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Effect of Different Method GnRH-$PGF_{2\alpha}$ Treatment on Induced of Estrus and Pregnancy in Breeding Season in Awassi Ewes

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Abstract: The object of this study was to investigate, the effect of $PGF_{2\alpha}$ administrated on 5 and 7 d following GnRH treatment on estrus and pregnancy during the breeding season in awassi ewes. A total of 60 ewes 3-5 years old were used in this experiment. The ewes were divided into 3 groups (20 ewes/group) in breeding season. First group (control) was treated with the PG-10 day protocol consisting of 2 intramuscular (i.m.) doses of $PGF_{2\alpha}$ analogue (250 μg of D-Cloprostenol/dose). The second group received intramuscular injection of GnRH analogue (busereline) and 5 day later an i.m. injection of $PGF_{2\alpha}$. In the 3 groups were treated with GnRH followed, 7 days later, by an i.m. injection of 250 μg of prostaglandin $F_{2\alpha}$. After the detection of estrus, the ewes were inseminated with 0.2 mL fresh sperm. Pregnancy was determined using ultrasonography on day 30 after the AI. Estrus response was 90% in Group 1, 90% in Group 2 and 35% in Group 3 ($p<0.001$). The pregnancy rate was 77.8% in Group 1, 83.3% in Group 2 and 42.8% in Group 3 ($p<0.05$). Lambing rate was 77.8% in Group 1, 83.3% in Group 2 and 42.8% in Group 3 ($p<0.05$). These results suggest that effect of induction estrus and pregnancy and lambing rate after GnRH and 5 days later an i.m. injection of prostaglandin $F_{2\alpha}$ were higher than GnRH and $PGF_{2\alpha}$ treatment followed 7 days injection of $PGF_{2\alpha}$. But comparable to $PGF_{2\alpha}$ treatment with 10 days intervals.

Key words: Ewes, GnRH, $PGF_{2\alpha}$, estrus induction, intramuscular, ultrasonography

INTRODUCTION

Estrus synchronization can be an effective means of increasing the proportion of ewes that become pregnant over a short time, resulting in more uniform lamb crops. Precise control of estrus cycles requires the manipulation of both follicular growth and luteal life Span (Husein and Kridli, 2003). The most frequently used estrus synchronization protocols, based on the use of equine chorionic gonadotropin after a pretreatment with progesterone or progestagen intravaginal devices (Evans and Maxwell, 1990; Menchaca *et al*., 2004).

Prostaglandin $F_{2\alpha}$ a great many of strategies have been developed in order to control ovarium function in sheep. Estrus synchronization can be controlled by manipulating either follicular development or Corpus Luteum (CL) (Husein and Kridli, 2003). Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) can be applied to the ewe to provide estrus synchronization (Gordon, 1997; Husein and Kridli, 2003). $PGF_{2\alpha}$ in sheep causes CL regression (Rubianes *et al*., 2003). $PGF_{2\alpha}$ applications are ineffective since CL does not respond to it between 0-2 days of sexual cycle. Likewise, since CL is luteolized spontaneously between 14-17th days, $PGF_{2\alpha}$ maintains ineffective. For this reason, it is applied in the form of double luteolitic dose with 9 days intervals in order to synchronize estrus and ovulation (Gordon, 1997). In fixed Timed Artificial Insemination (TAI) and natural breeding, the pregnancy rate in $PGF_{2\alpha}$ applications, for synchronizing estrus, are found low (Boland *et al*., 1978). Apart from this, in double dose $PGF_{2\alpha}$ applications applied with 10-14 days intervals, fixed timed artificial insemination applications are not preferred on big herds as a useful method (Evans and Maxwell, 1990; Menchaca *et al*., 2004). Estrus and ovulation formed following $PGF_{2\alpha}$ application can change depending on the existence of follicle developed in ovarium during the application of exogenous hormone (Husein and Kridli, 2003). Alternative applications have been developed in order to manipulate follicular development and provide estrus synchronization. One of

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these is $PGF_{2\alpha}$ treatment made after 5 days following GnRH administration on the sheep (Beck *et al*., 1996). Administering exogenous GnRH in any phase of sexual cycle causes the increase of LH that causes ovulation or atresia and a new follicular wave is occured in 2-3 days following this application (Webb *et al*., 1992; Wolfenson *et al*., 1994).

During the ovulatory cycle in sheep, follicles having 2-3 mm diameters appear and reach to the maximum 4-12 mm (Bartlewski *et al*., 1999; Vinoles *et al*., 2001). It has been reported that during the appearance of a new follicular wave, many small follicles do not gather and small follicles are not pressured during a development phase of a follicular wave. Moreover, it is suggested that a strong follicular dominance does not exist in the follicular wave appearing once in every 3-5 days (Duggavathi *et al*., 2003).

$PGF_{2\alpha}$ treated after 5 days following, the GnRH application has the ability to luteolize CL formation (Beck *et al*., 1996). Our hypothesis is that $PGF_{2\alpha}$ done on day 7 following the tretment of GnRH will be effective in increasing reproductive efficiency by prolonging the luteal function.

The objective of the experiment was to examine whether GnRH + $PGF_{2\alpha}$ (5 or 7 day later) method gives a sufficient on the fertility, compared with standard the method in which double doses of $PGF_{2\alpha}$ are applied in breeding season.

MATERIALS AND METHODS

Experimental animals and design: The study was performed Harran University sheep breeding unit, Sanliurfa, Turkey (37°07'N, 38°49'E). The material of the study consists of 60 Awassi ewes from different ages (2-5 aged) in breeding season. The same feeding conditions were applied to all animals. The sheep were kept in half-open folds during the study.

Animals were divided into 3 equal groups. The 1st group was given twice, at 10 day interval, i.m. of injections 250 μg of cloprostenol an analogue of $PGF_{2\alpha}$ ($PGF_{2\alpha}$, dalmazine®, intervet, Turkey). The second group received intramusculary (i.m) 10 μg of buserelin, an analogue of gonadotropine releasing hormone (receptal®, intervet, Turkey) and 5 days later i.m injection of 250 μg of D-Cloprostenol. The 3rd group was received intramusculary i.m, 10 μg of buserelin and 7 days later i.m injection of 250 μg of D-Cloprostenol.

Determination of the estrus and insemination: The estrus cycle of each ewe was checked twice daily, using teaser rams, with a time interval of about 12 h with a 3 day period after second $PGF_{2\alpha}$ application. After the detection of estrus, the ewes were inseminated once during estrus and performance of artificial insemination was recommended between 12 and 24 h after detection of standing estrus. Pregnant ewes were determined using the realtime B-mode ultrasound (Scanner LC 100 Vet, Pie Medical, Netherlands) with the 6-8 MHz linear-array transrectal probe on day 30 following artificial insemination.

Parameters and statistical analyses: The reproductive parameters below were calculated in each study group:

$$\text{Estrus response}\left(\frac{\text{Number of estrus ewes}}{\text{Number of ewes}}\right)\times 100$$

$$\text{Pregnancy rate}\left(\frac{\text{Number of pregnancy ewes}}{\text{Number of inseminated ewes}}\right)\times 100$$

$$\text{Lambing rate}\left(\frac{\text{Number of lambs born}}{\text{Number of ewes inseminated}}\right)\times 100$$

The results were analyzed by χ^2 test. Statistical analyses were performed using SAS (1989).

RESULTS AND DISCUSSION

In estrus synchronization performed with double dose $PGF_{2\alpha}$, only CL causes regression and has effect on follicular development. The first GnRH injection initiates a new follicular development and forms a more homogenous follicular wave. GnRH and $PGF_{2\alpha}$ applied following GnRH synchronize luteal function (Beck *et al*., 1996). In this study, GnRH + $PGF_{2\alpha}$ applications were performed in Awassi ewes.

Estrus response, as seen in Table 1; were found as 90% in Group 1 (control) and in Group 2. The Estrus rates in Group 1 and 2 were found considerably higher than Group 3 ($p<0.001$). These findings are similar to the results reported by Beck *et al*. (1996), which $PGF_{2\alpha}$ applied after 5 days following GnRH provides high level estrus synchronization. In addition, the present study confirms that GnRH with $PGF_{2\alpha}$ administration 7 days later is able to synchronize estrus in ewes and showed that this treatment is less effective for estrus synchronization than other estrus synchronization protocol studied. Thus, it was assumed that CL was sensitive to $PGF_{2\alpha}$ on day 5 day after GnRH administration. Beck *et al*. (1996) reported high levels of oestrous synchronization and fertility using a treatment of GnRH followed by $PGF_{2\alpha}$ 5 days later during the breeding season. However, it was seen that this

Table 1: The effect of different synchronization method in Awassi ewes on estrous response, pregnancy rate and lambing rate

Parameters	Group 1 $PGF_{2\alpha}$ + $PGF_{2\alpha}$	Group 2 GnRH + $PGF_{2\alpha}$ (5 days)	Group 3 GnRH + $PGF_{2\alpha}$ (7 days)	p-value
No. Ewes	n = 20	n = 20	n = 20	
Estrous response	18/20 (90.0)[a]	18/20 (90.0)[a]	7/20 (35.0)[b]	<0.001
Pregnancy rate	14/18 (77.8)[a]	15/18 (83.3)[a]	3/7 (42.8)[c]	<0.050
Lambing rate	14/18 (77.8)[a]	15/18 (83.3)[a]	3/7 (42.8)[d]	<0.050

[a-d]Means in the same row with different superscripts differ significantly

sensitivity was considerably decreased in potential CL on day 7 day on ovarium. When exogenous GnRH is administered to the ewes in any phase of estrus cycle, it causes LH secretion. Thus, it could initiate a new follicular wave causing ovulation or atresia (Webb *et al*., 1992; Wolfenson *et al*., 1994). The luteal tissue formed after GnRH injection can perform $PGF_{2\alpha}$ luteolizing performed 5 days later (Beck *et al*., 1996; Husein and Kridli, 2003). GnRH + $PGF_{2\alpha}$ protocol (7 days), the fact that development phase of either luteal function or dominant follicle in luteolizing time could decrease estrus synchronization rate. The extending luteal function might change the estrogen/progesterone rate in follicles (Bartlewski *et al*., 2000).

Pregnancy rate was found as 77.8% in Group 1 and 83.3% in Group 2. The pregnancy rate in Group 1 and 2 were found considerably higher than Group 3 (35.72%) ($p<0.05$). The pregnancy rate obtained from the inseminations that were performed after synchronized estrus was found similar in double dose $PGF_{2\alpha}$ and GnRH + $PGF_{2\alpha}$ (5 days) (Table 1). Beck *et al*. (1996) observed a high pregnancy rate in cyclic ewes treated $PGF_{2\alpha}$ after 5 days following the GnRH. When we compare GnRH + $PGF_{2\alpha}$ (7 days) group with the other 2 groups with regard to the pregnancy rate, the results are considerably low. This case is arised from the animals in GnRH + $PGF_{2\alpha}$ (7 days) group show low estrus rate than the other groups. So, it can be said that $PGF_{2\alpha}$, applied on day 7 following GnRH application, causes increase in fertility. When each group is compared with regard to the pregnancy rate, no considerable difference was found among the groups (Table 1). It was suggested that the extension of the lifespan of the CL in the ewes caused increase in fertility (Evans *et al*., 2001). The fact that either pregnancy rate or breeding rate will be effective in increasing reproductive efficiency of $PGF_{2\alpha}$ performed on day 7 following GnRH application supports our hypothesis.

Lambing rate was found as 77.8% in Group 1, 83.3% in Group 2 and 35.72% in Group 3. The lambing rate in Group 2 were found significantly higher than Group 3 ($p<0.05$) depending on low estrus rate in Group 3.

CONCLUSION

These results suggest that effect of induction estrus and pregnancy and lambing rate after GnRH and 5 days later an i.m. injection of prostaglandin $F_{2\alpha}$ were higher than GnRH and $PGF_{2\alpha}$ treatment followed 7 days injection of $PGF_{2\alpha}$.

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