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# Timing Ovulation in Ewes Treated with Ovsynch Protocol by Different Times of $PGF_2\alpha$ Injection during the Breeding Season

Research Article

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## ABSTRACT

This study was carried out at Sakha Animal Production Research Station, during the period from Oct., 2009 to Sep., 2010. Forty Rahmani ewes were divided into three treatment groups: the 1st, 2nd and 3rd treatment groups were intramuscularly injected (Day 0) with 1 mL GnRH analogue followed by an intramuscular injection with 0.7 mL $PGF_2\alpha$ 5 (G1), 6 (G2) or 7 (G3) days later. A second dose of 1 mL GnRH analogue was given on day 7 (G1), 8 (G2) or 9 (G3), and artificially insemination of treated doze was carried out 24 h later, while the 4th group represented the control ewes which were allowed for natural mating from 1st to the end of January, the breeding season. Results show that one out of 10 ewes (10%) exhibited estrous activity in G1 versus 30% (3 out of 10 ewes) in both G2 and G3. Ewes in G1 treatment group showed highest ($P<0.05$) lambing rate (60%), followed by G2 (50%) and the lowest in G3 (40%), while, lambing rate of the controls was 60%. Litter size and fecundity were significantly the highest ($P<0.05$) in G1 (1.67/litter and 100.2%), followed by G2 (1.40/litter and 70%) and the lowest in G3 (1.25/litter and 43%), respectively. $P_4$ concentration was significantly ($P<0.05$) increased in all treatment groups as affected by the 1st GnRH injection, thereafter, it showed marked decrease in all treatment groups post-PGF2α injection. Post-2nd GnRH injection, $P_4$ level showed again a pronounced increase in all treatment groups. On day 21-24 post-mating, $P_4$ level showed the highest values in all treatment groups. Based on the foregoing results, using GnRH-PGF2-GnRH (GnRH, 0 d; $PGF_2\alpha$ 5 d later and GnRH 48 h later) protocol during breeding season can be used for synchronization of estrus and ovulation to reduce service and lambing interval of ewes in the large flocks.

KEY WORDS estrus, ewes, lambing, litter size, Ovsynch, progesterone.

## INTRODUCTION

Estrus synchronization of ewes has been accomplished using several methods with various degrees of success. The progesterone impregnated intravaginal sponges, left *in situ* for 12-17 days in the breeding season, is a widely used method. But, another equally effective method is two injections of $PGF_2\alpha$ at 11-day interval. Although, the two methods used during breeding season can give high/sufficient rates of synchronization and lambing, they have some disadvantages of being applied for a long period. Intravaginal sponges can usually induce inflammation with adherence to vaginal mucosa, following the withdrawal of the pessary; a rich discharge with unpleasant odour is usually observed (Larsson *et al.* 1991). Also, after the second $PGF_2\alpha$ administration in the method of two injections of PGF2α, there are no signs of estrus and follicular development in some ewes not responding to the second PGF2α application, and insufficient luteal tissue sensitive to $PGF_2\alpha$, because luteolysis

or formation of the corpus luteum (CL) may be late after the first $PGF_2\alpha$ injection (Alaçam, 1994).

In the cyclic animals, a follicular wave terminates when the dominant follicle either regresses or ovulates, leading to the start of a new wave of follicular growth. An injection of GnRH analogues 6 days prior to an injection of $PGF_2\alpha$, enhances the conception rate (Stevenson *et al*. 1996), increases number of synchronized animals, and reduces variability of time to estrus (Twagiramungu *et al*. 1992). This decrease may be explained by the initiation of a new follicular wave following injection of GnRH, which results in a new dominant follicle, being present at the time of $PGF_2\alpha$ injection (Pursley *et al*. 1998). The time in this method for synchronization procedures is much shorter than in the other methods such as progesterone ($P_4$) sponges, $P_4$ implant or double doses of $PGF_2\alpha$. This method does not require the use of $P_4$ sponges and is therefore, less laborious. In addition, side effects like unpleasant and rich odour or surgical processes in the method of implant have not been observed (Ataman and Aköz, 2006).

While, early studies demonstrated the success of GnRH-based protocols in synchronizing and inducing estrus in cycling and anestrous cattle, fewer studies have examined the use of GnRH in ewes. The GnRH based on out-of-season breeding protocols are aimed at providing a source of $P_4$ by inducing ovulation or luteinization of follicles. Many studies differed in timing, dosage, and method of treatment with GnRH. Several GnRH products that are commercially available included Cystorelin (Merial), Factrel (Fort Dodge Laboratories), OvaCyst (Vedco), and Fertagyl (Intervet) (Lamb, 2002).

There are several reasons for wishing to control the time of estrus/ovulation in the ewes. In small herds, it may be a question of not having a rams available to detect estrus in ewes that have to be transported to the male for breeding. In larger herds, AI can be applied on a fixed time basis when an accurate method of controlling estrus and ovulation are employed (Wildeus, 1999). Therefore, the objectives of this study were to determine the effectiveness of GnRH PGF2$\alpha$+GnRH protocols to synchronize estrus and (or) ovulation of ewes during the breeding season.

## MATERIALS AND METHODS

This study was carried out at Sakha Animal Production Research Station, belonging to Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture during the period from Oct. 2009 to Sept. 2010.

### Animals and experimental groups

Total of 40 Rahmani ewes (45-61±3.14 kg live body weight, 3-4 years old and 2-3 parities) from the flock of Sakha Animal Production Research Station were used in this study. Ewes were divided into three treatment groups and control group, 10 in each. In the 1st, 2nd and 3rd treatment groups (G1, G2 and G3), ewes were intramuscularly injected (Day 0) with 1 mL GnRH analogue (Receptal, Intervet International BV Boxmeer-Holland, each 1 mL of Receptal contained 0.0042 mg Buserelin acetate equivalent to 0.004 mg Buserelin) followed 5 (G1), 6 (G2) or 7 (G3) days later by intramuscular injection of 0.7 mL $PGF_2\alpha$ (Estrumate, Coopers Animal Health LTD, Berkhamsted-England, each 1 mL of Estrumate contained 263 µg Cloprostenol Sodium equivalent to 250 µg Cloprostenol). A second dose of 1 mL GnRH analogue was given on day 7 (G1), 8 (G2) or 9 (G3) and artificial insemination of treated does was carried out 24 h later for ewes in all of the treatments.

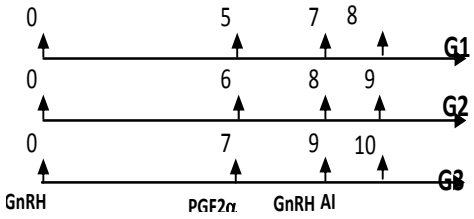


**Figure 1** GnRH-PGF2α-GnRH protocols by different times of $PGF_2\alpha$

On the other hand, 10 ewes without any treatment served as a control group and were allowed for natural mating during the same breeding season, while having no treatment at all. The control ewes were exposed to fertile ram from the contemporary to that of treatment ewes start time (1st Jan.) up to the end of the breeding season (end of Jan.).

### Housing and feeding system

All experimental ewes were housed in semi-open sheds in groups and fed concentrate feed mixture (CFM) and roughages. The average daily amount of the diet offered to each ewe was composed of 1.25 kg CFM (14% CP) beside 5 kg Egyptian clover during winter or 1.5 kg clover hay during summer.

### Semen dilution and artificial insemination

For all ewes in treatment groups, semen had been collected by the use of artificial vagina and diluted just before insemination. Immediately after semen collection, the fresh semen was taken for determination of sperm motility (Bane, 1982). Only ejaculates of >80% initial motility were diluted by Tris-yolk extender. Each 100 mL of Tris-yolk extender consisted of 3.025 g Tris, 1.675 g citric acid, 0.75 g glucose, 15 mL egg yolk, 1 mL antibiotics containing 100.000 IU penicillin and 100.000 µg streptomycin and distilled water up to 100 mL (Leboeuf *et al*. 2000).The extender was prepared and kept at 5 °C for 24 h before semen dilution. All chemicals used for the preparation of extender were

purchased from Sigma Chemical Company (P.O. Box 14508, Sant Louis, MO 63178, USA).

The extender was gently shacked and warmed up to 37 $^{o}$C using a water bath before semen dilution. The collected semen was diluted (1 part semen: 4 parts extender) at 37 $^{o}$C. In diluted semen, sperm concentration was about $300\times10^{6}$ sperm/mL and sperm motility remained over 70%. Insemination was carried out using a simple inseminating pipette with fine blunt bent end and a vaginal speculum. Semen was deposited into the cervix as far as possible (about 1 cm).

**Laparoscopic examination**

Laparoscopy (Wolf/8933/7 mm-made in USA with W German cens system) was used to visualize the ovarian structure changes 7 days post- insemination. Six fastened (16 hours before examination) ewes in each treatment group were examined for corpora lutea (CLs) count. The CLs were determined by the aid of scales on the stainless-steal rode used for the genital tract manipulation.

**Reproductive efficiency**

Estrous activity in term of number of responded ewes, and onset and the duration of estrus was recorded post-$PGF_2\alpha$ injection in all treatment groups. Also, lambing rate, and date and period of lambing were recorded. Fecundity rate (Number of born lambs/total number of treated ewes)×100 was calculated.

**Blood sampling**

Blood samples were taken morning before feeding via the jugular vein from all ewes into evacuated tubes (10 mL). The collected blood samples were separated to obtain serum by centrifugation of blood at 2500 rpm for 15 min. Serum was packed in labeled plastic tubes and stored at −20 $^{o}$C until assayed later for $P_4$ concentration. Blood sampling in all groups was just pre-treatment, one day post-1$^{st}$ GnRH, one day post-$PGF_2\alpha$, one day post-2$^{nd}$ GnRH (Day of AI) and on day 21 and 24 post-insemination (Pregnancy test).

**Progesterone assay**

Progesterone concentration was determined by Radioimmunoassay procedure in samples of selected 5 animals (3 ewes lambing+2 ewes of which did not conceived) of each treatment group. Quantitative determination of progesterone in serum samples was carried out using progesterone radioimmunoassay kit (catalog No. 1188 manufactured by Immunotech, France). The assay is based on competition reaction (Bojanic *et al*. 1991). Samples reaction (50 µL for progesterone) was incubated 1 h with $I^{125}$ labeled progesterone (500 µL), as tracer, in antibody-coated tube. After the incubation, the liquid contents of the tubes are aspirated and bound radioactivity is measured to determine progesterone in serum using automatic Mini-Gama counter (LKB 1275, USA). The sensitivity of the assay progesterone was 0.03 ng/mL, while, coefficient of variation was 4.3% for both progesterone intra- and inter-assay, respectively.

**Statistical analysis**

For onset and duration of estrus, litter size, number of CLs and $P_4$ concentration, one way analysis of variance was performed to obtain the effect of treatment. However, estrus, lambing and fecundity rates were performed using Chi square test. The obtained data was statistically analyzed according to Snedecor and Cochran (1982) using computer program of SAS system (2000). The significant differences among groups were carried out using Multiple Range Test of Dancan (1955). All significant differences were set at $P<0.05$.

## RESULTS AND DISCUSSION

**Estrous Activity Post-$PGF_2\alpha$ Injection**

Estrus response to $PGF_2\alpha$ injection on day 5, 6 and 7 for ewes in G1, G2 and G3, respectively in terms of rate (%), time of incidence (h) and duration (h) of estrus is presented in Table 1. Results show that one out of 10 ewes (10%) exhibited estrous activity after $PGF_2\alpha$ injection in G1 versus 30% (3 out of 10 ewes) in both G2 and G3. This means the higher estrus rate of ewes in response to $PGF_2\alpha$ injection on day 6 or 7 than those injected on day 5 (30 vs. 10%, Table 1).

**Table 1** Estrus rate (%), time of incidence (h) and duration (h) of estrus after $PGF_2\alpha$ injection for ewes in different treatment groups*

| Treatment group | N | Estrus response to post-$PGF_2\alpha$ injection | | | |
|---|---|---|---|---|---|
| | | Induction rate | | Onset of estrus (h) | Estrous duration (h) |
| | | n | % | | |
| G1 | 10 | 1 | 10 | 42.0±0.00$^{b}$ | 30.0±0.0 |
| G2 | 10 | 3 | 30 | 46.0±1.63$^{ab}$ | 34.0±3.2 |
| G3 | 10 | 3 | 30 | 49.3±1.08$^{a}$ | 32.0±1.6 |

*The means that have at least one common letter within the same column, do not have significant difference (P>0.05).

Although, one ewe exhibited estrus in G1, she showed the shortest time of incidence (42 h) and duration (30 h) of estrus after $PGF_2\alpha$ injection versus moderate time of incidence (46 h) and the longest duration (34 h) of estrus in G2 as well as the longest time of incidence (49.3 h) and moderate duration (32 h) of estrus in G3 (Table 1).

In the present study, estrus rate was lower than the one obtained by some authors. In this respect, estrus rate was 93.3% (Ataman and Aköz, 2006) or 90.9% (Beck *et al*. 1996) after the GnRH-PGF2α (100 µg of busereline and 5 later injection of 0.294 mg of Triaprost tromethamine, an analogue of PGF2α), while estrus rate was 86.6% (Ataman

and Aköz, 2006; Fritzgerald *et al*. 1985) or 100% (Öztürkler *et al*. 2003) after double PGF2α (9 days interval), respectively. The observed reduction in the present results might be due to the breed, dose and PGF2α analogue and time of treatment during the year (season).

**Lambing performance**

Results in Table 2 show that the lowest response to $PGF_2\alpha$ injection in G1 was significantly (P<0.05) associated with the highest lambing rate (60%), followed by G2 (50%) and the lowest in G3 (40%). On the other hand, lambing rate of the control group (60%) was similar to that of G1 (60%). In addition, there are slight differences in lambing period (the period from the 1st to last lambing) among treatment groups, being 6 d in both G1 and G3 versus 4 d in G2. However, a pronounced difference was observed between the control group from one side and the treated groups on the other side (18 vs. 4-6 days, Table 2).

**Table 2** Lambing rate (%) and lambing period (day) of ewes in different treatment groups and control group*

| Group | N | Lambed ewes | | Lambing period | |
|---|---|---|---|---|---|
| | | n | % | Date (from-to) | Duration (day) |
| G1 | 10 | 6 | 60[a] | 01/06-06/06 | 6 |
| G2 | 10 | 5 | 50[ab] | 01/06-04/06 | 4 |
| G3 | 10 | 4 | 40[b] | 01/06-06/06 | 6 |
| Control | 10 | 6 | 60[a] | 26/05-12/06 | 18 |

*The means that have at least one common letter within the same column, do not have significant difference (P>0.05).
N: Total number of ewes.

The present lambing rates are similar to 63.0% as reported by Das *et al*. (2000) and lower than 82% (Husein and Kridli, 2003) in ewes treated with $P_4$-GnRH-PGF2α treatment, 100% (Horoz *et al*. 1999) in ewes treated with PGF2α at 9-d interval, 85.3 and 81.8% (Ataman and Aköz, 2006) in ewes treated with GnRH-$PGF_2\alpha$ protocol and double PGF2α, respectively, and 88.8 and 92.5% (Beck *et al*. 1996) in ewes treated with 4 µg of the GnRH agonist (Buserelin) followed, 5 days later, by an injection of 100 µg of cloprostenol or with two injections of $PGF_2\alpha$ (125 µg cloprostenol, 11-days apart). However, the obtained results are higher than 43.75% reported by Simonetti *et al*. (2000). The resetting follicular development could produce a new dominant follicle that contains an oocyte of greater potential fertility, which would lead to greater embryonic survival. An injection of GnRH analogues 5 d prior to an injection of PGF2α, enhanced the conception rate and higher synchronization rate than double PGF2α injection (Mihm *et al*. 1999).

**Litter size and fecundity**

Results in Table 3 show significant differences in litter size (LS) and fecundity rate (FC) among treatment groups. The LS and FC were significantly (P<0.05) the highest in G1 (1.67/litter and 100%), followed by G2 (1.40/litter and 70%) and the lowest in G3 (1.25/litter and 50%), respectively. In comparing treatments with control group, ewes in G1 showed higher LS and FC than the control one (1.67 and 100 vs. 1.50 and 90%, respectively), but the differences were not significant.

**Table 3** Litter size and fecundity of ewes in different treatment groups and control group*

| Treatment group | N | Born lambs | | Fecundity (%) |
|---|---|---|---|---|
| | | Number | Litter size | |
| G1 | 10 | 10 | 1.67[a] | 100.0[a] |
| G2 | 10 | 7 | 1.40[ab] | 70.0[b] |
| G3 | 10 | 5 | 1.25[b] | 50.0[c] |
| Control | 10 | 9 | 1.50[a] | 90.0[a] |

*The means that have at least one common letter within the same column, do not have significant difference (P>0.05).
N: Total number of ewes.

In spite of the marked reduction in LS and FR in G2 and G3 as compared to the control, the positive effect of treatment was observed in terms of reducing lambing period as a result of synchronizing ovulation and timing of AI in G2 and G3 (Table 2). In accordance with the present results, Beck *et al*. (1996) found that LS was 1.69 and 1.74 for ewes treated with GnRH-$PGF_2\alpha$-GnRH protocol or double $PGF_2\alpha$ 11-days apart.

The low fertility rate is primarily attributed to factors including breed, heredity, environment, management and the reproductive soundness of the ewes (Husein and Kridli, 2002). Ovulation, fertilization and early embryonic mortality rates are also among the factors influencing the litter size (Beck *et al*. 1994). Of these factors embryonic mortality has been considered to be the greatest limitation to reproductive efficiency across mammalian species and has been estimated between 25 and 60% (Roberts *et al*. 1985). Early embryonic mortality usually occurs during the first 3 weeks of gestation which results in pregnancy rates ranging from 16 to 76% (Nephew *et al*. 1994).

**Ovarian structure day 7th post-insemination**

Laparoscopy examination on Day 7th post-insemination in term of number of CLs and follicles on the right and left ovaries of ewes in different treatment groups is shown in Table 4. The examination of the right ovaries shows that the number of CLs was significantly (P<0.05) greater in G1 than that of G2 and G3 (1.16 vs. 0.33 and 0.33/ovary). The observed greater CLs in G1 was associated with the presene

of three CLs on right ovary and increasing number of ewes bearing CLs (4 out of 6 ewes) averaging 66.7% of all the examined ewes versus presence of one CL on the ovary of 33.3% in ewes of both G2 and G3. However, the differences in number of follicles among treatment groups were not significant (Table 4).

The examination of the left ovaries showed that the differences in number of CLs were not significant. However, the number of follicles was greater in G1 and G3 than that of G2 (1.16 and 1.0 vs. 0.16/ovary, respectively). The observed increase in follicular number of ewes in G1 and G3 was associated with presence of 2-4 follicles on right ovary. Based on the total number on both right and left ovaries, the differences among treatment groups in number of follicles were not significant. Ewes in G1 showed the greatest total CLs number (2.16/ewe) and were followed by those in G2 (1.5/ewe). Meanwhile, ewes in G3 showed the least CLs number (Table 4).

In agreement with the present results, El-Gohary (2006) reported that the total number of CLs ranged between 1.1 and 1.4 CL for Rahmani ewe. In pregnant ewes up to d 26 post-mating, follicular waves have also been documented, based on ultrasonographic observations. It is worthy noting that no follicle≥3 mm was observed on the ovaries in sheep. This local inhibition of the CL on follicular dynamics is sustained, in the ovary that bore the CLs during pregnancy, for up to 4 weeks after parturition (Bartlewski *et al*. 2000). Progesterone profile: Results in Table 5 revealed that $P_4$ level was significantly ($P<0.05$) the highest in G2 (3.12 ng/mL), followed by G1 (1.50 ng/mL) before treatment and the lowest in G3 (0.73 ng/mL). These levels may indicate that most ewes in G1 and G2 were in luteal phase, while those in G3 were in follicular phase. Post-1$^{st}$ GnRH injection, $P_4$ level significantly ($P<0.05$) increased in all treatment groups, being the highest in G2 (5.40 ng/mL), moderate in G2 (4.13 ng/mL) and the lowest in G1 (3.27 ng/mL). Such trend may indicate higher response to GnRH injection of ewes in all treated groups, being in luteal phase and reflecting nearly synchronization of the reproductive status of ewes in all the treatment groups (Table 5). Similar trend was observed by Beck *et al*. (1996), who showed that treatment with GnRH resulted in higher plasma $P_4$ concentrations.

Post-$PGF_2\alpha$ injection, $P_4$ level significantly ($P<0.05$) decreased to the minimal values in all treatment groups, being less than 0.5 ng/mL without significant differences among treatment groups. Such reduction may indicate higher response to $PGF_2\alpha$ injection in term of CLs regression induced after ovulation by 1$^{st}$ GnRH injection. Post-2$^{nd}$ GnRH injection, $P_4$ level again showed significant increase in all treatment groups ($P<0.05$). This elevation in $P_4$ level was associated with the initiation of new CLs as affected by 2$^{nd}$ GnRH injection, but the differences in $P_4$ level among groups were not significant (Table 5).

Sheep and cattle, as the other domestic species, show two stages of ovarian antral follicle development (Mihm and Bleach, 2003). First, a slow growth phase which is believed to be independent of gonadotropins (Cahill, 1981; Lussier *et al*. 1987), second, a fast growth phase that requires gonadotropin support, and is usually described as a follicle wave (Sunderland *et al*. 1994). Recruitment refers to the synchronized growth of a group of ovarian antral follicles that eventually gain the ability to fully respond to endocrine (gonadotrophic) stimuli. Selection is the process by which only a limited numbers of these cohorts of follicles are rescued from atresia and continue to grow to an ovulatory size. Dominance is a characteristic of a large selected ovarian antral follicle (dominant follicle) of a wave or cohort of follicles that permits its survival and further development in an endocrine environment suppressive to other co-existing follicles (subordinate follicles). Follicle emergence or follicular wave emergence is the beginning of the growth of a group of antral follicles from the minimum recordable size that subsequently ovulate or undergo atresia (Ginther *et al*. 1996).

The marked increase in $P_4$ concentration in ewes of all treated groups following GnRH administration could be due to the sudden release of LH, leading to ovulation or luteinization of dominant follicles of the present wave (Örsan *et al*. 2007). The pulsatile or tonic mode of LH release is generated in response to pulsatile GnRH release from the hypothalamus (Levine *et al*. 1982). Pulsatile LH release prevails at all reproductive states in ewes, including the period before, during and after the preovulatory surge of gonadotropins (Rawlings and Cook, 1993), and it is also present in ovariectomized ewes. In cyclic ewes, low-amplitude pulses occur from 1 to 6 times in a 6 h period (Goodman *et al*. 1981). An increase in tonic LH secretion during the proestrous period results from an increase in pulse frequency (Baird, 1978). An increment in basal (non-pulsatile) LH release during the surge was also suggested (Rawlings and Cook, 1993).

On day 21-24 post-mating, $P_4$ level showed the highest values above 1 ng/mL in all treatment groups as a result of pregnancy incidence, being higher in G2 (7.58 ng/mL and G3 6.56 ng/mL) than in G1 (4.13 ng/mL) (Table 5).

Within both groups of ewes, plasma $P_4$ concentrations were different in pregnant ewes, but were almost at a normal $P_4$ profile. Normal $P_4$ profiles in ewes induced to ovulate 35 d postpartum was observed by Wallace *et al*. (1992). Corpora lutea (CLs) secrete $P_4$ later with respect to the LH surge and at a lower rate than CLs formed after subsequent ovulations (Schirar *et al*. 1989). It would also appear that basal and LH stimulated $P_4$ secretion is suppressed in luteal

**Table 4** Number of CLs and follicles on ovaries of treated ewes post 7 days of insemination*

| Treatment group | Right ovary | | Left ovary | | Total | |
|---|---|---|---|---|---|---|
| | CLs | I9Follicles | CLs | Follicles | CLs | Follicles |
| G1 | 1.16±0.43$^{a}$ | 1.66±0.30 | 1.00±0.23 | 1.16±0.59$^{a}$ | 2.16±0.36$^{a}$ | 2.50±0.73 |
| G2 | 0.33±0.19$^{b}$ | 2.00±0.52 | 1.17±0.15 | 0.16±0.15$^{b}$ | 1.50±0.20$^{ab}$ | 2.16±0.64 |
| G3 | 0.33±0.19$^{b}$ | 1.33±0.30 | 1.00±0.23 | 1.00±0.33$^{a}$ | 1.17±0.28$^{b}$ | 2.33±0.45 |

*The means that have at least one common letter within the same column, do not have significant difference (P>0.05).

**Table 5** Progesterone concentration (ng/mL) during different treatment periods in different treatment groups*

| Treatmenttime | Treatment group | | |
|---|---|---|---|
| | G1 | G2 | G3 |
| Pre-1$^{st}$ GnRH | 1.50±0.260$^{Cb}$ | 3.12±0.142$^{Ca}$ | 0.73±0.012$^{Dc}$ |
| Post-1$^{st}$ GnRH | 3.27±0.311$^{Bb}$ | 5.40±0.489$^{Ba}$ | 4.13±0.866$^{Bab}$ |
| Post-PGF2α$^{*}$ | 0.45±0.001$^{D}$ | 0.47±0.002$^{E}$ | 0.45±0.001$^{D}$ |
| Post-2$^{nd}$ GnRH | 1.00±0.581$^{CD}$ | 1.68±0.383$^{D}$ | 1.14±0.218$^{C}$ |
| Post-mating (21-24 d) | 4.13±0.017$^{Ab}$ | 7.58±0.443$^{Aa}$ | 6.56±0.422$^{Aa}$ |

* Pre-2$^{nd}$ GnRH.
A, B…E: The means that have at least one common letter within the same column, do not have significant difference (P>0.05).
a, b and c: The means that have at least one common letter within the same row, do not have significant difference (P>0.05).

cells collected following the first postpartum ovulation in the ewe (Braden *et al*. 1989).

In sheep, this rise takes the form of an increase of frequency of the pulsatile LH discharges to hourly (Barid, 1978), thus producing a progressive four- to five-fold increase in mean serum LH concentrations which persists for 2-3 days (Karsch and Foster, 1981).

The mentioned increase in tonic LH secretion is required for the growth and development of the preovulatory ovarian follicles and the stimulation of the developing follicles to secrete more and more oestradiol ($E_2$) possibly by promoting biosynthesis of the androgenic precursors for oestradiol (Goodman *et al*. 1981; Karsch and Foster, 1981).

During the estrous cycle, the negative feedback effect of oestradiol on GnRH secretion is limited by elevated $P_4$ (Barid and Scaramuzzi, 1976; Karsch *et al*. 1979) and oestradiol may inhibit pulse amplitude by an action on the pituitary to decrease its response to GnRH (Goodman and Karsch, 1980).

Since OV-Synch protocol is now applicable for cows, studying the implementation of this protocol for ewe is valuable. Based on the foregoing results, using GnRH-PGF2α-GnRH protocol (0-5-7) during the breeding season pregnancy rates were greatest for ewes synchronized with the OV-Synch (G1), providing additional evidence that inseminating ewe at a fixed time may provide greater preg nancy rates than with estrous detection and reduce lambing period for flocks of greater number of ewes.

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