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RESEARCH ARTICLE

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COMPARISON BETWEEN SHORT- AND LONG-TERM OF ESTRUS SYNCHRONIZATION WITH DIFFERENT DOSES OF PMSG IN NOEMI EWES

Al-Sharari, M.M.M and Mohamed, Ali*

Department of animal production, Qassim University, College of Agriculture and Veterinary Medicine, Buraidah 6622, Saudi Arabia

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*Corresponding author:

Viviane dos Santos Marques

ABSTRACT

Two experiments were performed on 60 ewes and 7 Noemi rams were used. Two and 4 groups were designed in Experiment I and II respectively. Each group has 10 ewes. In experiment I, intra-vaginal sponges, containing 40 mg of FGA, were left in the vagina of groups control and G1 for 5 d. All ewes received GnRH at time of sponge insertion and time of sponge removal; ewes received prostaglandin F_{2α} and only PMSG hormones at the doses of 500 IU in G 1. Experiment II, the protocol followed the same protocol of Experiment I for control and G1. As for G2 and G3 followed protocol in G1 but the dose of PMSG were 700 in G2 and 1000 IU in G 3. Ewes were naturally mated 36 hours after sponge removal. All ewes in both Exp I and II showing estrus, except one ewe not showing estrus in control group of Exp II. In Exp I the pregnancy rates in control and G1 were 40 % and 70 %, respectively. However, in Exp II the pregnancy rate were 50, 90, 90 and 70% in control, G1, G2 and G3, respectively. The mean time from sponge out to show estrus were longer in control (55.9±1.9 and 58.44±3.5 h. in Exp I and II, respectively) compared to other groups. The difference was statistically significant (P<0.05). The mean litter sizes were higher in G1, G2 and G3 of Exp II which are 1.33, 1.55 and 1.71, respectively. The difference was not significant to Exp I. It was concluded that short term treatment using FGA and PMSG administration in the Noemi ewes may be useful to condense estrus and in order to increase pregnancy rate.

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INTRODUCTION

Reproduction is the most important factor in determining profitability in a ewe-lamb enterprises. To obtain lambs of ewes twice in one year or three times in two years (Goulet and Castonguay 2002; Bazer et al. 2007). Estrus synchronization results in an increase of offspring per year, as it reduces the lambing interval and can be handled regardless of the season in the small ruminants (Wei et al., 2016). Synchronization of estrus in sheep has been achieved by the use of intravaginal sponges containing synthetic progestagens. Several synchronization treatments incorporate an injection of pregnant mare serum gonadotropin (PMSG) at the end of the progestagen treatment in order to obtain a more precise and reliable synchronization of estrus since it increases the occurrence of ovulation and improves the post-treatment fertility in small ruminants (Mohamed Ali, 2014a). The most common method is to combine progestagen for 12 days and inject PMSG at sponge withdrawal (Mohamed Ali, 2014a). This method is widely used in experiments as well as with farmers and give acceptable and sometimes good results in the fertility rate.

However, this method has been accompanied with low first cycle conception rate and poor fertility especially under semi-arid conditions, and also poor fertility in the case of improper human handling of animals (Mohamed Ali et al., 2014). In addition to this method, after a long period of placing the sponge in the vagina, has some undesirable drawbacks such as attach the sponge to the vaginal wall as a result of inflammation and unpleasant odorous discharge and sometimes bloody secretions after sponge removal (Ataman and Akoz 2006). Several attempts have combined hormones to improve the method of estrus synchronization so that it is easy and fast for farmers and researchers. The key in this method is to inject a gonadotropin releasing hormone (GnRH) when inserting the sponge, which is to get rid of the mature follicles, to inject prostaglandins is to luteolysis the corpus luteum, and inject the PMSG to increase the mature of follicles for ovulation. Progesterone can prevent ovulation during the period in which spontaneous luteolysis may occur in animals. PMSG is produced from the endometrial cups of the uterine wall of the pregnant mare and has an effect similar to the follicle stimulating hormone (FSH) and the luteinizing hormone (LH) so that giving it to female animals improves reproductive efficiency,

increases the number of ovulated follicles and fertility rate, especially in anestrus ewes. Therefore, it is used in veterinary medicine to control the reproductive activity of female animals. Through a study conducted by Hackett, et al. (1981) found that 83% of ewes became pregnant with viable foetuses after injecting 500 IU of PMSG. Another study of Santos et al. (2010) had a estrus rate of more than 86% and improved fertility. In present study, PMSG will give different concentrations higher than the previous studies, which are 500, 700 and 1000 IU, to find out its effect on the estrus rate, fertility rate and the number of lamb per lambing in Noemi ewes. There is currently a new market of PMSG from the Chinese company, which is of recent production, where it is tested with local ewes with different concentrations, as mentioned previously. Therefore, the objectives of this study were to determine the reproductive performance (lambing rate and litter size) of adult ewes naturally mated at a synchronized estrus in relation to duration of sponge treatment and dose of PMSG.

MATERIALS AND METHODS

A total of 60 cyclic Noemi ewes, 3±5 years of age and 45-55 kg body weight, were used in this study. The experiment was conducted during July to September 2020 at the Agriculture and Veterinary Research Center, Qassim University. The animals were managed under the same conditions on one farm. They were kept under semi-shaded yards, offered barley, alfalfa hay, clean water and integrated mineral licks. Experiment I (short-term treatment), ewes (n=20) were equally subdivided into two groups; control group (n=10) and G1 group (n=10). Ewes were vaginally inserted with a sponges impregnated with 40 mg of flourogestone acetate (FGA; Ceva, Santa Animals, Libourne, France) and left for 5 days. For control group, at sponge insertion all ewes were received 1mL gonadotropine releasing hormone (0.0042 mg of GnRH; Argoselina, Laboratorios Argos S.R.L; Argentina) and at sponge out the ewes were injected 125 µg of prostaglandin F_{2α}(PGF; Estrumate, Merck Animal Health, Desoto, KS, USA). The protocol of G1 group followed the protocol of control group, except all ewes were received 500 iu of pregnant mare gonadotrophine (PMSG; Sansheng®, Ningbo Sansheng Pharmaceutical Co. Ltd, Ningbo, China) at sponge removal. In Experiment II (long term-treatment), ewes (n=40) were equally subdivided into four groups (control, pmsg500, pmsg700, and pmsg1000 groups). All ewes were vaginally inserted with a sponges impregnated with 40 mg of flourogestone acetate (FGA) for 12 days. These ewes were received 1 ml GnRH at sponge inseration and injected with PGF at sponge removal. At sponge removal, all ewes were injected (i.m.) with 500 IU, 700 IU and 1000 IU of PMSG for G1, G2, and G3, respectively.

In Experiment I and II, at the onset of estrus, natural mating was carried out by the use of fertile rams in a ratio of 1:5. The males were introduced in the herd after pessary removal for a total period of 96 h. the duration from sponge removal until estrus exhibition was recorded. After 30 days of natural mating, pregnancy was confirmed by ultrasound (Esaote, pie medical, Netherlands). Percentage of ewes lambing to natural matting at the synchronized estrus (lambing rate) and number of lambs born per female lambing (litter size) were used as measures of reproductive performance. Lambing was determined by daily observation and identification of ewes in small lambing plots. Statistical Analysis: Data were analysed using a statistical software SPSS release 21.0. Reproductive parameters among 6 group treatments were compared by ANOVA, and chi-square. Data are presented as arithmetic mean ± SEM. Significance was considered at P<0.05.

RESULTS

Table 1 shows the effect of synchronization treatment on estrus response in the Noemi ewes. In the Experiment I (short-term treatment), all ewes (100%) exhibited standing for NM. Ewes in G1 given the PMSG have shown estrus shortly (after 48.80 h) after sponge removal as compared with those in control (55.90 h).

In case of no given PMSG (control), nine out of 10 ewes exhibited standing NM during 49-60 h, however, in G1 (8 ewes) intensifying the estrus duration within short time (36-48 h) (Figure 1).

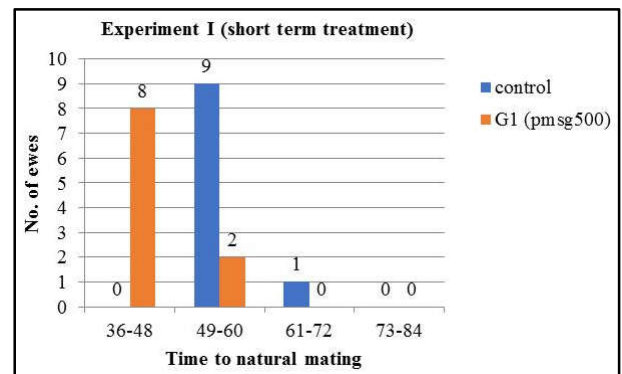


Figure 1. Distribution of ewes for NM over time following synchronization treatment in experiment I

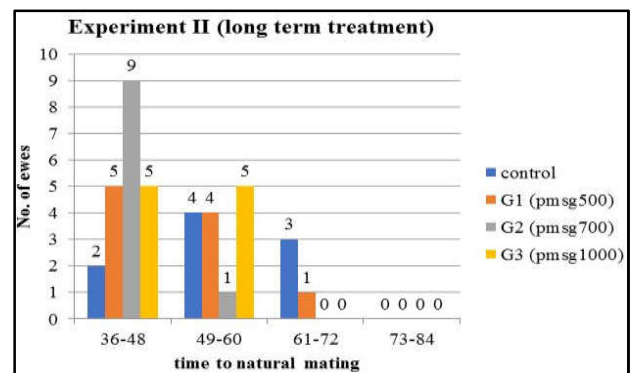


Figure 2. Distribution of ewes for NM over time following synchronization treatment in experiment II

The difference between groups were statistically significant ($P>0.05$). The pregnancy rate after synchronization treatment in the control and G1 were 40.00 and 70.00 %, respectively. Despite this enhancement, there was no statistical difference. In Experiment II (long-term treatment), all ewes (100%) exhibited standing for NM except one from control group. Ewes in the control group had estrus between 36 to 72 hour, with an average of 58 hours, unlike the ewes in the G1, G2 and G3 showed estrus between 36 to 60 hours, with an average of 48-50 hours (Figure 2). In Table 1, increasing PMSG doses tended to increase pregnancy rate in G1, G2 and G3 (90, 90 and 70%, respectively) than control group (50%). Despite this enhancement, there was no statistical difference. Comparing short term treatment with long term treatment on the pregnancy rates was in a favor of long term treatment (75%) which was higher than short term treatment (55%). However, the difference was not statistically significant. Table 2 shows the number of lamb for each birth, as there was a numerical increase in the Experiment II compared to the Experiment I, as well as an increase in the number of twins births in the experiment II, which led to an increase in the litter size.

DISCUSSION

PMSG has long-lasting LH- and FSH-like effects on the follicle. PMSG can enhance the recruitment of small follicles, reduce atretic follicles and also stimulate estradiol and progesterone secretion. In consequence, it improves the reproductive performance and increases ovulation and pregnancy rates (Mohamed Ali, 2014b). The results of the application experiment also testified that time to standing matting, estrus rate, pregnancy rate and lambing rates of progestagen/PMSG protocol were effective treatment protocols. This indicated that progestagen/PMSG (500 and 700 IU) were an optimum scheme for estrus synchronization in Noemi ewes.

Table 1. Effects of estrus synchronization on mean time to natural mating and pregnancy rate in Noemi ewes

| Groups | No. of ewes | No. of ewes standing for male (%) | Mean time from sponge removal to NM (hrs) | No. of pregnant ewes (%) |
|--|-------------|-----------------------------------|---|--------------------------|
| Experiment I (short-term treatment) | | | | |
| Control | 10 | 10 (100) ^a | 55.90±1.92 ^b | 4 (40.00) ^a |
| G1 (Pmsg500) | 10 | 10 (100) ^a | 46.40±1.80 ^a | 7 (70.00) ^a |
| Total | 20 | 20 (100) ^A | | 11 (55.00) ^A |
| Experiment II (long-term treatment) | | | | |
| Control | 10 | 9 (90.00) ^a | 58.44±3.52 ^b | 5 (50.00) ^a |
| G1 (Pmsg500) | 10 | 10 (100) ^a | 49.60±3.16 ^a | 9 (90.00) ^a |
| G2 (pmsg700) | 10 | 10 (100) ^a | 42.40±2.16 ^a | 9 (90.00) ^a |
| G3 (pmsg1000) | 10 | 10 (100) ^a | 47.60±2.01 ^a | 7 (70.00) ^a |
| Total | 40 | 39 (97.5) ^A | | 30 (75.00) ^A |

^{a,b}: Values with different superscripts in the same column and experiment differ significantly at P<0.05, Chi-square and kruskal-Wallis one-way ANOVA.

^{A,B}: Values with different superscripts in the same column between experiments differ significantly at P<0.05, Chi-square.

Table 2. Reproductive performance of ewes naturally mated relation to duration of sponge and PMSG dose level

| Parameters | Experiment I | | Experiment II | | | |
|-------------------------------|--------------|----------|---------------|----------|----------|----------|
| | Control | G1 | Control | G1 | G2 | G3 |
| no. of ewes | 10 | 10 | 10 | 10 | 10 | 10 |
| No. of ewes lambing (%) | 4 (40.0) | 7 (70.0) | 6 (60.0) | 9 (90.0) | 9 (90.0) | 7 (70.0) |
| No. of lamb | 5 | 8 | 7 | 12 | 14 | 12 |
| No. of twins per lambing (%) | 1 (25) | 1 (14) | 1 (16) | 3 (33.3) | 5 (55.5) | 3 (50) |
| No. of triple per lambing (%) | 0 | 0 | 0 | 0 | 0 | 1 (14.2) |
| Litter size | 1.25 | 1.14 | 1.16 | 1.33 | 1.55 | 1.71 |

The findings were highly consistent with early reports of Wei et al. (2016) and Titi et al., (2009). The results of Timurkan and Yildiz (2005) indicated that the pregnancy rates were 90.62%, 93.75% and 100% in ewes intramuscularly injected with PMSG 500, 600 and 750 IU, respectively, at the time of intravaginal sponges removal. The use of FGA plus PMSG is the best choice to improve the fertility rate in ewes (Wei et al. 2016). This is likely associated with the doses of eCG. The different doses of PMSG had different effects on the induction of ovulations. Five hundred IU eCG was insufficient to cause multiple ovulation in dairy heifers, but doses of more than 1000 IU induced multiple ovulation of dairy Cow (Bellows & Short 1972). Moreover, Noemi sheep probably have a good response to PMSG treatment especially at high doses 700 – 1000 IU. Therefore rate of ovulation is affected by the PMSG dose level employed. An adequate dose of PMSG improves prolificacy, thus produces multiple gestations. Since similar results have not been reported on this sheep species, further researches should be conducted to provide a better understanding of reproductive endocrine effects of progestagen/PMSG treatment in ewes. However, a high doses of PMSG can an increase in fetal or lamb mortality. So, to avoid non-desirable fetal losses and large litter sizes, the dose level of such gonadotropin has to be precisely adjusted according to the season of the year and the physiological state of the ewes (Simonetti et al., 2002).

Several methods for controlling ovarian activity and focusing on improving fertility in small ruminants. Most of the methods of unifying estrus are to combine progestin for 12 days with PMSG and are widely used in experiments as well as with breeders and give acceptable and sometimes good results in the rate of fertility. In the present study, the administration of GnRH 5 days before the administration of PGF_{2a} might be to induce ovulation/luteinization of the dominant follicle, thus inducing follicular turnover. Progestagen sponges applied on the day of GnRH administration and withdrawn on the day of PGF_{2a} administration prevented the occurrence of estrus either before or after the PGF_{2a} administration. The luteal tissue that forms as a result of the GnRH administration is responsive to PGF_{2a} and is capable of undergoing luteolysis (Titi et al., 2009). This was the case in sheep as progesterone concentrations declined following PGF_{2a} administration as mentioned by Titi et al., (2009). The lambing results in Noemi ewes were acceptable and more comparable to previous studies (Husein and Kridli 2002; Alminer et al. 2005; Titi et al., 2009). These studies have reported relatively low lambing rates from mating during the induced estrus in Awassi ewes. In present study the estrus rate were 100 to 97 % of ewes showing standing for mating in both short and long term treatment, respectively.

The administration of GnRH is always effective in inducing ovulation when the mature follicle present in the ovary. Thus the response to GnRH is dependent upon the stage of the cycle at which it is administered (Geary et al. 2000; Alminer et al. 2005). In study by Titi et al., (2009), about half of the ewes had active corpora lutea at the time of GnRH administration. Because of that, the administration of GnRH to ewes was less effective in inducing a great drop in progesterone as that observed in goats (Titi et al., 2009). Geary et al. (2000) reported that during the final stages of the estrous cycles cows failed to ovulate in response to GnRH administration. The authors further reported that most of these cows express estrus prior to the PGF_{2a} administration. This is where the importance of progestagen supplementation lies. The administration of a progestagen source between the GnRH and PGF_{2a} treatments may be effective in delaying estrus and ovulation and allowing for better synchrony (Lamb et al. 2001). When PGF_{2a} is administered and sponges are removed, ewes encounter luteolysis and/or sudden progesterone withdrawal, which should enhance follicular development and estrus expression. In this regard, PMSG administration at sponge removal contributed to improving in lambing rate and fecundity in G1 (both experiment), G2 and G3 ewes via enhanced follicular development and more mature follicles will be ovulated. A 5-day GnRH-PGF_{2a} treatment protocol has been reported for estrus synchronization in sheep (Husein and Kridli 2003; Titi et al., 2009). Husein and Kridli (2003) showed that a 5-day GnRH-PGF_{2a} can be effective in inducing estrus outside the breeding season in sheep only when preceded by progestagen sponges. In the present study, it appears that the incorporation of this protocol plus PMSG at sponge removal improved follicular development in sheep. This progestagen plus PMSG promoted growth of more dominant follicles and enhanced the lambing rate.

CONCLUSION

In conclusion, 5-days progestagen sponges with GnRH at sponge insertion and PGF_{2a} plus PMSG at sponge removal can be effective in synchronizing estrus and improving fecundity in sheep. Such combination may yield same responses that using the traditional progestagen sponges for 12 days and PMSG.

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