Research Article

Gonadotrophin stimulation of ewes that are not pregnant following multiple matings during the season

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Abstract: In this study, the aim was to obtain pregnancy following synchronization and to increase profitability in ewes that did not become pregnant during the breeding season after at least 3 matings. Nonpregnant Middle Anatolian Merino ewes (n = 117) were given a progesterone sponge for 9 days. At the time of sponge removal, the ewes received 250 IU pregnant mare serum gonadotropin (PMSG) + 125 μg prostaglandin F2α (PGF2α). Two days later, they were injected with either human chorionic gonadotropin (hCG) (Group I, n = 48) or gonadotropin-releasing hormone (GnRH) (Group II, n = 43), or remained as a control (Group III, n = 26). The ewes were allowed to mate at estrus following the PGF2α injection. Pregnancy diagnosis was performed on day 30 after mating by ultrasonography. Group ratios for estrus (58.3%, 55.8%, 53.8%), conception (78.6%, 87.5%, 14.3%), lambing (90.9%, 90.5%, 50.0%), multiple births (15.0%, 31.6%, 0.0%), and average lamb number for birth (1.15%, 1.31%, 1.0%) were reported. In conclusion, the gonadotropin-supplemented protocols used here were found to be useful in ewes that were not pregnant after multiple matings during the breeding season in increasing the profitability of flocks with this problem.

Key words: Breeding season, gonadotropin-releasing hormone, human chorionic gonadotropin, Merino

1. Introduction

Sheep breeding in Turkey is mostly done for producing meat from lamb production. Therefore, each ewe is important in this business; however, most sheep breeds, such as Merino and Akkaraman, are low prolific. These breeds usually have low ovulation rates but are very well adapted to the environmental conditions in Turkey. A low pregnancy rate in ewes has many causes such as a poor follicular pool, low ovulation rates (1,2), fertilization failures (3,4), and embryonic and fetal losses (4–6). As ovulation and fertilization failures may happen in 10% of ewes, major pregnancy losses occur during the first 3 weeks of pregnancy, which covers oviductal development, maternal recognition of pregnancy, and attachment of the embryo to the uterus (7–9).

One of the factors contributing to the problems above may be the inadequate development of a follicle, which leads to either ovulation failure or poor development of the corpus luteum (CL) and therefore impaired progesterone synthesis or bad quality embryos that fail to result in pregnancy. As a result, to alleviate these problems, animals are supplemented with exogenous hormones to ensure ovulation and better CL and embryo development. For this purpose, there are two commonly used hormones: gonadotropin-releasing hormone (GnRH) and human chorionic gonadotropin (hCG). These 2 hormones have previously been applied to ewes either at the time of mating/estrus or after insemination, such as on day 4, 7, or 12, to ensure ovulation and increased progesterone production via accessory CL development (10–13). hCG mimics the effect of the luteotropic luteinizing hormone (LH) (14) and induces ovulation of the follicle, which possesses the LH receptors. It could be used at the time of estrus or at diestrus. Therefore, it helps to ensure ovulation, development of a better CL, and increased progesterone production. Earlier studies have shown the beneficiary effects of hCG use on increased lambing rates when used either at mating or after insemination (10,12).

Similarly, GnRH is also beneficial in improving pregnancy rates with a similar aim as hCG. It has been used in ewes at the time of mating or later, after mating during diestrus (13,15–18). Instead of acting on LH receptors, GnRH directly stimulates the release of LH within hours after application (19). In Awassi ewes, GnRH induced multiple births when applied at the time of insemination (13). GnRH has also resulted in a decrease in early pregnancy loss when applied at mating or 7 days after mating in Rahmani ewes (16). GnRH demonstrates this effect through an increased ovulation ratio, better luteinization of the dominant follicle, and a better

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progesterone environment for the benefit of the embryo (20,21).

This study was designed to evaluate the fertility of ewes that had difficulties becoming pregnant after at least 3 fertile matings. The aim was to prevent these ewes from being culled. As the sheep population has constantly been decreasing and good quality ewes with specific characteristics of any breed, such as those in this study, are especially important, nonpregnant ewes must be given more chances to become pregnant and stay in the breeding stock. The aim was to ensure ovulation through the application of either GnRH or hCG in ewes and to compare the efficiency of these gonadotropins on these ewes to induce pregnancy and improve the lambing ratio.

Since the ewes used in this study did not become pregnant after at least 3 matings following natural estrus, we applied a common synchronization program based on progesterone and then applied a gonadotropin, either hCG or GnRH, at a fixed time in all ewes (i.e. 48 h after sponge removal). We then aimed to investigate the pregnancy outcome and the timing of gonadotropin injection relative to mating time and its effect on pregnancy.

2. Materials and methods

2.1. Animals and experimental procedure

Anatolian Merino ewes were housed in the Bahri Dağdaş International Agricultural Research Institute in Konya, Turkey. The present study is the result of scientific evaluations of the routine management procedures of this institute. The ewes were fed a standard diet (grazing in the pasture for 6 h per day and fed 0.5 kg of alfalfa, 0.2 kg of wheat stalk, and 0.5 kg of 14HP-2500ME concentrate) and water was given ad libitum. Ewes (n = 117), diagnosed nonpregnant in the last examination, were given an intravaginal progesterone sponge (Chronogest, 30 mg FGA, İntervet, İstanbul, Turkey) for 9 days and injected with PGF2α (125 µg, Dalmazin, 125 mcg d-cloprostenol, Vetas, Turkey) and PMSG (250 IU, Folligo, Intervet International, Boxmeer, the Netherlands) at the time of sponge removal (Figure). Then, 48 h later, the ewes were injected with either hCG 150 IU (Group I, n = 48; Chorulon, İntervet, İstanbul, Turkey) or GnRH (Group II, n = 43; 4 mcg Receptal, İntervet, İstanbul, Turkey), or with nothing (Group III, control, n = 26). Ewes were monitored for heat 24 h after sponge removal by teaser rams (n = 4), and those detected in heat were mated with fertility-proven rams (n = 10). Pregnancy diagnosis was performed by transrectal ultrasonography 30 days after the mating (Scanner 480, Pie Data Medical, Maastricht, the Netherlands). The time of mating, number of pregnancies at first diagnosis, lambing times, and number of lambs were recorded for the evaluations.

2.2. Statistical analysis

Estrus, pregnancy, lambing, and fecundity ratios were analyzed using a Z-test with the Minitab 16 program.

3. Results

Estrus detection ratios were not significantly different between the groups (Table 1). Conception rates were 78.6% for the hCG group and 87.5% for GnRH group and did not significantly differ (P ≥ 0.05); however, the rate was 14.3% for the control group, and this was significantly lower than that of Groups I and II. About 90% of the ewes that were pregnant on day 30 gave birth in both Groups I and II (P ≤ 0.05), which was significantly higher than the 50% observed in Group III. A total of 49 lambs were obtained (23 from the hCG group, 25 from the GnRH group, and 1 from the control group). Detailed results are presented in Table 1. The day the ewes were in heat and the number of ewes detected in heat and subsequently mated are presented in Table 2. Ewes were detected in heat within 4 days after sponge removal. Those detected in heat were mated and data related to pregnancy outcomes are given in Table 2.

4. Discussion

The repeat breeder problem is commonly explained for cows that do not become pregnant after at least 3 inseminations without an obvious pathological problem. However, for sheep, there is no research related to this problem. This study focuses on ewes that were not pregnant after multiple matings while in heat although they previously had fertile seasons. Since ewes are seasonal breeders, they have limited opportunity to become pregnant. Those that are not pregnant during the breeding season are culled from the flock. In our study, the ewes were specially kept as they were genetically selected animals because of their traits and their pregnancies were important. However, a certain number of ewes in each season had difficulties getting pregnant and therefore did not produce lambs. Although these animals were detected to be in heat and were mated, the reason why they did not become pregnant was unclear. Their failure to become pregnant could be a result of ovulation problems due to either a lack of a competent dominant follicle or because of a gonadotropin surge, which means these animals had fertilization problems (4). Another reason could be an embryonic loss at early stages as these animals showed that they were in heat during later periods. In ewes, embryonic loss was found to be about 9%, and the frequency of the embryonic deaths were the highest between days 18 and 26 (5,7). Even though fertilization takes place, many factors could affect embryonic development. Impaired CL development can induce a low progesterone environment, leading to poor embryo development (4,5,22). Ovulation
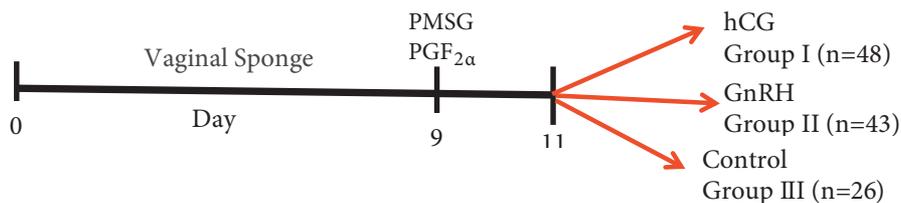
Figure. Application method.

Table 1. Parameters evaluated in the study.

	hCG	GnRH	Control	P-value hCG-GnRH	hCG-control	GnRH-control
N	48	43	26			
Estrus ratio (%)	58.3 (28/48)	55.8 (24/43)	53.8 (14/26)	ns	ns	ns
Conception (%)	78.6 (22/28)	87.5 (21/24)	14.3 (2/14)	ns	0.001	0.001
Lambing (%)	90.9 (20/22)	90.5 (19/21)	50.0 (1/2)	ns	0.001	0.001
Number of lambs	23	25	1	ns	0.028	0.023
Fecundity	1.15 (23/20)	1.31 (25/19)	1.0 (1/1)	ns	ns	ns
Multiple births (%)	15.0 (3/20)	31.6 (6/19)		ns		

Table 2. Number of ewes, days of heat, and mating distribution.

	hCG group			GnRH group			Control		
Day	Estrus	Conception	Birth	Estrus	Conception	Birth	Estrus	Conception	Birth
1	11	10	10	9	7	7	10	1	1
2	9	7	6	9	9	8	3	1	-
3	7	4	4	6	5	4	1	-	-
4	1	1	0	-	-	-	-	-	-
Total	28	22	20	24	21	19	14	2	1

from an aged dominant follicle could lead to fertilization with a disadvantageous embryo (23,24). As these animals were in heat at multiple times without a pregnancy, the factors mentioned above could have been the major problem in the flock we studied. Therefore, the purpose of this study was to reintroduce such animals back to the flock instead of culling them. For this reason, these animals were synchronized with a common protocol (progesterone + PMSG + PGF2α), and gonadotropin support was supplemented at a fixed time for all animals. The ewes were injected with either hCG or GnRH 48 h after sponge removal, and the ewes were mated at detected estrus.

In all groups, the estrus detection rate was less than 60%, which is comparably lower than reported rates after any synchronization protocols in the breeding season (11,13). However, considering that these animals are

physically challenged and the time of the study was around the end of the breeding season, the estrus rate could be acceptable. Since hCG or GnRH were applied at a fixed time in all ewes, it could be concluded that the ewes not in heat had not even developed a dominant follicle on which the gonadotropins would work. However, information on whether these animals were in heat at the next possible time was not recorded afterward, and they were not sampled for progesterone to see whether a CL was developed with the possibility in mind that they had undetectable heat.

Those that showed to be in heat were allowed to mate upon detection; therefore, some ewes were mated before or after or right on the day of the hCG or GnRH injections. The ewes came into heat 4 days after sponge removal, and Table 2 presents the number of ewes for each day for estrus, pregnancy, and lambing. Some of the ewes were in estrus the day before hCG or GnRH treatment

and were allowed to mate. However, the next day's injection could have ensured that these ewes had ovulated. Physiologically, their time of ovulation coincided with the gonadotropin injections. Data from Table 2 indicate that a fixed-time gonadotropin supplementation is reasonable as treatments have been given at the time of mating for individual ewes (12,13,16,17). The approach taken here probably could decrease the labor and time spent for the procedure; however, it may increase the cost if the number of ewes that do not show heat is high, which would entail unnecessary injections.

The conception rates were 78.6% for the hCG group and 87.5% for the GnRH group. The lambing rate for these animals was about 90%. Fecundity ratios were 1.14 and 1.29 for the hCG and GnRH groups, respectively. All of these results were significantly higher than those observed in the control group. Since these are low prolific breeds, high fecundity was not expected. Gonadotropin supplementation was performed at a time when only ovulatory follicles were normally expected to be present in the ovaries. Hashem et al. (11) clearly explained that on the day of estrus, the ovaries only have the follicles that are already selected and have become dominant; therefore, GnRH or hCG injections could induce ovulation but not stimulate the development of a new follicular pool or the growth of subordinate follicles (25). In the present study, the treatments appeared to ensure ovulation in these animals as the day 30 conception rates were high and only 2 ewes in each group lost their pregnancy at later stages.

Hashem et al. (16) and Khan et al. (12) applied GnRH or hCG at the time of mating and reported that these treatments increased the ovulation rates and improved embryonic viability. Peters et al. (26) suggested that gonadotropin supplementation at the time of insemination in cows induced ovulation and better luteinization. Furthermore, Khan et al. (12) showed that hCG at the time of mating induced conceptus growth. This could be due to a direct effect on oocyte maturation, resulting in

much healthier embryos (12) and better luteinization, which leads to higher progesterone production by the CL. Hashem et al. (16) observed that GnRH treatment in ewes caused a significant increase in the circulating progesterone concentrations. Lashari and Tasawar (17) reported similar results in Lohi sheep, in which GnRH administration on the day of mating increased serum progesterone. Confirming the suggestion by Peters et al. (26), GnRH or hCG supplementation at estrus probably induces better luteinization of the follicle cells to form a good quality CL, which produces higher amounts of progesterone. All of these effects would induce healthier and better-developed embryos, and the uterus would also be positively affected by the implantation.

None of the parameters evaluated differed between the hCG and GnRH groups in the present study. hCG directly mimics LH functions (27), while GnRH stimulates a release of LH (28); therefore, it was anticipated that the expected outcome from this application would be similar. A considerable number of ewes did not come into heat, meaning that the synchronization protocol did not help these animals to develop ovarian activity; however, once the ewes came into heat, the addition of gonadotropins probably ensured ovulation and embryonic development. It is a proven fact that in sheep hCG or GnRH supplementation leads to increased fertility by increasing the lambing rate, either due to higher embryo numbers or a decreased embryonic loss when injected at the time of mating or later in diestrus (12,13,16).

In conclusion, based on the results of this study and of previous reports, supplementation with gonadotropins is a useful option to increase fertility in ewes. Furthermore, in ewes such as those used in this study, hCG or GnRH could be used as a convenient way to induce ovulation and pregnancy. Considering the fact that this study was performed near the end of the breeding season, the results obtained are valuable.

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