



Contents lists available at BioMedSciDirect Publications

## International Journal of Biological & Medical Research

Journal homepage: [www.biomedscidirect.com](http://www.biomedscidirect.com)



### Original Article

# Efficiency of short time protocols based on combined FGA, PGF2 $\alpha$ , GnRH and eCG treatments on oestrus synchronization and reproductive performance of kermani ewes during the breeding season

Nasroallah Moradi Kor<sup>a\*</sup>, Kaveh Mohammadi Khanghah<sup>b</sup>, Ali Veisi<sup>c</sup>

<sup>a</sup> Young Researchers Club, Baft Branch, Islamic Azad University, Baft, Iran, <sup>b</sup> Department of Animal Reproduction, Razi University of Agricultural Sciences and Natural Resources, Kermanshah, Iran, <sup>c</sup> Department of Animal Breeding, Razi University of Agricultural Sciences and Natural Resources, Kermanshah, Iran.

#### ARTICLE INFO

##### Keywords:

Estrus Synchronization,  
Kermani sheep,  
breeding season,  
eCG

#### ABSTRACT

The objectives of the present study were to determine the efficiency of short time protocols based on combined FGA, PGF2 $\alpha$ , GnRH and eCG treatments on oestrus synchronization and reproductive performance of kermani ewes during the breeding season. Seventy-five multiparous non-lactating kermani ewes weighing  $45 \pm 0.5$  kg with a body condition score ( $3.6 \pm 0.1$ ) were allocated into five groups. The treatment are shown in article body. The ewes were checked for oestrus by teaser rams at 8 h intervals. The ewes were hand-mated (male to female ratio 1:5) at the beginning of oestrus and subsequently 12 h intervals until the end of oestrus. The percentages of female in estrus and the interval to oestrus (h after treatment), fertility and prolificacy rate were recorded. The obtained data were analyzed using the GLM procedure of SAS system. Treatments had no significant effect on incidence of oestrus, however, ewes received T4 tended to show a lower oestrus. Hormonal treatments significantly affected the interval to oestrus which was earlier in treated control ewes than in T<sub>4</sub> and T<sub>2</sub> ( $P < 0.05$ ). Fertility rate tended to be higher in T<sub>3</sub> and T<sub>2</sub> groups compared to that of control group also Prolificacy rate was higher in T<sub>4</sub> group. Results of the present study showed that PGF2 $\alpha$ -FGA-eCG as well as FGA-PGF2 $\alpha$ -eCG short term 5-day treatments is effective in synchronizing oestrus in ewes during the breeding season. Also GnRH-PGF2 $\alpha$ -eCG 5-day treatment has been found effective to provide high level of oestrus followed by acceptable levels of fertility after natural mating.

© Copyright 2010 BioMedSciDirect Publications IJBMR -ISSN: 0976:6685. All rights reserved.

### 1. Introduction

Estrus synchronization or the induction of estrus is a valuable management tool for increasing the pregnancy rate in ewes [1]. Modern sheep husbandry has improved the efficiency of extensive production and controlled the reproductive process for intensive production [2]. The most economically important trait in sheep production is reproduction and it can be manipulated using hormonal treatments [3]. Several techniques have been developed to induce out of season estrus in sheep, allowing farmers to raise and provide the market lambs year round.

Intravaginal devices containing different types of progestogens, maintained in situ during 12–14 days, associated with gonadotrophin administration is the most widely were used. An extension of the lifespan of the ovulatory follicle, as a consequence of low concentrations of progesterone, may be associated with a low viability of the ovulated oocyte [4]. The fertility of the ewe is affected in a dose dependent manner by fluorogestone acetate [5] or progesterone in intravaginal devices [6]. Treatment with intravaginal sponge impregnated with (FGA) for a period of 10-16 days and intramuscular injection of (PMSG) at intravaginal device removal, have been successfully used to improved the reproductive performance in ewes [7, 8]. It has been shown that the administration of gonadotropins such as equine chorionic gonadotropin (eCG) stimulates follicular growth and increases ovulation rate and fertility and induces a tighter synchrony of ovulation in both anestrous and cycling sheep [9]. The use of AI is facilitated after estrous synchronization programs which induce

\* Corresponding Author : Nasroallah Moradi kor  
Member of Young Researchers Club,  
Baft Branch, Islamic Azad University, Baft,  
Iran. Phone: +98137684047,  
E-mail address:  
[Moradikor@iaubaft.ac.ir](mailto:Moradikor@iaubaft.ac.ir)

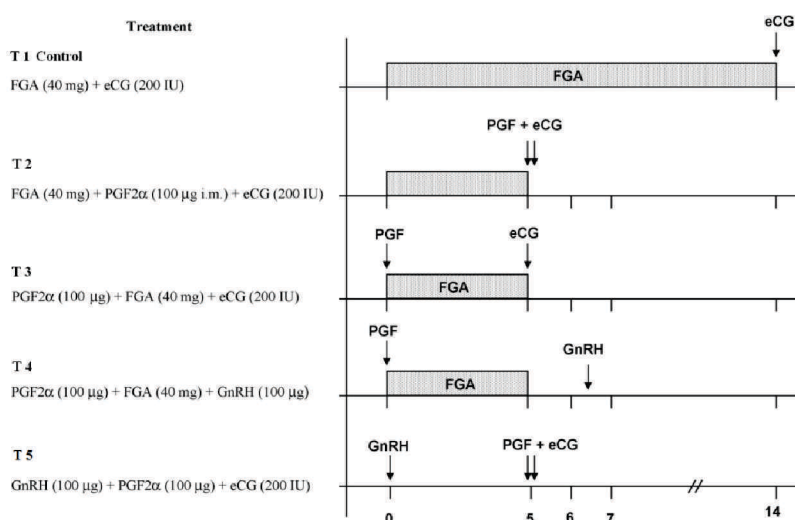
© Copyright 2010 BioMedSciDirect Publications. All rights reserved.

tight estrus during a short period of time, and improve pregnancy and prolificacy rates. During the breeding season, when goats are actively cycling, estrus can be synchronized with PGF<sub>2</sub> $\alpha$  or one of its analogues, such as cloprostenol [10]; however, the number of observations in different breeds is still insufficient for allowing firm conclusions [11]. The most extensively researched method [12] is the use of vaginal sponges impregnated with 40 to 50 mg of flugestone acetate FGA. [13] showed that induction of estrus in indigenous Damascus goats, outside the breeding season by using medroxyprogesterone acetate (MAP) plus injection of eCG (equine chorionic gonadotropin) at the time of sponge removal resulted in estrous response of 100%; conception rate and fecundity were 65.8% and 195.2% respectively. Oestrus and ovulation synchronization technologies in ewes are mainly based on the control of corpora lutea lifespan with prostaglandin or using progestagen/progesterone treatments plus equine chorionic gonadotropin (eCG) for 12–14 days [10, 14]. Prostaglandin alone does not provide an acceptable synchrony of oestrus, which depends on the stage at the time of the oestrus cycle when prostaglandin-induced luteolysis [15, 16]. Moreover, this method is only applicable in cyclic females. Progestagen treatment systems are quite long and do not meet the expectations of dairy ewe farmers because products (milk and derived cheeses) cannot be used during the treatment period. Therefore, in order to shorten the treatment period, a luteolytic dose of PGF<sub>2</sub> $\alpha$  can be injected at the end or 24–48 h before the end of progestogen treatment [6, 14]. However, such methods have resulted in a high fertility rate variability. Improved knowledge of ovarian dynamics [17, 18] and wave-like patterns with the presence of follicular dominance [19, 20, 21] indicate possibilities to control follicular development by synchronization of the follicular wave that gives rise to the preovulatory follicle, and synchronize oestrus and ovulation. In cattle, GnRH injections promote LH release thus inducing either ovulation or atresia of the dominant follicle followed by the appearance of a new follicular wave in a synchronous way [22, 23]. Treatments with a combination of GnRH plus PGF<sub>2</sub> $\alpha$  have been used to control ovarian follicular and luteal function and increase the precision of oestrus and ovulation synchronization in reproductive management programs [24]. Therefore, the present investigation was conducted to compare the efficiency of combined progestagen, PGF<sub>2</sub> $\alpha$ , GnRH and eCG treatments in order to develop a short time protocol for synchronizing oestrus in kermani ewes before mating during the breeding season.

## 2. Materials and Methods

This experiment was conducted on Rezvan junior college farm in kerman provinces (latitude 300 18 / N, longitude 570 7 / E, altitude 1755 m) during the breeding season from September to February 2010. The average ambient temperature during experiment was 30 to 35° C. The annual rainfall in this region ranges from 140 to 155 mm, with an erratic distribution throughout the year. The animals were submitted to examination for general clinical condition, sanitary and reproductive health. This experiment aimed at evaluating the efficiency of short time protocols based on combined FGA, PGF<sub>2</sub> $\alpha$ , GnRH and eCG treatments on oestrus synchronization and reproductive performance following natural service. Seventy-five multiparous non-lactating kermani ewes weighing 45  $\pm$  0.5 kg with a body condition score (3.6  $\pm$  0.1) were allocated into five groups (Fig. 1).

**Fig. 1. Experimental design and timeline for treatment administration.**



The groups are subsequently defined by letters represented the sequence and combination of treatments used: (T1) control = FGA (fluorogestone acetate intravaginal sponges, 40 mg, Intervet, Milano, Italy) for 14 days + eCG (Ciclogonina, Intervet, Milano), 200 IU i.m. at sponge removal (s.r.). (T2) = FGA (40 mg, 5 days) + PGF<sub>2</sub> $\alpha$  (Cloprostenol, ICI, Estrumate, Schering-Plough, Milano) 100 $\mu$ g i.m. s.r. (Day 5) + eCG (200 IU i.m. s.r., Day 5). (T3) = PGF<sub>2</sub> $\alpha$  (100 $\mu$ g ICI, Day 0) + FGA (40 mg, 5 days) + eCG (200 IU i.m. s.r., Day 5). (T4) = PGF<sub>2</sub> $\alpha$  (100 $\mu$ g ICI, Day 0) + FGA (40 mg, 5 days) + GnRH (a GnRH analogue, Fertagyl, Intervet, Milano, Italy, 100 $\mu$ g given i.m. 30 h after s.r.). (T5) = GnRH (100 $\mu$ g i.m., Day 0) + PGF<sub>2</sub> $\alpha$  (100 $\mu$ g ICI, Day 5) + eCG (200 IU i.m., Day 5). The ewes were checked for oestrus by teaser rams at 8 h intervals. The ewes were hand

-mated (male to female ratio 1:5) at the beginning of oestrus and subsequently 12 h intervals until the end of oestrus. At lambing, fertility (number ewes lambing/number ewes treated) and prolificacy (number lambs born/number ewes lambing) were recorded. Results of experiments were analyzed using the GLM procedure of the SAS system [25]. Differences among treatments in the interval between FGA or PGF2 $\alpha$  injection and oestrus and ovulation time were analyzed by least-squares analysis of variance, and the differences between the treatment groups were compared by Student's t-test. Percentages of animals in oestrus, fertility and prolificacy were compared by using the chi-square test.

### 3. Results and discussion

Seven ewes were excluded from the data due to the loss of intravaginal sponges (N= 7) or accidental trauma (N= 3 ewes). Treatments did not differ in the incidence of ewes in oestrus (Table 1) although (T<sub>4</sub>) ewes tended (P < 0.10) to show a lower incidence of ewes in oestrus (71.4%, 10/14) compared to treatment Groups and control Groups (Table 1). Hormonal treatments affected (P < 0.05) the interval to oestrus which was earlier in treated control ewes (30.3 $\pm$ 4.4 h) than in T<sub>4</sub> (36.3 $\pm$ 4.0 h; P < 0.05) and T<sub>2</sub> (43.6 $\pm$ 8.1 h; P < 0.01) (Table 1). Fertility rate tended (P < 0.10) to be higher in T<sub>3</sub> (93.3%) and T<sub>2</sub> (87.5%) Groups, if compared with the control Group (T<sub>1</sub>). Prolificacy rate was higher in T<sub>4</sub> Group (173%; P > 0.05) (Table 2).

**Table 1: Influence of synchronization treatments on oestral responses of adult kermani ewes.**

Treatment groups	Treated ewes	Ewes in oestrus	Onset of estrus
T <sub>1</sub> (Control)	16	15 93.7	30.3 $\pm$ 4.4Ac
T <sub>2</sub>	16	14 87.5	43.6 $\pm$ 8.1Bb
T <sub>3</sub>	15	14 93.3	35.0 $\pm$ 8.0d
T <sub>4</sub>	14	10 71.4	36.3 $\pm$ 4.0d
T <sub>5</sub>	14	13 92.8	33.9 $\pm$ 8.4a

The results of this study indicate that the incidence of females in oestrus following PGF2 $\alpha$ -FGA-eCG, GnRH-PGF2 $\alpha$ -eCG or FGA-PGF2 $\alpha$ -eCG short time treatments was similar to that of FGA-eCG long term control regimen. Mean interval to oestrus delayed in T<sub>2</sub> treated ewes. The efficiency in synchronizing estrus of T<sub>1</sub> 5-day protocol is given by the fact that progestagen prevents new corpora lutea formation [6, 14, 26] while PGF2 $\alpha$  ensures the luteolysis 5 days later and eCG improves the synchronization. Incidence of oestrus in T<sub>2</sub> treated ewes found in this study (87.5%) is similar to those obtained in other studies following longer control treatments [27] and a long term traditional control treatment [16].

**Table 2 : Influence of synchronization treatments on reproductive performance of adult kermani ewes**

Treatment groups	Treated ewes	Fertility Rate %	Prolificacy %
T <sub>1</sub> (Control)	16	10/16 62.5	155.5
T <sub>2</sub>	16	14/16 87.5 <sup>a</sup>	146.1
T <sub>3</sub>	15	14/15 93.3 <sup>A</sup>	161.6
T <sub>4</sub>	14	6/14 42.8 <sup>Bb</sup>	173.0
T <sub>5</sub>	14	10/14 71.4	165.5

Different superscripts in columns differ significantly; P<0.05.

The delayed interval to oestrus observed in (T2) might be attributed to the status of corpora lutea at the time of induced PGF2 $\alpha$  luteolysis as well as to the stage of follicular development at the time of prostaglandin administration. Previous studies have demonstrated a great variability in the occurrence of oestrus related to the stage or the day of the cycle at which PGF2 $\alpha$  was given [15, 16]. In this study, the efficiency of (T2) in inducing high level of synchronization is comparable to that observed in goats [28], where a PGF2 $\alpha$  injection at the time of the CIDR device insertion for 5 days promotes the growth of a large follicle, which is aged about 5 days at end of treatment. This treatment resulted into a pregnancy rate of 80% after natural service [28], similar to the fertility rate observed in the present study (87.5%). In the current study fertility rates after natural mating following short term 5 days (T2) and (T3) protocols were similar and higher than in long-time (14 days) treatment control group, despite its high percentage of ewes in oestrus. The reasons for this maybe might be attributable to the sub-luteal serum progesterone concentration observed at the end of the long term treatment with progestogen [4]. Ungerfeld and Rubianes [6] showed that high levels of short time progestagen priming (6 days), followed by low doses of eCG, are able to control follicular turnover and to determine good levels of fertility. It is likely that the induction of luteolysis by PGF2 $\alpha$  injected at the time of the insertion of FGA sponges (T2) ensured high levels of progestogen during the short term progesterone priming. Moreover, an improving oestrus synchronization has been observed by synchronizing the follicular wave to luteolysis [29], with subsequent good fertility, as probably happened for the most of T2 and T1 treated ewes in this trial. The efficiency of GnRH-PGF2 $\alpha$ -eCG treatments in inducing a high level of oestrus (91.7%) matches the results of [30] in Welsh Halbred ewes treated with a combination of buserelin and PGF2 $\alpha$  (90.9%) or those of in GnRH-PGF2 $\alpha$  - eCG Akkaram cross breed treated ewes (93.7%). This outcome also corresponds to the results (85%) obtained in cattle by using a combined buserelin-PGF2 $\alpha$  treatment [31]. Again, the level of synchronization is comparable with that obtained with progestagen pessaries observed in this study (control Group) and in others [15]. GnRH injection at random stages of the oestrus cycle, promoting LH surge, generally induces ovulation/luteinisation of the dominant follicle followed by a new follicular wave and a derived new dominant follicle with the best oocyte quality [32]. Moreover it has been shown that the corpora lutea is responsive to PGF2 $\alpha$  since Day 3 of the oestrus cycle [28]. The overall results of this study suggest that GnRH injection is able to ensure the most animals to be responsive to PGF2 $\alpha$  5 days after its administration, while prostaglandin induces a regression of corpora lutea or luteinised follicles induced by GnRH. The fertility rate following natural mating in GnRH-PGF2 $\alpha$ -eCG treated ewes was lower (66.7%) than in PFe (92.3%) or FPe (86.7%) Groups. Speculatively this might be explained by considering that eCG may induce reduced fertility [14], probably due to a development of an anovulatory follicular cyst or a failed fertilization or loss of embryos. However, the fertility rate of GnRH-PGF2 $\alpha$ -eCG treated ewes is comparable with the results obtained in other studies with progestagen pessaries or with PGF2 $\alpha$  at 9-day or 12-day intervals [33]. PGF2 $\alpha$ -FGA-GnRH treatment resulted into the lowest percentages of females coming into oestrus, followed by the lowest

fertility rate after natural service. The reasons for this might be ascribed to an early injection of GnRH with a subsequent LH early surge which blocks the ovarian steroidogenesis [34] and prevents the occurrence of oestrus. Moreover, it has been reported that GnRH administration may induce a premature ovulation or less functional corpora late [35] with a subsequent reduced fertility.

#### 4. Conclusion

In conclusion this study has shown that PGF2 $\alpha$ -FGA-eCG as well as FGA-PGF2 $\alpha$ -eCG short term 5-day treatments is effective in synchronizing oestrus in ewes during the breeding season and could be used as a valid alternative to FGA-eCG long term protocol for synchronizing oestrus under field condition in flocks where natural mating were used. Also GnRH-PGF2 $\alpha$ -eCG 5-day treatment has been found effective to provide high level of oestrus followed by acceptable levels of fertility after natural mating. This short time method is more advantageous for its reduced costs, furthermore it is less laborious because it does not require the use of progestagen sponges and avoids potential pathological diseases (pessary retention, vaginitis).

#### Acknowledgments

The authors are grateful to Mr. Mohammad Ali Mohammadi Najad for his useful and kindly help. The authors also would like to have a special thank to the director of the Rezvan junior college farm for providing the research facilities.

#### 5. References

- [1] Moradi Kor N, Sadeghi S, Ziaei N. Comparison reproductive Performance in Kermani ewes Treated with two synchronization methods and Subsequent eCG treatment out of the breeding season. International Journal of Biological & Medical Research. 2012;3(2): 1485-1489.
- [2] Zarkawi M, Al-Merestani MR, Wardeh MF. Induction of synchronized oestrus and early pregnancy diagnosis in Syrian Awassi ewes, outside the breeding season. Small Rumin Res. 1999; 33: 99-102.
- [3] Atsan T, Emsen E, Yaprak M, Dagdemir V, Diaz CAG. An economic assessment of differently managed sheep flocks in eastern Turkey. Ital. J. Anim. Sci. 2007; 6, 407-414.
- [4] Vinales C, Meikle A, Forsberg M, Rubianes E. The effect of subluteal levels of exogenous progesterone on follicular dynamics and endocrine patterns during the early luteal phase of the ewe. Theriogenology. 1999; 51, 1351-1361.
- [5] Allison AJ, Robinson TJ. The effect of dose level of intravaginal progestagen on sperm transport, fertilization 350 IU of eCG n and lambing in the cyclic Merino ewe. J. Reprod. Fertil. 1970; 22, 515-531.
- [6] Ungerfeld R, Rubianes E. Effectiveness of short term progestogen primings for the induction of fertile oestrus with eCG in ewes during late seasonal anoestrus. Anim. Sci. 1999; 68, 349-353.
- [7] Gomez-Brunet A, Santiago-Moreno J, Montoro V, Garde J, Pons P, Gonzalez-Bulnes A,opez-Sebastian A.L. Reproductive performance and progesterone secretion in estrus-induced Manchega ewes treated with hCG at the time of AI. Anim. Reprod. Sci. 2006; doi:10.1016/j.smallrumres.04.015.
- [8] Moradi Kor N, Ziaei N, Esfandiari Pour E. Comparison of reproductive performance in Raieni goats following different estrous synchronization methods and subsequent eCG treatment during the natural breeding season. Global Veterinaria. 2011; 7(6): 618-624.
- [9] Dogan I, Nur Z. Different estrous induction methods during the non-breeding season in Kivircik ewes. Vet. Med. 2006; 51(4): 133-138.
- [10] Gordon I. Controlled reproduction in sheep and goats. 1st Edn., Vol. 2., CAB International, Wallingford, UK. 1997; PP: 351-439.
- [11] Moradi kor N, Ziae N. The effect of PGF2 $\alpha$  administration and subsequent eCG treatments on the reproductive performance in mature Raieni goats during the breeding season. Asian Journal of Animal and Veterinary advance. 2012; 7(1): 94-99.

- [12] Bearden HJ, and Fuquay, JW. Applied animal reproduction. 5th Edn., Prentice-Hall, Inc., New Jersey. 2000; P: 230.
- [13] Zarkawi M, Al-Merestani MR, Wardeh MF. Induction of synchronized estrus in indigenous Damascus goats outside the breeding season. *Small Rumin. Res.* 1999; 33: 193-197.
- [14] Martemucci G, D'Alessandro AG. Strategie di miglioramento dell'efficienza riproduttiva negli ovini. Sincronizzazione farmacologia degli estri e delle ovulazioni (Strategies for improve reproductive efficiency in sheep: pharmacological synchronization of estrus and ovulation). *Agricoltura Ricerca* 1999; 183, 3-18.
- [15] Martemucci G, Toteda F, Manchisi A, Gambacorta M, Bellitti E. Effetto luteolitico nella pecora di un analogo di PGF $2\alpha$  (cloprostenolo) in relazione allo stadio del ciclo sessuale (Luteolytic effect in ewe of an analog of PGF $2\alpha$  (cloprostenol) given at different stages of the oestrus cycle). *Zoot. Nutr. Anim.* 1986; 12, 487-492.
- [16] Martemucci G, Manchisi A, Toteda F, Gambacorta M, Bellitti E. Manifestazioni dell'estro in pecore trattate con prostaglandina F $2\alpha$  naturale o progestagene (FGA) e PMSG. Alcune osservazioni sulle performances riproduttive dopo inseminazione artificiale (Occurrence of oestrus in ewes treated with prostaglandin F $2\alpha$  or progestagen (FGA) and PMSG. Some observations on lambing performance after artificial insemination). *Zoot. Nutr. Anim.* 1987; 13, 39-49.
- [17] Ravindra JP, Rawlings NC, Evans ACO, Adams GP. Ultrasonographic study of ovarian follicular dynamics in ewes during the oestrus cycle. *J. Reprod. Fert.* 1994; 101, 501-509.
- [18] Ginther OJ, Kot K, Wiltbank MC. Associations between emergence of follicular waves and fluctuations in FSH concentrations during the estrous cycle in ewes. *Theriogenology.* 1995; 43, 689-703.
- [19] Noel B, Bister JL, Paquai R. Ovarian follicular dynamics in Suffolk ewes in different periods of the year. *J. Reprod. Fert.* 1993; 99, 695-700.
- [20] Gibbons JR, Kot K, Thomas DL, Wiltbank MC, Ginther OJ. Follicular and FSH dynamics in ewes with a history of high and low ovulation rates. *Theriogenology.* 1999; 52, 1005-1020.
- [21] Evans ACO, Duffy P, Hynes N, Boland MP. Waves of follicles development during the estrous cycle in sheep. *Theriogenology.* 2000; 53, 699-715.
- [22] Webb R, Gong JG, Rusbridge SM. Control of ovarian function in cattle. *J. Reprod. Fert.* 1992; (Suppl. 45), 141-156.
- [23] Peters AR, Ward SJ, Warren MJ, Gordon PJ, Mann GE, Webb R. Ovarian and hormonal responses of cows to treatment with an analogue of gonadotrophin releasing hormone and prostaglandin F $2\alpha$ . *Vet. Rec.* 1999; 144, 343-346.
- [24] Bo GA, Baruselli CS, Martinez MF. Pattern and manipulation of follicular development in *Bos indicus* cattle. *Anim. Reprod. Sci.* 2003; 78, 307-326.
- [25] SAS/STAT TM Guide for Personal Computers, Version 8. 1 Edn. SAS Institute Inc., 1999/2000. Cary, NC.
- [26] Wildeus S. Current concepts in synchronization of estrus: sheep and goats. *J. Anim. Sci.* 2000; 77, 1-14.
- [27] Fitzgerald JA, Rugless AJ, Steliflug JN, Hansel W. A seven-day synchronization method for ewes using medroxyprogesterone acetate (MAP) and prostaglandin F $2$ . *J. Anim. Sci.* 1985; 61, 465-469.
- [28] Rubianes E, Menchaca A. The pattern and manipulation of ovarian follicular growth in goats. *Anim. Reprod. Sci.* 2003; 78, 271-287.
- [29] Hariadi M, Broomfield D, Wright PJ. The synchrony of prostaglandin-induced estrus in cows was reduced by pretreatment with HCG. *Theriogenology.* 1998; 49, 967-974.
- [30] Beck NFG, Jones M, Davies B, Peters AR, Williams SP. Oestrus synchronization in ewes: the effect of combining a prostaglandin analogue with a GnRH agonist (buserelin). *Anim. Sci.* 1996; 62, 85-87.
- [31] Twagiramungu H, Guilbault LA, Proulx J, Villeneuve P, Dufour JJ. Influence of an agonist of GnRH (buserelin) on estrus synchronization and fertility in beef cows. *J. Anim. Sci.* 1992; 70, 1904-1910.
- [32] Mhim M, Curran N, Hyttel P, Boland MP, Roche JF. Resumption of meiosis in cattle oocytes from preovulatory follicles with a short and a long duration of dominance. *J. Reprod. Fert.* 1999; 116, 293-304.
- [33] Martemucci G, Gambacorta M, Bellitti E, Manchisi A, Toteda F. Controllo dell'ovulazione con PMSG e GnRH in pecore trattate con progestagene (Ovulation control by PMSG and GnRH in ewes treated with progestagen). *Zoot. Nutr. Anim.* 1984; 10, 11-22.
- [34] Hay MF, Moor RM. Functional and structural relationships in the graafian follicle population of the sheep ovary. *J. Reprod. Fert.* 1975; 45, 583-593.
- [35] Keisler DH, Keisler LW. Formation and function of GnRH-induced subnormal corpora lutea in cyclic ewes. *J. Reprod. Fert.* 1989; 87, 265-273.