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Comparison of fertility parameters in Romanov sheep synchronized with progesterone-based protocol plus PMSG or GnRH

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ABSTRACT

Objective: The aim of this study was to compare the fertility parameters in response to pregnant mare serum gonadotropin (conventional treatment) or gonadotrophin-releasing hormone (alternative treatment) in Romanov sheep subjected to a 7-d short-term protocol during non-breeding season.

Materials and Methods: All sheep (n:57) were subjected to short-term synchronization protocol. Intravaginal sponge impregnated with 20 mg fluorogestone acetate was inserted for 7 days and all sheep received 125 µg cloprostenol at sponge removal. Sheep were randomly assigned to receive no additional treatment (CON, n:16), 240 IU pregnant mare serum gonadotropin (PMSG, n:24) at sponge removal or 10 µg buserelin acetate (GnRH, n:17) at 30 h after sponge removal. Natural mating was performed following detection of estrous with fertile eight Romanov rams. Estrous response, pregnancy rate, lambing rate, and litter size were compared among groups.

Results: Estrous response and pregnancy rate were 86% and 75.4% in all sheep, respectively. Estrous response was numerically higher about 7% ($p>0.05$) in treatment groups (PMSG, 87.5%; GnRH, 88.2%) than CON (81.2%). However, pregnancy rate was numerically higher ($p>0.05$) in PMSG (83.3%) than GnRH (70.6%) and CON (68.7%). Similarly, lambing rate in the PMSG (79.1%) was approximately 15% numerically greater ($p>0.05$) than in GnRH (64.7%) and CON (62.5%). In addition, litter size in PMSG (2.1) was also numerically higher ($p>0.05$) than GnRH (1.9) and CON (1.9).

Conclusion: The use of GnRH provided similar estrous response compared to use of PMSG in Romanov sheep synchronized with short-term protocol. However, use of PMSG provided numerically higher pregnancy rate, lambing rate, and litter size than use of GnRH. Considering the serious ethical concerns and animal welfare for the production of PMSG, it is necessary to use alternative gonadotropins. Comprehensive studies are needed to compare the fertility parameters between application of PMSG and GnRH in Romanov sheep.

Keywords: *PMSG, GnRH, Fertility, Romanov, Sheep*

INTRODUCTION

Raising lambs for slaughter during winter allows the farmers to take advantage of the higher prices of lamb products (Abecia et al., 2012). The majority of sheep breeds perform different reproduction activities depending on season, feeding regime, latitude/longitude, the length of the photoperiod (Doğan et al., 2006). Reproduction of sheep is commonly controlled with hormone-based synchronization protocols including progesterone releasing devices/sponges (Guner et al., 2022).

Intravaginal device/sponge impregnated with progestagen is used for 12 to 14 days (long period) to mimic luteal phase of estrous cycle in sheep (Ungerfeld and Rubianes, 2002). However, similar or better fertility rates was reported after a short-term (5 to 7 days) progesterone-based synchronization protocol by reducing long-term progesterone exposure in previous studies (Vinoles et al., 2001; Martinez-Ros et al., 2019a; Guner and Saat, 2021).

It is well known that pregnant mare serum gonadotropin (PMSG) is frequently used to stimulate the estrous behavior and ovulation at progesterone sponge/device removal (Bruno-Galarraga et al., 2021). However, the production of PMSG from pregnant mares is considered to be a serious ethical concern (Vilanova et al., 2019). Current ethical concerns on animal welfare may lead to ban the production and using PMSG in future (Santos-Jimenez et al., 2020). Additionally, previous studies reported that repeated use of PMSG associated with PMSG neutralizing antibodies decreased fertility in sheep (Roy et al., 1999; Maurel et al., 2003; Guner et al., 2022). There have been inconsistency fertility rates after the use of gonadotropin releasing hormone (GnRH) as alternative gonadotropin instead of PMSG in progesterone-based synchronization protocols in different breeds (Reyna et al., 2007; Martemucci and D'Alessandro, 2011; Silva et al., 2015; Martinez-Ros and Gonzalez-Bulnes, 2019; Santos-Jimenez et al., 2020). There have been limited studies reporting fertility after progesterone-based synchronization protocol in Romanov sheep (Macías-Cruz et al., 2013; Martinez-Ros et al., 2019a). Besides, there has been no report related to efficacy of different gonadotropin (PMSG or GnRH) on fertility in Romanov sheep in literature. The objectives of the present study were to determine the efficacy of GnRH as alternative gonadotropin on estrous response and pregnancy rate in Romanov sheep.

MATERIALS and METHODS

The experimental procedures were approved by the Siirt University Animal Care Committee (Reference No. 2019-06).

Animals and management

This study was conducted on a total of 65 Romanov sheep, between the ages of 1-4, during non-breeding season (April-May) and housed under the same care conditions at Siirt University Goat Research and Application Center (37° 56' N, 41° 56' E). Sheep were fed with 3 kg of lentil hay and 400 g concentrate feed per head per day. Flushing was not applied to the sheep throughout the study.

Study design

Romanov sheep (n:65) were subjected to short-term synchronization protocol (Martinez-Ros et al., 2019a) and an intravaginal sponge containing 20 mg of fluorogestone acetate (Chronogest®, İntervet, Turkey) was inserted for 7 days. However, study was completed with 57 animals due to the absence of sponge before the sponge removal in 8 sheep. Sheep (n:57) were allocated to three groups including two treatments and one control group. All sheep received 125 μg cloprostenol (Estrumate®, İntervet, Turkey) at the time of sponge removal. No additional hormone was administered after application of 125 μg cloprostenol in the control group (CON, n:16). Sheep in PMSG group (n:24) received 240 IU of pregnant mare serum gonadotropin (PMSG; Chronogest PMSG®, İntervet, Turkey) at sponge removal. Sheep in GnRH group (n =17) received 10 μg buserelin acetate (GnRH, Receptal®, Intervet, Turkey) at 30 h after sponge removal. Estrous detection was made by teaser Romanov rams, starting 24 h after the sponge withdrawal for 3 days. Eight rams, known to be fertile, were used for natural breeding. A pregnancy diagnosis was performed via transrectal ultrasound (Easi-Scan equipped with a 4.5 MHz - 8.5 MHz; IMV, USA) at 30 days post natural mating. Number of single, twins or triplets was determined to calculate the litter size which is defined as number of lambs/total number of sheep that lambing.

Statistical analysis

The SPSS® 25.0 package program (SPSS Inc., Chicago, IL, USA) was used in the statistical analysis. Chi-square test was used to compare the estrous response, pregnancy rate, lambing rate, and litter size. The significance level was considered at $p<0.05$ for all analyzes.

RESULTS

The overall estrous response was 86.0% after short-term progesteron-based synchronization protocol in Romanov sheep in the present study. Considering the difference among groups, estrous response was numerically about 7% higher ($p>0.05$) in treatment groups (PMSG, 87.5%; GnRH, 88.2%) than control group (81.2%). Irrespective of

groups, overall pregnancy rate was 75.4% in all sheep. Although there was no statistical difference (p>0.05) in the pregnancy rate among groups, PMSG group (83.3%) was numerically higher than GnRH (70.6%) and CON (68.7%) groups (Table 1). The pregnancy loss interval from the first pregnancy examination to lambing was 7.0% (3/43) in this study. Similar to pregnancy rate, lambing rate in the PMSG group was approximately 15% numerically greater (p>0.05) (79.1%) than GnRH (64.7%) and CON (62.5%) groups (Table 1). There was no difference (p>0.05) in the percentage of single (20%, 70%, 10%), twin (36.4%, 36.4%, 27.2%), and triplets (21%, 47.4%, 31.6%) among CON, GnRH and PMSG groups, respectively. Irrespective of groups, the percentage of Romanov sheep that gave birth single (25%), twin (50%), and triple (25%) lamb. The number of lambs, in sheep that gave birth, ranged from 1 to 3 and the mean of litter size was 2.00±0.09. Litter size was numerically higher (p>0.05) in PMSG group (2.1) than GnRH (1.9) and CON (1.9) groups.

Table 1. Reproductive parameters in Romanov sheep received different gonadotropins with progesterone-based synchronization protocol

Reproductive Parameters	CON (n:16)	GnRH (n:17)	PMSG (n:24)	P value
Estrous detection rate (%)	81.2 (13)	88.2 (15)	87.5 (21)	NS
Pregnancy rate (%)	68.7 (11)	70.6 (12)	83.3 (20)	NS
Lambing rate (%)	62.5 (10)	64.7 (11)	79.1 (19)	NS
Litter size	1.9 (19/10)	1.9 (21/11)	2.1 (40/19)	NS

CON: sheep received no treatment after sponge removal, GnRH: sheep received 10 μg busereline acetate at 30 h after sponge removal, PMSG: sheep received 240 IU pregnant mare serum gonadotropin at the time of sponge removal, NS: not significant

DISCUSSION

Romanov is one of the most prolific breeds that provides higher reproductive efficiency in Turkey (Kutluca Korkmaz and Emsen, 2016). As in Turkey, farmers have practically implemented the crossbreeding the purebred Romanov sheep with other domestic sheep to maximize productivity in many countries (Đuričić et al., 2019; Murphy and Freking, 2021). However, Romanov is aseasonally polyestrous breed and distribution of lambing was not equal the throughout year (Đuričić et al., 2022). Therefore, progesterone-based synchronization protocol is widely used to gain higher income with more lamb production for slaughtering in Romanov sheep during winter (Murphy and Freking, 2021).

Pregnant mare serum gonadotropin is commonly applied in conjunction with progesterone-based synchronization protocols to increase estrous response, ovulation rate, pregnancy rate, litter size in sheep (Abecia et al., 2012). Unlike to common dose of PMSG (500 IU) in non-prolific breeds (Barrett et al., 2004), using a low dose of PMSG (250-300 IU) is sufficient to provoke estrous behavior and multiple lambing in prolific breeds such as Romanov (Macías-Cruz et al., 2013). Additionally, using low dose of PMSG decrease the cost of synchronization protocol (Macías-Cruz et al., 2013). Besides, the major concern for PMSG is collection of high blood from pregnant mares with unethical condition (Vilanova et al., 2019) and the development of PMSG neutralizing antibodies following repetition use (Guner et al., 2022). Therefore, recent studies focused on the determination of the efficacy of alternative gonadotropin such as gonadotropin-releasing hormone (GnRH) in conjunction with progesterone-based synchronization protocol in sheep (Martinez-Ros and Gonzalez-Bulnes, 2019; Santos-Jimenez et al., 2020.

In the present study, estrous response was 86% following short-term (7 d) progesterone-based synchronization protocol during non-breeding season in this study. Consistent with our results, higher estrous response ranging from 77.1 to 100% was reported after short or long-term progesteron-based synchronization protocol in different breeds (Ataman et al., 2006; Ustuner et al., 2007; Martinez-Ros et al., 2019a; Guner and Saat, 2021). Similar to our results, estrous response was higher (100%) following progesterone-based protocol in crossbreed Romanov sheep (Macías-Cruz et al., 2013). Additionally, estrous response was numerically about 7% higher in treatment groups (GnRH and PMSG) than in control group. Consistent with our results, Cavalcanti et al. (2012) reported that GnRH administration at 24 h after

short-term protocol did not change (95.2% vs. 100%) the estrous response compared to the control group (Cavalcanti et al., 2012). Martinez-Ros and Gonzalez-Bulnes (2019b) and Santos-Jimenez et al. (2020) reported that estrous response was equal (89.5%) or similar (88.9% vs. 94.5%, respectively) after either short-term CIDR-56h-GnRH or CIDR-PMSG protocol.

Contrary to previous reports, administration of GnRH after sponge removal statistically reduced the estrous response from 92.3 to 66.6% (Martemucci and D'Alessandro, 2011) and from 90 to 30% (Silva et al., 2015) in different breed sheep synchronized short-term protocol. Administration of GnRH is recommended at least 24–36 h after progestagen removal or luteolysis. However, acceleration of LH surge within 1–4 h post-administration may induce premature ovulation of pre-ovulatory follicles and may not allow secrete adequate estradiol for estrous behavior (Silva et al., 2015; Martinez-Ros and Gonzalez-Bulnes, 2019b). Similar estrous response demonstrated that administration of GnRH at 30 h after sponge removal did not suppress estrous behavior compared to PMSG in Romanov sheep in this study. Unlike Romanov (aseasonally polyestrous breed), application of GnRH is not recommended due to inadequate follicle development during non-breeding season in non-prolific sheep. Considering the importance of breed (Romanov) differences, Ben Saïd et al. (2007) reported that prolific breeds (Romanov) require a very small estradiol signal to induce estrous behavior and ovulation rates compared to non-prolific breeds. However, small difference in estrous response in treatment groups compared to control group could have resulted from less estradiol response needed to stimulate estrous behavior n Romanov sheep.

In previous studies reported that long-term progesterone-based synchronization protocols lead to extension of the lifespan of the ovulatory follicle, sub-luteal progesterone concentration at sponge removal, higher risk of vaginitis, and low fertility rates (Vinoles et al., 1999; Ungerfeld and Rubianes, 2002). In a comprehensive study conducted on 1750 sheep (Menchaca et al., 2018), a higher pregnancy rate in 6 days protocol (43.5%) compared to 14 days protocol (37.8%) confirmed the reports of previous studies. Although short-term synchronization protocols offer similar or higher fertility rates, short-term protocols are less widely used by practitioners due to unaware of these detrimental effects compared to long-term protocols (Menchaca et al., 2018; Martinez-Ros et al., 2019a; Uriol et al., 2019). Several retrospective studies revealed that Romanov breed had primarily superior fertility rate (from 92 to 95.9%) during breeding season (Casas et al., 2005; Đuričić et al., 2019; Đuričić et al., 2022). The pregnancy rate was 75.4% after short-term synchronization protocol regardless of groups in this study. Similar to our results, Martinez-Ros et al. (2019a) determined the pregnancy rate as 78.9% following short-term (7-day) progesterone-based synchronization protocol in Segureña×Romanov breed. In the present study, the pregnancy rate was higher than previous report (65%) that was carried out 12-day progesterone-based synchronization protocol in Romanov × Pelibuey breed (Macías-Cruz et al., 2013). Our result was within the range of pregnancy rates (66.7 to 85.7%) after short-term progesterone-based synchronization protocol in non-prolific different breeds such as Merino-Akkaraman, Kıvırcık, and Awassi in Turkey (Ataman et al., 2006; Özyurtlu et al., 2011; Guner and Saat, 2021).

Considering the pregnancy rate among groups, numerically higher pregnancy rate was obtained in PMSG group (83.3%) than those in GnRH (70.6%) and CON (68.7%) in this study. Similar to higher pregnancy rate, lambing rate and litter size was also numerically higher in PMSG group compared to other groups in this study. It was reported that Romanov breed had three times more granulosa cells in their preovulatory follicles than other prolific breeds (Ricordeau et al., 1990; Macías-Cruz et al., 2013). Low PMSG doses could be sufficient to not only increase estradiol levels by improving growth of antral and non-ovulatory follicles but also postovulatory luteal function (Macías-Cruz et al., 2013; Gonzalez-Bulnes et al., 2020). However, GnRH has no effect on growth or regression of subordinate or ovulatory follicles (Reyna et al., 2007). Additionally, there has been limited information related to fertility results by comparing the GnRH and PMSG in progesterone-based synchronization protocol. Similar to our results, Martinez-Ros and Gonzalez-Bulnes (2019b) reported that pregnancy rate was approximately 10% (68.4% vs. 57.9%) lower with administration of GnRH at 56 h after sponge removal compared to PMSG. Although the time of GnRH application was equal in our study, the use of GnRH drastically decrease the pregnancy rate from 92.3% to 33.3% compared to use of PMSG in crossbreed Altamurana sheep (Martemucci and D'Alessandro,

2011). Most reports indicated that use of GnRH (at ~48 h after sponge removal) was co-administered with PMSG instead of single administration of GnRH. However, the pregnancy rate decreased by approximately 10% (Zonturlu et al., 2018) or 20% (Cavalcanti et al., 2012) in sheep received GnRH and PMSG compared to single administration of PMSG in synchronization protocol.

CONCLUSION

In conclusion, alternative gonadotropins are required instead of using PMSG due to ethical concerns in PMSG production and welfare of pregnant mares in progesterone-based synchronization protocol in sheep. The use of GnRH at 30 h after sponge removal was sufficient to show similar estrous behavior compared to low dose PMSG in Romanov sheep. However, numerically higher pregnancy rate, lambing rate, and litter size were obtained with the use of PMSG in sponge removal compared to use of GnRH. Further comprehensive studies are needed to compare the fertility parameters between PMSG and GnRH in Romanov sheep.

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