the same row are statistically significantly different (*P*<0.05)

TABLE IV
Twin, triplet, and multiple pregnancy rates and statistical evaluation

Multiple Pregnancy	Group 1	Group 2	Group 3	Total
Positive[1]	40[a] (73%)	33[a] (69%)	33[a] (70%)	106 (47.7%)
Negative[1]	33[a] (45.2%)	36[a] (52.2%)	47[a] (58.8%)	116 (52.3%)
Total	100	100	100	300

[1]: number of animals and (percentage), [a,b]: varied characters in the same row are statistically significantly different (*P*<0.05)

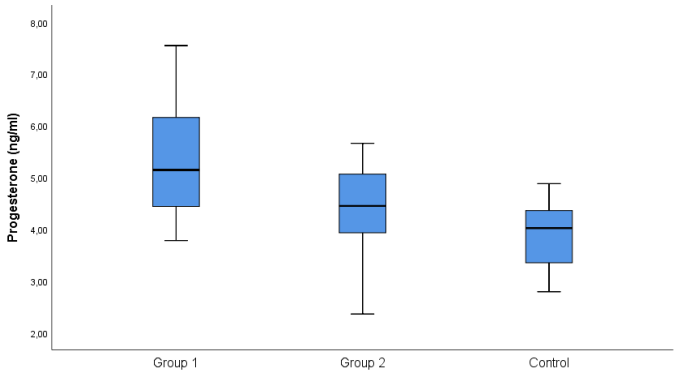

FIGURE 1. Progesterone level in blood samples taken on the 22nd day of pregnancy: Group 1 median 5.14 (3.78–7.55), group 2 median 4.45 (2.36–5.66), and control median 4.02 (2.79–4.88)

All lambs were weighed after birth. Live birth weights were evaluated separately for singletons, twins, and triplets. For singleton lambs, live birth weight was significantly higher in group 1 compared to group 3 (control) (*P*=0.04) (FIG.2).

When the live birth weight of twin lambs were compared, there was a significant difference between group 1 and group 3 (control) (*P*<0.001) and between group 2 and group 3 (control) (*P*<0.001) (FIG. 3).

When the live birth weights of triplet lambs were compared, a significant difference was found between all groups (*P*<0.001) (FIG. 4).

At the neonatal stage, daily body weight gains were compared between singleton lambs. When daily body weight gain was higher in group 1 compared to group 3 (*P*=0.003), there was no difference between group 1 and group 2. On the other hand, when daily body weight gain was higher in group 2 compared to group 3 (*P*=0.01), there was no difference between the groups for twin and triplet lambs (FIG. 5).

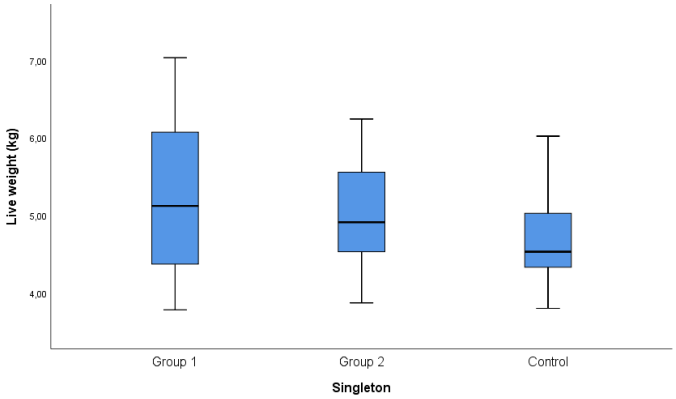

FIGURE 2. Live birth weight comparison of singleton lambs

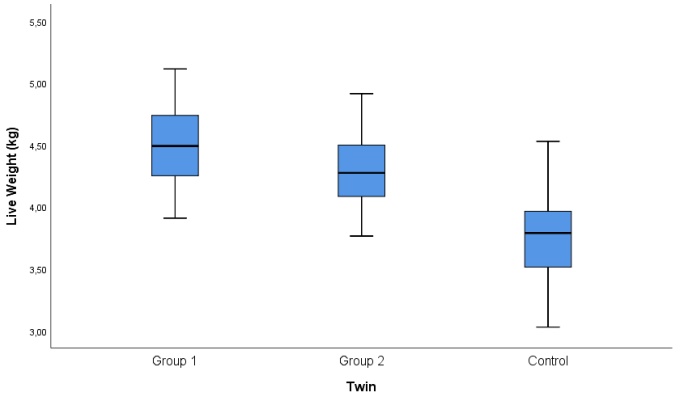

FIGURE 3. Live birth weight comparison of twin lambs: *P*<0.001 between group 1 and control, *P*<0.001 between group 2 and control; group 1 median 4.49 (3.91–5.12), group 2 median 4.27 (3.77–4.92), and control median 3.79 (3.03–4.53)

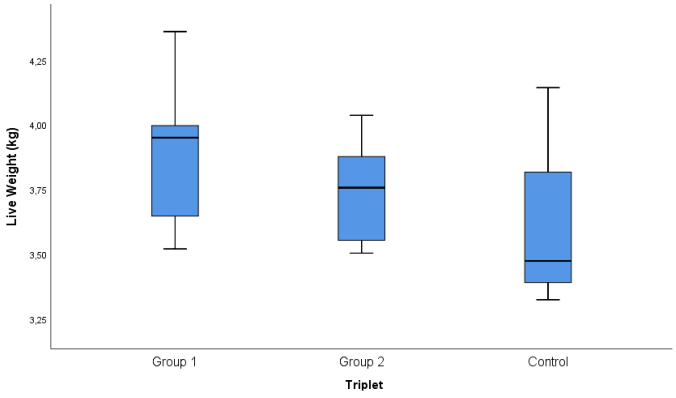

FIGURE 4. Live birth weight comparison of triplet lambs: *P*<0.001 between all groups; group 1 median 3.95 (3.52–4.36), group 2 median 3.75 (3.50–4.04), and control median 3.47 (3.32–4.14)

At the time of parturition, the placentas of singleton lambs that had been completely expelled were weighed and compared between groups. Only one ewe with a singleton birth demonstrated retention. According to this comparison, the placenta weights of both groups 1 and 2 were significantly higher than the placenta weight of the control group (*P*<0.001)(FIG. 6).

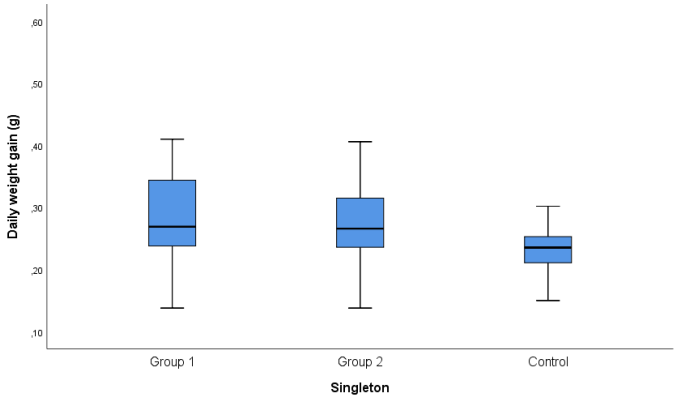

FIGURE 5. Comparison of daily body weight gain in the neonatal stage for singleton lambs: Group 1 median 0.26 (0.14–0.51), group 2 median 0.26 (0.14–0.41), and group 3 (control) median 0.23 (0.15–0.32)

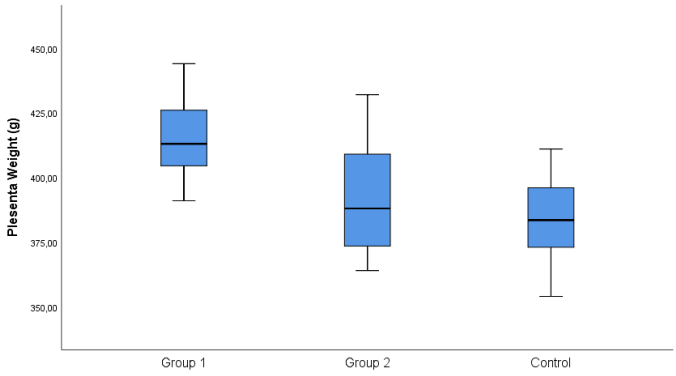

FIGURE 6. Comparison of placenta weights between the groups for singleton lambs

In the follicular wave dynamics in small ruminants, it is assumed that the follicle reaches dominance in the first and ovulatory (last wave before ovulation) waves. Interventions on follicles at these stages to increase Progesterone levels are considered successful [12]. It is known that increasing Progesterone levels improve maternal acceptance and placentation, and when this activity is established, live birth weight increases [14]. For this purpose, the aim of the present study was to create an accessory CL or increase the secretion volume by inducing luteotrophic activity on the existing CL by applying hCG on different d of the subsequent cycles of sexually stimulated and mated ewes.

It was determined that 250 IU of hCG administrated on postmating d 5 had no effect on Progesterone levels and also did not affect reproductive performance in goats [12]. However, there are studies indicating that hCG administered on the 7th d after mating contributes to the development of the accessory CL and increases P_4 levels [15]. In the present study, postmating hCG administration significantly increased P_4 levels in blood samples taken on the 22nd d of pregnancy. This luteotrophic activity was thought to occur through the creation of an accessory CL or by supporting an existing CL.

Similarly, in a study where hCG was administered on different d after mating (d 1, 7, and 12), hCG administration on d 7 increased P_4 levels and lambing rate compared to the control group [16]. It was thought that the success of administering hCG to the group on d 7

after mating was achieved by targeting the Graafian follicle of the first wave of follicular dynamics, as in the present study. In another study with similar results, hCG administered in the late phase of the cycle (d 12) after mating did not contribute to reproduction [17].

In addition to these, factors affecting live birth weight in the prenatal stage are also important for viability and development after birth [18, 19]. One of the factors is the level of P_4 in the material during pregnancy, which was found to be directly proportional to birth weight [20]. The results obtained in the present study confirm this view.

The body composition of ewes during mating and their nutritional status during gestation also have significant effects on live birth weight [19]. Accordingly, the ewes were evaluated before the current study was started and no difference was found in their condition. Any possible difference in nutrition was eliminated by giving equal rations to the groups. Therefore, the contribution to live birth weight and neonatal development was entirely attributed to increased P_4 levels and placental development during the gestation period.

P_4 level was increased with postmating hCG administration, and live birth weight also increased in direct proportion to P_4 level. Subsequently, the daily live weight of the lambs was monitored during the neonatal stage. Accordingly, it was found that the daily body weight gain of the lambs in the postmating hCG–administered groups was significantly higher than the control group in parallel with the increase in live birth weight.

Although the live birth weight increased in the treatment groups, there was no difference in the rate of difficult deliveries between the groups. It was determined that postmating short-term P_4 administration increased P_4 levels in early pregnancy but did not affect birth weight. This has been associated with the fact that the increase in P_4 is not ubiquitous throughout the pregnancy but is limited to only one period [21]. In the present study, postmating hCG administration increased live birth weight. This was attributed to an increase in P_4 that extended throughout the entire pregnancy.

In another study, it was determined that daily 25 mg P_4 administration starting from the 36th h after mating increased the nutrients in feto-placental fluids in the later stages of pregnancy [22]. The results obtained in the present study support this finding. The increase in live births and placenta weight was associated with increased P_4 levels, which improved fetal nutrition. In this respect, the results obtained in the present study are consistent with those of a previous study indicating that increased P_4 levels improve placentation and providing better fetal nutrition [13]. In a previous study, it was determined that hCG applied on post-mating d 11 and controlled intravaginal releasing device (CIDR) protocols applied between post-mating d 7–19 increased maternal P_4 level (in blood samples taken on d 12–17) and improved reproductive performance in sheep [23]. In the same study, post-mating hCG and CIDR treatments did not change the birth weights of singletons and quadruplets but did change the birth weight of twins. In their study, Rostami *et al.*, found that direct supplementation of P_4 after mating (by applying CIDR protocol) did not increase P_4 levels on d 22 of pregnancy, whereas post-mating hCG administration did [23]. It was believed that exogenous P_4 administration could have a negative impact on endogenous P_4 secretion [24]. Therefore, the authors aimed to increase endogenous Progesterone levels during the critical phase of pregnancy.

There are also studies suggesting that postmating hCG administration did not improve reproductive parameters [25, 26]. In the present study, postmating hCG administration increased P_4 levels and lamb numbers.

At the same time, it was determined that posmating hCG administration reduced lamb mortality after birth until weaning [23]. The results obtained in both studies are consistent in terms of P_4 value, but the neonatal mortality rate did not differ between the groups in the current study.

CONCLUSION

According to the findings of the present study, administration of hCG after mating enhances offspring development during pregnancy in ewes by increasing P_4 levels and placentation. Optimum efficiency can be achieved when hCG administrations are made approximately on the fifth d after mating. It was also found that postmating hCG administration increases live birth weight and contributes to offspring development in the neonatal stage.

ACKNOWLEDGMENT

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Statement of animal rights

This study was approved by the Animal Research Ethics Committee of Cumhuriyet University with the decision no. 361 on 11.11.2020.

Conflict of interest statement

The authors have no relevant financial or non-financial interests to disclose.

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