African Journal of Biotechnology Vol. 10(57), pp. 12363-12367, 28 September, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1870
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

The effects of pregnant mare serum gonadotropin (PMSG) injection a day prior or at controlled intravaginal drug-releasing (CIDR) removal on multiple births in Afshari ewes

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Accepted 18 August, 2011

According to the importance of lambing rate to profitability of sheep holders, this trial investigated the effects of pregnant mare serum gonadotropin (PMSG) injection a day prior or at controlled intravaginal drug-releasing device (CIDR) removal on multiple births in synchronized Afshari ewes. 16 cycling, multiparous fat-tailed Iranian Afshari ewes, weighing 66.5±2.5 kg, were used in the trial. The ewes were randomly allocated in equal numbers (n = 8) to two treatment groups. The estrous cycles were synchronized using CIDR's inserted for a period of 14 days. In a group (PMSG/ d -1) of ewes, PMSG (300 IU) intramuscularly (IM) was injected a day (day = -1) prior to CIDR removal (day 0 = day of CIDR removal) and another group (PMSG/ d 0) received PMSG at CIDR removal (day 0). 48 following CIDR removal (day 1), GnRH (50 µg) was administered to half of each group of ewes and ewes were mated with the proven rams. Ovarian follicular activity was monitored with the aid of transrectal ultrasonograpy: on the day of CIDR removal (day 0) and a day later (at estrus, day 1). Pregnancy was diagnosed with the aid of transabdominal ultrasonograpy 30 days after mating. Following PMSG (day = -1) treatment, the mean number of large follicles on both ovaries increased from CIDR removal to estrus (1.50±0.61 vs. 2.78±0.69) (P < 0.05). Lambing rate in PMSG/ d -1+GnRH, PMSG/ d -1 noGnRH, PMSG/ d0+GnRH and PMSG/ d0 noGnRH groups was 175, 125, 175 and 150% respectively. The results show that PMSG injection a day prior or at CIDR removal, caused large follicles development, but no significant difference was seen between the two treatment lambing rates and also, the lambing rate increased in group treated with GnRH.

Key words: Pregnant mare serum gonadotropin (PMSG), gonadotropin-releasing hormone (GnRH), Afshari ewes, lambing.

INTRODUCTION

Lambing rate is a very important and profitable factor for sheep holders in semi-intensive production systems. It has been observed that in these holding systems, rams are able to mate ewes at almost all around the year. Exogenous hormones and synchronization methods are used to maintain and increase lamb production all around the year (Rosado et al., 1998). One injection of PMSG can stimulate follicular growth, and higher ovulation rate in outside the breeding season (Greyling and van Niekerk, 1989).

Gonadotropins such as PMSG (Dogan and Nur, 2006), follicle-stimulating hormone (FSH) (Boscos et al., 2002) and gonadotropin-releasing hormone (GnRH) (Akif Cam and Kuran, 2004) treatment increase the number of follicles, ovulation rate and litter size. PMSG and

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progesterone analogues are used vastly to control fertility in cyclic and anestrous ewes, and to synchronize estrus for fixed-time artificial insemination (Buckrell et al., 1994).

When no PMSG was injected during estrus synchronization, ovulations occur later and with a longer period of time (Barrett et al., 2004).

In an experiment, PMSG injection at 24 h prior to sponge removal or at sponge removal, caused the increase lambing, multiple birth and fecundity rates compared to ewes given PMSG 24 h after sponge removal (Koyuncu and Ozis Alticekic, 2010). GnRH is another hormone that is used vastly for ovulation induction at estrus. It is reported that GnRH administration immediately after AI may increase the multiple birth rate of synchronized Awassi ewes during breeding season (Turk et al., 2008).

Walker et al. (1986) reported that injection of PMSG + GnRH had a desirable effect on ewes' reproductive performance. They suggested that an administration of 50 µg GnRH with 1500 IU PMSG increase the fertility and ovulation rates in ewes. The purpose of this trial was to determine the effects of PMSG administration prior or at CIDR removal with or without GnRH injection on lambing rate of Afshari ewes.

MATERIALS AND METHODS

The experiment was conducted during the breeding season (October to February), at the Zanjan University farm located in Zanjan city.

Synchronization protocol

16 cycling, multiparous fat-tailed Iranian Afshari ewes, weighting 66.5±2.5 kg, were used in the trial. The ewes were randomly allocated in equal numbers (n = 8) to two treatment groups. All animals had not previously been used for any eCG treatment, superovulation or as embryo recipients in a MOET program. The estrous cycles were synchronized using CIDR's (EAZI-BREED™, CIDR®, NewZealand) inserted for a period of 14 days. In a group (PMSG/ d -1) of ewes, PMSG (300 IU; BIONICHE; Australia) intramuscularly (IM) was injected a day (day = -1) prior to CIDR removal (day 0 = day of CIDR removal) and another group (PMSG/ d 0) received PMSG at CIDR removal (day 0). 48 h following CIDR removal (day 2), GnRH (50 µg; Cystorelin®; CEVA Aante Animal; France) was administered (IM) to half of the ewes of each group and ewes were mated with the proven rams. All animals were provided with water and manually fed alfalfa hay supplemented with grain pellets (CNCPS, 2003), *ad libitum*.

Ultrasonography studies

Ovarian follicular activity was monitored by transrectal ultrasonography (Piemedical, Falco100; Holland, 8 MHz), at CIDR removal and at estrus. The ultrasonographic scanning of both ovaries was recorded using a MP4 player (Marshal X720, China). In brief, during the ultrasonographic evaluations, ewes were kept in a darkened room and restrained in a fostering crate in the standing position. After introducing a hydro soluble contact gel into the rectum (to enhance the ultrasound transmission), the probe was placed into the rectum with the transducer oriented perpendicularly with the abdominal wall. When the urinary bladder was surpassed and the uterine horns were located, the probe was rotated laterally clockwise for 90° and counter-clockwise for 180° to evaluate both ovaries and their structures. One experienced operator performed all the recordings. The ovaries were scanned in several planes to identify all visible follicles (>1 mm in diameter). All follicles larger than 1 mm were counted and classified according to their diameter in one of the following classes: small (≤2 mm), medium (3 mm) and large (≥ 4mm) follicles. Pregnancy was diagnosed with the aid of transabdominal ultrasonograpy 30 days after mating.

Blood sampling and progesterone analysis

Blood samples were collected in early pregnancy diagnosis on day 30 and on the middle pregnancy diagnosis on day 75 from the jugular vein into EDTA tubes. Blood samples were centrifuged (in 3000 rpm for 15 min), and plasma was harvested and stored at -20°C until progesterone assay. Concentrations of progesterone were determined by ELISA kit (Monobind®; USA) with 0.1 ng/ml sensitivity.

Statistical analyses

The number of ovarian follicles (that is, small, medium and large follicles) and progesterone concentrations were analyzed using the mixed procedure of SAS (9.1). Mean comparison was performed by least square mean method. The analysis included sources of variation due to treatment groups, days (repeated measures) and their interactions. The differences were compared by Tukey test. Effects of weight and age of ewes were added as a covariate to the model. The percentage data were analyzed using the chi-square test. Significant differences between treatments were determined at the $P < 0.05$ level. Data were expressed as the mean±SEM, unless otherwise stated.

RESULTS

The follicular responses following two groups of PMSG treatment are shown in Table 1. The results of ultrasonography showed that the mean number of small follicles on the right, left and both (right+left) ovaries between the two treatment groups (PMSG/ d -1 vs. PMSG/ d 0) was not different ($p>0.05$) on the day of CIDR removal (day 0) and at estrus (day 1).

The mean number of medium follicles on the left ovary in PMSG/ d -1 group was significantly lower ($p<0.05$) than in the PMSG/ d 0 group at CIDR removal. No significant difference ($p>0.05$) was observed in the mean number of medium follicles on the right and both ovaries between the two treatment groups as well as on days 0 at CIDR removal and on day 1 at estrus (Table 1). Following PMSG treatment on day -1, the mean number of large follicles increased in PMSG/ d 0 group ($p<0.05$) from day 0 to day 1 (Table 1).

All ewes show estrus behavior within 48 to 54 h after CIDR removal, and then four rams were introduced randomly to each group. The ewes were diagnosed pregnant by ultrasonography 30 days after ram induction. After lambing, the lambing rate in PMSG/ d -1 + GnRH

Table 1. The mean (±SEM) number of small, medium and large follicles in the two treatment groups on the day of CIDR removal (day 0) and at estrus (day 1).

Parameter	CIDR removal (day 0)		Estrus (day 1)	
	PMSG /d -1	PMSG/ d 0	PMSG/ d -1	PMSG/ d 0
Small follicles				
Right ovary	1.57±0.47	1.04±0.66	1.45±0.50	2.54±0.66
Left ovary	1.27±0.36	1.49±0.50	1.40±0.38	1.24±0.50
Total	3.00±0.66	2.49±0.94	2.19±0.71	3.74±0.94
Medium follicles				
Right ovary	1.61±0.38	1.26±0.54	1.28±0.39	1.01±0.54
Left ovary	1.39±0.30[a]	2.24±0.42[b]	1.58±0.32	1.49±0.42
Total	3.05±0.49	3.49±0.68	2.61±0.57	2.49±0.68
Large follicles				
Right ovary	0.66±0.40	0.60±0.56	1.56±0.42	0.70±0.56
Left ovary	0.86±0.29	0.90±0.41	0.92±0.31	1.25±0.41
Total	1.50±0.61	1.49±0.85	2.78±0.69[b]	1.99±0.85

Different superscripts (a,b) in the same row within an ovary indicate a significant difference ($P < 0.05$). Different superscripts (c,d) in the same row within the two treatment groups indicate a significant difference ($P < 0.05$).

Table 2. The mean progesterone concentrations at early pregnancy (EP) and middle pregnancy (MP) and lambing rate of the two groups.

Group	P4 concentration		Lambing rate (%)	Total (%)
	EP	MP		
PMSG/ d -1 + GnRH	6.12±2.45	10.03±2.45	175	150
PMSG/ d -1 noGnRH	5.62±2.18	8.77±2.18	125	
PMSG/ d 0 + GnRH	6.96±3.03	8.38±3.03	175	162.5
PMSG/ d 0 noGnRH	5.01±3.22	8.85±3.22	150	

EP = Early pregnancy (day 30); MP = middle pregnancy (day 75).

group ewes (175%) was more ($p<0.05$) than that in ewes who received no GnRH (125%). Lambing rate in PMSG/ d 0 + GnRH group ewes (175%) was also more ($p<0.05$) than that in no GnRH received ewes (150%). Progesterone concentration of blood samples increased significantly ($P < 0.001$) in all treatment groups from early to mid-pregnancy (Table 2) but no significant differences was observed between the treatment groups at early and mid-pregnancy.

DISCUSSION

In this study, ewes' ovary evaluation was conducted via transrectal ultrasonography and during ovaries detection, all ewes had standing position that was more comfortable for them than dorsal recumbency (Riesenberg et al., 2001; Kermani et al., 2010). According to Dogan and Nur (2006) findings, PMSG stimulates the number of follicles and this caused more follicular development; the results are in line with our findings. In this study, PMSG administration at day 1 and day 0 had desirable effects on ovarian enlargement and responses (Riesenberg et al., 2001).

Higher doses of PMSG is effective in increasing the ovulation rate and one injection of PMSG prior to sponge removal (–24 and –48 h) increased the percentage of ewes in estrus (98%), shortened the interval to estrus (29±1.6 h) and influenced the onset of estrus within 36 h (60%), compared with ewes treated with PMSG at the time of sponge removal (Barrett et al., 2004).

The results obtained from this experiment showed that in the two period of PMSG treatment [PMSG (-1) and PMSG (0)] the mean number of small follicles on left, right and both ovaries was not significantly different in CIDR removal and estrus days. Ali (2007) reported that

the injection of 300 IU PMSG, 48 h before or at sponge removal had no effect on ovulation rate and these findings are in agreement with our outcomes. The mean number of medium sized follicles at CIDR removal day on the left ovary was more than that of the right ovary, and it was significantly different ($P<0.05$) in the two times PMSG administration. We can attribute it to the effect of PMSG on heightening the size of antral follicles (Walker et al., 1986) but the mean number of medium sized follicles on the right ovary, at estrus day and the total of both were not different significantly ($P>0.05$); that could be due to the same effect of the two times PMSG administrations on ovulation rate (Zeleke et al., 2005).

In our observations, the mean number of large follicles at estrus day on the right ovary with PMSG d-1 treatment and on the left ovary with PMSG d0 treatment was more, but it was not significantly different, and also the mean number of total follicles on both ovaries was not significantly different between the treatments. However, the average number of total large follicles in PMSG d-1 treatment increased significantly ($P<0.05$) at estrus. Our findings are in agreement with that of Cline et al. (2001). Also, the effect of PMSG administration dosage can be affected by variation in breeds genetic and different breeds have variable responses (Ali, 2007; Ince and Karaca, 2009).

Ewes' lambing rates in this study were affected by the time of treatment (Table 2). The highest lambing rate was observed in PMSG (d-1) + GnRH and PMSG (0) +GnRH groups (175%), but in PMSG (d-1) and PMSG (0) groups that did not received GnRH, the lambing rate declined to 125 and 150% respectively in agreement with previous reports (Jabbour et al., 1991; Leyva et al., 1998). The importance of GnRH in combination with PMSG on fecundity in another study (Turk et al., 2008) shows that using GnRH immediately after artificial insemination in sheep, caused raise in litter size by synchronization with combination of progestagen, PMSG and PGF2α. GnRH Injection had a desirable effect on ovulation synchronization when administrated at 20 and 24 h after progesterone sponges removal, and also Luteinising hormone (LH) surge was created 2 h after its injection in season or out of the reproductive season (Reyna et al., 2007).

After measuring blood samples progesterone concentration, the mean progesterone concentrations at early pregnancy were significantly lower than that of the middle pregnancy ($P<0.01$) and these results was similar to those of other studies (Theodosiadou et al., 2004), but no significant difference was seen between the mean progesterone concentration of treatment groups at early and middle pregnancy.

In conclusion, using estrus synchronization by progesterone CIDR accompanied with two periods of PMSG injection due to all ewes estrus synchronization, the mean number of large follicles following PMSG d-1 treatment increased significantly ($P<0.05$), but the mean lambing rate of the two treatment groups was not significantly difference (150% vs. 162.5%). Also, GnRH administration is effective for the increase of ovulation rate and consequently lambing rate.

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