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EFFECTS OF GNRH AGONIST (CINNARELIN) ON REPRODUCTIVE PERFORMANCE IN SYNCHRONIZED IRANIAN CROSSBRED EWES DURING THE BREEDING SEASON

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ABSTRACT

This study was carried out to investigate the efficiency of GnRH on reproductive performance of synchronized Iranian crossbred ewes during the breeding season. The ewes (n = 90) were treated with CIDR containing 30 mg progesterone for 14 days and were injected with 400 IU PMSG at the time of removal of the CIDR. The ewes were then divided into three equal groups of 30 ewes each. One milliliter of water soluble was administered to each ewe in the Group 1 (control) at the time of both the CIDR withdrawal and AI. In the group 2 approximately 12.5 µg of GnRH (CinnaRelin) were injected to each ewe immediatly after AI. In the 3 Group, 12.5 µg GnRH were injected to each ewe on 11 to 13 days post-insemination. Intracervical AI with diluted fresh semen was performed once at 12 hours following the estrus onset. Lambing and multiple birth rates were significantly higher ($P<0.05$) in the Group 2 (123.3 % and 45.8 %) in comparison to other groups. Pregnancy and fecundity rates tended to be higher in the Group 2 (80 % and 154.2 %) and the Group 3 (76.6 % and 130.4 %), when compared with the control Group (66.6 % and 110 %). However, the differences among any groups in terms of the number of twin lambs, gestation period, lambs birth weight and male and female lamb rate were statistically insignificant ($P>0.05$). In conclusion, GnRH administration improved the reproductive performance of the ewes when administrated at the time of AI or during the midluteal phase after AI.

Key words: Iranian crossbred ewes; lambing rate; GnRH; synchronization

INTRODUCTION

The Arkhar-Merino is a sheep breed which was produced by crossbreeding between wild Arkhar rams with ewes of the Novocaucasian Merino, Précoce and Rambouillet breeds (Ernst and Dmitriev, 2007). The goal of the crossbred sheep production in the northwest Iran was genetic improvement of local wool trait breeds. The most economically important trait in sheep production is reproduction and it can be manipulated using hormonal treatments (Atsan *et al*., 2007). Hormonal treatment to control ovulation and reproduction is a prerequisite for successful breeding and increasing the number of pregnant females (Husein *et al*., 2005), resulting in a short breeding period and more uniform newborn crop (Husein and Kridli, 2003). Gonadotrophin releasing hormone (GnRH) plays a key role in the reproductive function and has a potential for fertility control in mammals (Diskin *et al*., 2002). In many studies where GnRH was used, it was aimed to increase the progesterone levels (P4) in serum, to decrease the early embryonic deaths and as a result to increase the pregnancy rates. GnRH injection causes a predictable release of luteinizing hormone (LH) and a significant increase in serum P4 (Stevenson *et al*., 1993). In addition, it is reported that GnRH injection on 11 to 14 days (during the mid luteal phase) after artificial insemination (AI) increased serum concentrations of P4 and caused a tendency toward higher pregnancy rates in cattle (Hansen, 2002). It is thought that GnRH treatment may increase the chances of embryo survival by improving luteal function and/or interfering with the luteolytic mechanism (Beck *et al*., 1994; Cam *et al*.,

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2002). The application of GnRH reduced the variation in the timing of the LH surge for goats, improving the synchrony of ovulation (Pierson *et al*., 2003). The GnRH administration at the time of oestrus increased serum concentrations of P4 and improved pregnancy rates in cows (Ullah *et al*., 1996). Because of decreasing Iranian crossbred ewe population in recent years (Jafari, 2008), the strategies which can improve population of this animal can be profitable for farmers and conserving this animal. This study is the first to report the GnRH treatment on reproductive indices of Arkhar-Merino ewes reared in the northwest Iran. Hence, this study was designed to determine the effects of single administration of GnRH treatment at the time of AI or during the mid luteal phase after AI on their pregnancy rate and lambing performance in Iranian crossbred ewes synchronized with CIDR+PMSG technique during the breeding season.

MATERIAL AND METHODS

Hormonal preparations

Controlled internal drug release with 30 mg of progesterone, a progestagen analogue (InterAg, Hamilton, New-Zealand), PMSG (folligon; Intervet International B.V., Boxmeer, the Netherlands) and GnRH (each ml of GnRH contains 5μg, CinnaRelin; CinnaGen Biopharma Co, Tehran, Iran) were purchased from Intervet Drug Industry (Tehran, Iran).

Animals and location

In the present study, a total of 120 clinically healthy, free of reproductive disorders and once lambed adult Iranian crossbred ewes (forty-five Arkhar-Merino× Ghezel (AM-GH); forty-five Arkhar-Merino×Moghani (AM-MG), 2-5 years old, weighing 40-50 kg), and six healthy adult crossbred rams (3-4 years old, weighing 65-75 kg) were used. The study was carried out in breeding season (June/September, 2011) at the agricultural research station, University of Tabriz, East Azerbaijan province at 38°07´N, 46º29´E and altitude of 1567 m. The ewes were kept indoors at night and outdoors most of the day. Indoors, the ewes were fed a concentrated diet based on cottonseed meal, barley and wheat bran having 2450 kcal ME and 14 % crude protein through the experimental period. Water and a mineral supplement was available *ad libitum*.

Treatment schedule

The ewes (n = 90) were treated with CIDR containing 30 mg progesterone for 14 days and were injected with 400 IU PMSG at the time of the CIDR removal. The ewes were then divided into three equal groups of 30 ewes each. One milliliter of water soluble was administered to each ewe in the Group 1 (control) at time of both the CIDR withdrawal and AI. Approximately 12.5 μg of GnRH (CinnaRelin) was injected to each ewe in the Group 2 immediatly after AI. In the Group 3, 12.5 μg of GnRH was injected to each ewe on 11 to 13 days post-insemination. In order to determine the time of the estrus onset, all the ewes were monitored every 6 hours from 12 to 96 hours following CIDR withdrawal using six teaser rams equipped with an apron.

Semen collection, processing and insemination of the ewes

Semen was collected from all the rams with aid of an artificial vagina, the ejaculates were held in a warm water bath at 37°C until their assessment. Each ejaculate was immediately evaluated to determine motility of the semen. Sperm motility was identified as the percentage of sperm cells that demonstrated progressive motility, from 0 to 100 %, by a qualified and experienced investigator. Semen was placed on a heated (37°C) glass slide and scoring was performed at microscopic magnification of 200×. The mean of the two successive estimates was used as the final motility score. Spermatozoa concentration was determined by a hemocytometric method using the standard haemocytometer (Improved Neubauer, Deep 1/10 mm, Labart, Germany) slide and dilution pipette designed for counting red blood cells (Gur *et al*., 2005). All the ejaculates having forward progressive motility more than 70 %, were then pooled and diluted at the 1 : 1 ratio (semen : diluents) at 37°C with the skim milk extender. Hence, 1000 IU sodium G penicillin and 1000 μg dihydrostreptomycine sulphate was added to 1 ml of the diluted semen. The diluted semen was kept at 37°C in a water bath until insemination. Each ewe was intracervically inseminated at 12 h following the estrus onset using specific insemination catheter containing 0.5 ml of the diluted semen (approximately 2×10^8 spermatozoa/ml).

Pregnancy diagnosis

Pregnancy was diagnosed by transabdominal ultrasound examination at day 57 after AI. The ultrasound equipment used was a real-time scanner (Shimasonic, Model SDL-32C; Shimadzu Deutschland GmbH, Duisburg Germany). The probe was lubricated with ultrasonic gel (Ultrasonic Gel, Virbac Australia Pty Ltd, Peakhurst, NSW) and placed in contact with the abdomen in order to visualize the foetus on the screen. The number of ewes carrying at least one live foetus was recorded (Reyna *et al*., 2007).

Statistical analysis

Values concerning gestation period and lamb birth weight were compared by one-way analysis of variance (ANOVA) and *post hoc* Tukey-HSD test (SAS, 2008). The chi-squared test was performed to determine the differences among all groups concerning the other reproductive traits measured. Statistical significance was proved at P<0.05.

Pregnancy, lambing, and fecundity rates were calculated as follows (Zeleke *et al*., 2005):

$$\text{pregnancy rate} = \frac{\text{ewes lambing}}{\text{ewes inseminated}} \times 100$$

$$\text{lambing rate} = \frac{\text{lambs born}}{\text{ewes inseminated}} \times 100$$

$$\text{fecundity rate} = \frac{\text{lambs born}}{\text{ewes lambing}} \times 100$$

RESULTS AND DISCUSSION

The values concerning reproductive parameters, gestation period and lambs birth weight are presented in Table 1. Lambing and multiple birth rates were significantly higher ($P<0.05$) in Group 2 (123.3 % and 45.8 %) in comparison to Group 3 (100 % and 26 %) and Group 1 (73.33 % and 10 %, control Group). When the number of single lambs was statistically compared only a significant difference ($P<0.05$) between the Groups 1 (90 %) and 2 (13 %) was recorded. Pregnancy and fecundity rates tended to be higher in the Group 2 (80 % and 154.2 %) and the Group 3 (76.6 % and 130.4 %), when compared with the Group 1 (66.6 % and 110 %). However, the differences among any of the groups in terms of the number of twin lambs, gestation period, lamb birth weight and male and female lamb rate were statistically insignificant ($P>0.05$).

Table 1: Reproductive performance of ewes after different treatment protocols (Group 1: progestagen + PMSG + post-insemination NaCl; Group 2: progestagen + PMSG + insemination GnRH; Group 3: progestagen + PMSG + post (11 to 13 days) – insemination GnRH)

Variable	Group		
	1	2	3
Number of ewes	30	30	30
Pregnancy rate (%)	66.6	80	76.6
Lambing rate (%)	73.33[b]	123.3[a]	100[b]
Fecundity rate (%)	110	154.2	130.4
Multiple birth rates (%)	10 % (2/20)[b]	45.8 % (11/24)[a]	26 % (6/23)[b]
Number of lambs			
Single	18 (90 %)[a]	13 (54.2 %)[b]	17 (73.9 %)[a]
Twin	2 (10 %)	9 (37.5 %)	5 (21.7 %)
Triplet		2 (8.3 %)	1 (4.4 %)
Gestation period (days)	149-157	148-155	149-153
Lambs birth weight (kg)	5.1 ± 0.4	4.3 ± 0.4	4.6 ± 0.4
Female lamb rate (%)	45.4 (10/22)	51.4 (19/37)	50 (15/30)
Male lamb rate (%)	54.6 (12/22)	48.6 (18/37)	50 (15/30)

Data are expressed as mean ± S.M.E.

[a, b] values within a rows having different superscripts differ significantly ($P<0.05$)

Little is known regarding the reproductive indices of Arkhar-Merino rams and ewes under normal environmental conditions in Iran. In the present study in attempt to reduce embryonic morality and hence to improve reproductive performance of the Iranian crossbred ewes, a single administration of GnRH (12.5 µg) treatment was applied during the breeding season. The results obtained in this study showed that administration of GnRH at the time of AI and on 11 to 13 days post-insemination (during the mid luteal phase) improves lambing and twinning rates in synchronized ewes. These results are in agreement with the previous reports in ewes, where GnRH administration improved reproductive performance (Safranski *et al*., 1992; Turk *et al*., 2008). However, some studies have reported that GnRH administration had no effect on reproductive performance in dairy cows (Ryan *et al*., 1994; Tefera *et al*., 2001). Differences in the effectiveness of GnRH therapy on reproductive performance reported by others could be related to the genetics, age and different GnRH analogues or their doses.

In the present study a significant effect of exogenous GnRH treatment on number of single lambs of ewes was observed in the Groups 1 and 2 ($P<0.05$). GnRH, used alone, induced a closely synchronized LH surge 2 hours after intramuscular injection during the breeding (Rubianes *et al*., 1997a). The beneficial effect of GnRH supplementation at time of insemination increases conceptus growth (Kleemann *et al*., 1994; Lashari and Tasawar, 2007). This would have helped to improve embryo survival as larger conceptuses produce more IFN-tau, thereby more effectively suppressing the luteolytic mechanism and allowing more time for the establishment of pregnancy (Nephew *et al*., 1994). Reyna *et al*. (2007) reported that GnRH application had a positive effect in synchronizing the time of ovulation but had no effect on the growth or atresia of the ovulatory or subordinate follicles.

Administration of GnRH resulted in a rapid increase in plasma LH concentration and also in an increase in plasma progesterone concentrations in sheep (Beck *et al*., 1994; Cam *et al*., 2002). A functional courpus Luteum (CL) is required for the maintenance of pregnancy (Howell *et al*., 1994). The number of accessory CLs is increased with the administration of GnRH (Beck *et al*., 1994; Cam *et al*., 2002). Although plasma LH and P4 concentration and the number of accessory CLs was not determined in the present study, the effect of GnRH on embryo survival in ewes may occur through GnRH-stimulated LH surge stimulating production of progesterone by the CL and/or causing ovulation and the formation of accessory CLs. GnRH also promotes the formation of an accessory CL when injected at dioestrus (Stevenson *et al*., 1996). Injection of GnRH may have stimulated the transformation of small cells to large cells which had a higher basal secretion rate of P4 (Stevenson *et al*., 1993; De Rensis and Peters, 1999). The results of the present study showed that GnRH administration on the day of insemination improved rate and number of lambs born. This is in agreement with the previous findings of Peters *et al*. (1992); Cam *et al*. (2002); Khan *et al*. (2007) and Lashari and Tasawar (2007; 2010), who observed positive effect of GnRH administration on the day of mating on embryo survival in sheep and dairy cows. GnRH treatment on the day of insemination can increase pregnancy rate in cows by 6 to 7 % (Peters *et al*., 1992).

During the first 3 weeks of pregnancy, 30-40 % of fertilized eggs are lost in sheep and goats (Nancarrow, 1994; Michels *et al*., 1998). Of this total loss, 70-80 % occurs between days 8 and 16 after insemination (Sreenan *et al*., 1996), the majority of this is the result of embryo mortality (Beck *et al*., 1996). Fertilization failure accounts for only 5-10 % of losses (Wilmut *et al*., 1986). One of the major causes of embryonic loss is thought to be the inadequate luteal function (Nancarrow, 1994). Results of the present study showed that the treatment of ewes with CinnaRelin (a GnRH analogue) once during the mid luteal phase after AI (on days 11 to 13 days post-insemination) improved pregnancy rates of Iranian crossbred ewes during the breeding season. This is in agreement with the findings of Batavani and Eliasi (2004) and Macmillan and Thatcher (1991) that GnRH administration results in an increase in pregnancy rates in river buffaloes and dairy cattle, respectively, whereas others reported no beneficial effect in cattle (Jubb *et al*., 1990; Jayakumar and Vahida, 2000). Alacam *et al*. (1999) studied the effect of gonadorelin (a GnRH analogue) administered on day 12 post-mating in a small number of Angora goats and reported that GnRH administration increased pregnancy rate, kidding rate and litter size. The results of the present study are in agreement with these findings. When GnRH analogue was given on the day 12 after AI in cows, serum progesterone concentration was not affected, while serum LH levels increased significantly compared to controls (Yildiz *et al*., 2009). The response to GnRH depends on the phase of the cycle at which the hormone is administered (Geary *et al*., 2000).

There was no significant difference in lamb birth weight and gestation period between the GnRH treated and the control groups in the present study. This result is in concert with the trial of Turk *et al.* (2008) in Awassi ewes during the breeding season. It has been reported that progesterone supplementation increases subsequent fetal growth (Kleemann *et al*., 1994). Therefore, it is possible that the GnRH administration may also stimulate subsequent fetal growth through enhanced luteal activity. Additionally, no statistically significant differences were observed among any groups in terms of the male and the female lamb rate, which agreed with the result obtained by Turk *et al*. (2008) in Awassi ewes.

The results of the present study demonstrated that administration of exogenous GnRH (12.5 μg) significantly affected reproductive performance of ewes when administered at the time of AI or during the mid luteal phase after AI. Increased pregnancy rate and litter size in GnRH administered ewes are thought to be the results of GnRH effect on increasing embryo survival through enhanced luteal function. Additionally, this technique (CIDR+PMCG) is an effective tool for estrus synchronization in Iranian crossbred ewes at the northwest Iran during the breeding season.

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REFERENCES

ALACAM, E. – GUVEN, B. – AYAR, A. – SABAN, E. 1999. Ankara Kecilerinde gonadorelin uygulamalarinin kan progesteron, ostradiol 17 duzeyleri ile bazi fertilite parametrelerine etkisi (effect of gonadoreline administration on blood progesterone, oestradiol 17 concentration and some fertility parameters in Angora goats). *Turk. J. Vet. Anim. Sci*., vol. 23, 1999 p. 77-81.

ATSAN, T. – EMSEN, E. – YAPRAK, M. – DAGDEMIR, V. – DIAZ, G. A. G. 2007. An economic assessment of differently managed sheep flocks in eastern Turkey. *Ital. J. Anim. Sci*., vol. 6, 2007, p. 407-414.

BATAVANI, R. A. – ELIASI, K. 2004. Effect of GnRH analogue (Gonadorelin) on pregnancy rates in river buffaloes. *Iranian J. Vet. Res*., vol. 10, 2004, p. 81-85.

BECK, N. F. G. – JONES, M. – DAVIES, B. – MANN, G. E. – PETERS, A. R. 1996. The effect of GnRH analogue (buserelin) treatment on day 12 post-mating on ovarian structure and plasma progesterone and oestradiol concentration in ewes. *J. Anim. Sci*., vol. 63, 1996, p. 407-412.

BECK, N. F. G. – PETERS, A. R. – WILLIAMS, S. P. 1994. The effect of GnRH agonist (buserelin) treatment on day 12 post mating on the reproductive performance of ewes. *Anim. Prod*. vol. 58, 1994, p. 243-247.

CAM, M.A. – KURAN, M. – YILDIZ, S. – SELCUK, E. 2002. Fetal growth and reproductive performance in ewes administered GnRH agonist on day 12 post-mating. *Anim. Reprod. Sci*., vol. 72, 2002, p. 73-82.

DE RENSIS, F. – PETERS, A. R. 1999. The control of follicular dynamics by PGF2α, GnRH, hCG and oestrus synchronization in cattle. *Reprod. Dom. Anim*., vol. 34, 1999, p. 49-59.

DISKIN, M. G. – AUSTIN, E. J. – ROCHE, J. F. 2002. Exogenous hormonal manipulation of ovarian activity in cattle. *Dom. Anim. Endocrin*., vol. 23, 2002, p. 211-228.

DREW, S. B. – PETER, A. R. 1994. Effect of buserelin on pregnancy rates in dairy cows. *Vet. Rec*., vol. 134, 1994, p. 267-269.

ERNST, L. K. - DMITRIEV, N. G. 2007. Sheep: transbalkan finewool. *Ftp: //ftp.fao.org /docrep/ fao/009/ ah759e/ah 759e16.pdf.*

GEARY, T. W. – DOWNING, E. R. – BRUEMMER, J. E. – WHITTIET, J. C. 2000. Ovarian and estrous response of suckled beef cows to select synch estrous synchronization protocol. *Prof. Anim. Sci*., vol. 16, 2000, p. 1-5.

GREYLING, J. P. C. – VAN DER NEST, M. 2000. Synchronization of oestrus in goats: dose effect of Progestagen. *Small Rumin. Res*., vol. 36, 2000, p. 201-7.

GUR, S. – BOZKURT, T. – TURK, G. 2005. Short term effects of dexamethasone on hyaluronidase activity and sperm characteristics in rams. *Anim. Reprod. Sci*., vol. 90, 2005, p. 255-263.

HANSEN, P. J. 2002. Embryonic mortality in cattle from the embryo's perspective. *J. Anim. Sc*i., vol. 80, 2002, p. 33-44.

HUSIN, M. Q. – ABABNEH, M. M. - HADDAD, S. G. 2005. The effects of progesterone priming on reproductive performance of GnRH–PGF2 a-treated anestrous goats. *Reprod. Nutr. Dev*., vol. 45, 2005, p. 689-698.

HUSIN, M. Q. – KRIDLI, R. T. 2003. Effect of progesterone prior to GnRH–PGF2a treatment on induction of oestrus and pregnancy in anoestrous Awassi ewes. *Reprod. Dom. Anim*., vol. 38, 2003, p. 228-232.

JAFARI, K. 2008. Agriculture Organization of East Azerbaijan Province in Iran. *http://Gostareshonline.ir/.*

JAYAKUMAR, C. – VAHIDA, A. M. 2000. Effects of administration of a gonadotrophin releasing hormone analogue at mid-cycle post-insemination. *Indian Vet. J*., vol. 77, 2000, p. 454-455.

JUBB, T. F. – ABHAYARATNE, D. – MAIMO, J. – ANDESON, G. A. 1990. Failure of an intramuscular injection of an analogue of gonadotrophin releasing hormone 11 to 13 days after insemination to increase pregnancy rates in dairy cattle. *Aust. Vet. J*., vol. 67, 1990, p. 359-361.

KHAN, T. H. – BECK, N. F. – KHALID, M. 2007. The effects of GnRH analogue (buserelin) or hCG (Chorulon) on day 12 of pregnancy on ovarian function, plasma hormone concentrations, conceptus growth and placentation in ewes and ewe lambs. *Anim. Reprod. Sci*., vol. 102, 2007, p. 247-257.

KLEEMANN, D. O. – WALKER, S. K. – SEAMARK, R. F. 1994. Enhanced fetal growth in sheep administered progesterone during the first three days of pregnancy. *J. Reprod. Fertil*., vol. 102, 1994, p. 411-417.

LASHARI, M. H. – TASAWAR, Z. 2007. The effect of GnRH or hCG given on day of mating on ovarian function in Lohi sheep at Multan. *Pakistan. Biol. Reprod*., vol. 7, 2007, p. 177 (Abst).

LASHARI, M. H. – TASAWAR, Z. 2010. The Effect of GnRH Given on Day of Mating on Ovarian Function and Reproductive Performance in Lohi Sheep. *Pakistan. Vet. J*., vol. 30 (1), 2010, p. 29-33.

MACMILLAN, K. L. – THATCHER, W. W. 1991.

Effects of an agonist of gonadotrophin releasing hormone on ovarian follicles in cattle. *Biol. Reprod*., vol. 45, 1991, p. 883-889.

MICHELS, H. – VANMONTFORT, D. – DEWIL, E. – DECUYPERE, E. 1998. Genetic variation of prenatal survival in relation to ovulation rate in sheep: a review. *Small Rumin. Res*. vol. 29, 1998, p. 129-142.

NANCARROW, C. D. 1994. Embryonic mortality in the ewe and doe. In: Zavy, M. T., Geisart, R. D. (Eds.), Embryonic Mortality in Domestic Species. CRC Press, London. p. 79-97.

NEPHEW, K. P. – CARDENAS, H. – Mc CLURE, K. E. – OTT, T. L. – BAZER, F. W. – POPE, W. F. 1994. Effects of administration of human chorionic gonadotropin or progesterone before maternal recognition of pregnancy on blastocyst development and pregnancy in sheep. *J. Anim. Sci*., vol. 72, 1994, p. 453-458.

PETERS A. R. – DREW S. B. – MANN G. E. – LAMMING G E. – BECK N. F. (1992). Experimental and practical approaches to the establishment and maintenance of pregnancy. *J. Physiol Pharmacol*., vol. 43, 1992, p. 143-152.

PIERSON, J. T. – BALDASSARRE, H. – KEEFER, C. L. – DOWNEY, B. R. 2003. Influenced of GnRH administration on timing of the LH surge and ovulation in dwarf goats. *Theriogenology*, vol. 60, 2003, p. 397-406.

REYNA, J. – THOMSON, P. C. – EVANS, G. – MAXWELL, W. M. 2007. Synchrony of ovulation and follicular dynamics in merino ewes treated with GnRH in the breeding and non-breeding seasons. *Reprod. Dom. Anim*., vol. 42, 2007, p. 410-417.

RUBIANES, E. – UNGERFELD, R. -VIÑOLES, C. – RIVERO, A. – ADAMS, G. P. 1997. Ovarian response to gonadotropin treatment initiated relative to wave emergence in ultrasonographically monitored ewes. *Theriogenology*, vol. 47, 1997, p. 1479-1488.

RYAN, D. P. – SNIJDERS, S. – CONDON, T. – GREALY, M. – SREENAN, J. - O'FARRELL, K. J. 1994. Endocrine and ovarian responses and pregnancy rates in dairy cows following the administration of a gonadotrophin releasing hormone analog at the time of artifical insemination or at mid-cycle post insemination. *Anim. Reprod. Sci*. vol. 34, 1994, p. 179-191.

SAFRANSKI, T. J. – LAMBERSON, W. R. – KEISLER, D. H. 1992. Use of melengestrol acetate and gonadotropins to induce fertile estrus in seasonally anestrous ewes. *J. Anim. Sci*., vol. 70, 1992, p. 2935-2941.

SAS. (2003). SAS Users Guide. SAS Institute Inc. Cary, NC.

SREENAN, J. M. – DISKIN, M. G. – DUNNE, L. 1996. Embryonic mortality: the major cause of reproductive wastage in cattle. In: Proceedings of the 47th Annual Meeting of the European Association of Animal Production, Lillehammer, August 1996.

STEVENSON, J. S. – PHATAK, A. P. – RETTMER, I. M. M. O. – STEWART, R. E. 1993. Postinsemination administration of receptal: follicular dynamics, duration of cycle, hormonal responses, and pregnancy rates. *J. Dairy Sci*., vol. 76, 1993, p. 2536-2547.

STEVENSON, J. S. – KOBAYASHI, Y. – SHIPKA, M. P. – RAUCHHOLZ, K. C. 1996. Altering conception of dairy cattle by gonadotropin-releasing hormone preceding luteolysis induced by prostaglandin F2α. *J. Dairy Sci*., vol. 79, 1996, p. 402-410.

TEFERA, M. – CHAFFAUX, S. – THIBIER, M. – HUMBLOT, P. 2001. A short note: Lack of effect of post-AI hCG or GnRH treatment on embryonic mortality in dairy cattle. *Livest. Prod. Sci*. vol. 71, 2001, p. 277-281.

TURK, G. – GUR, S. – SONMEZ, M. – BOZKURT, T. – AKSU, E. H. – AKSOY, H. 2008. Effect of exogenous GnRH at the before time of artificial insemination on reproductive performance of Awassi ewes synchronized with progestagen-PMSG-PGF2alpha combination. *Reprod. Dom. Anim*., vol. 43, 2008, p. 308-313.

ULLAH, G. – FUQUAY, J. W. – KEAWKHONG, T. – CLARK, B. L. – POGUE, D. E. – MURPHEY, E. J. 1996. Effect of gonadotropin-releasing hormone at estrus on subsequent luteal function and fertility in lactating Holsteins during heat stress. *J. Dairy Sci*. vol. 79, 1996, p. 1950-1953.

WILMUT, I. – SALES, D. I. – ASHWORTH, C. J. 1986. Maternal and embryonic factors associated with prenatal loss in mammals. *J. Reprod. Fertil*., vol. 76, 1986, p. 851-864.

ZELEKE, M. – GREYLING, J. P. C. – SCHWALBACH, L. M. J. – MULLER, T. – ERAMUS, J. A. 2005. Effect of PMSG dose on oestrus synchronization and fertility in Dorper ewes during the transition period. *Small. Rumin. Res*., vol. 56, 2005, p. 47-53.