African Journal of Biotechnology Vol. 10(57), pp. 12358-12362, 28 September, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1771
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Effect of gonadotropin-releasing hormone (GnRH) treatment on multiple births in Afshari ewes

Mohammad Ali Sirjani[1], Mohammad Hossein Shahir[1], Hamid Kohram[2, 3]* and Ahmad Zare Shahneh[2]

[1]Department of Animal Science, Faculty of Agriculture, University of Zanjan, Zanjan, Iran.
[2]Department of Animal Science, Faculty College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran.
[3]Depertment of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran.

Accepted 8 August, 2011

The purpose of this study was to investigate the effects of gonadotropin-releasing hormone (GnRH) administration on the induction of multiple births in synchronized Afshari ewes. 16 cycling, multiparous fat-tailed Iranian Afshari ewes, weighing 66.5 ± 2.5 kg, were used in the trail. Estrus was synchronized using controlled internal drug release CIDR's; inserted for 14 days. Pregnant mare serum gonadotrophin (PMSG) (300 IU) was injected to ewes a day before CIDR removal. The ewes was randomly allocated to two groups (n = 8). Synchronization treatment was initiated with a week interval in two groups (weeks 1 and 2). 24 h following CIDR removal (day 1), GnRH (50 µg) was administered to half of each group ewes (GnRH and control groups), and ewes mated with the proven rams. Pregnancy was diagnosed with the aid of transabdominal ultrasonograpy 30 days after mating. Ovarian follicular activity was monitored with the aid of transrectal ultrasonograpy on the days of CIDR removal and at estrus (approximately day 1). Following PMSG treatment, the mean number of large follicles on total ovaries increased from CIDR removal to estrus 1.48 ± 0.71 vs. 2.85 ± 0.82 (P < 0.05). Lambing rate in GnRH group (162.5%) was higher (P < 0.05) compared to the control group (125%). The mean weights of lambs born in the GnRH and control groups were 5.5 ± 0.1 and 5.2 ± 0.1, respectively.

Key words: GnRH, estrus synchronization, Afshari, ewe, lambing rate.

INTRODUCTION

During the follicular phase of ewe cycle estrus, estradiol has great impact on gonadotropin-releasing hormone (GnRH secretion); estradiol not only regulates the frequency and amplitude of GnRH pulses, but also the releasing pattern and induces sustained GnRH surge with qualitative changes (Evans et al., 1995). A dose of GnRH can increase the number of gonadotropin-dependent follicles, which grow up until pre-ovulatory phase in response to FSH (Lopez-Alonso et al., 2005). GnRH surge making simultaneous control the FSH and LH surge due to ovulation, but the second FSH surge was not affected (Evans et al., 1996; Bowen et al., 1998). Studies have shown that kisspeptin cells are the main connection between gonadal steroids and GnRH neurons. In ewes, pre-ovulation induction of LH / GnRH surge more likely involves the sequential action of estrogen on the arcuate nucleus + preoptic area (ARC + POA) cell populations. From the practical perspective, kisspeptin injection can be simultaneous LH surge in progesterone-primed cyclic ewes and reinitiate cyclicity in anoestrous (Caraty et al., 2010).

For estrus synchronization in ewes, sponges and implants impregnated with progesterone and / or its analogues as well as prostaglandin E2 (PGF2α), alone or in combination with gonadotropins are vastly used (Boscos et al., 2002). The use of GnRH has a positive effect on ovulation synchronization. Injection of this hormone at 20 and 24 h after progesterone sponges removal induced LH surge within 2 h after GnRH injection and had desirable effect on ovulation in breeding season and non-breeding season (Reyna et al., 2007).

Using GnRH immediately after artificial insemination in sheep caused a raise in litter size by synchronization with

*Corresponding author. E-mail: kohram@can.ut.ac.ir. Tel: +98-261-2248082. Fax: +98-261-2246752.

combination of progestagen, PMSG and PGF2α (Turk et al., 2008); it was also showed that a GnRH injection at 24 h after CIDR removal could enhance the number of embryos in multiple ovulation and embryo transfer (MOET) programs (Menchaca et al., 2009). Using PMSG caused an elevation in multiple birth and the proportion of ewes that have more than one lamb (Langford, 1982). This hormone heightened the size of antral follicles and in some cases, non-ovulatory follicles were produced and followed by increase in estrogen concentrations; generally using this hormone had a positive effect on ovulation and lambing rate (Barrett et al., 2004). Therapeutic use of GnRH or hCG on day 12 pregnancy can increase ovarian activity and improve conceptus growth and placental attachments in ewes (Khan et al., 2007).

The aim of this study was to examine the effects of GnRH injection, on the reproductive efficiency and multiple births in ewes synchronized by CIDR + PMSG.

MATERIALS AND METHODS

The experiment was conducted during the breeding season (November), at the Zanjan University farm located in Zanjan city. The site is situated at 48.31 ± 21°E longitude and 36.40 ± 13°E latitude and at an altitude of 1663 m above sea level. The mean annual temperature is 14°C and the annual rainfall in this region ranges from 350 to 380 mm.

Animals and synchronization program

16 cycling, multiparous fat-tailed Iranian Afshari ewes, weighing 66.5 ± 2.5 kg were used in the trail. Estrus was synchronized using CIDR's (EAZI-BREED™, CIDR®, NewZealand) inserted for 14 days. PMSG (300 IU; BIONICHE; Australia) was intramuscularly (IM) injected to ewes a day before CIDR removal. The ewes were randomly allocated into two period groups (n = 8) synchronization treatment initiated with a week interval in the group. 24 h following CIDR removal (day 1), GnRH (50 µg; CYSTORELIN®; CEVA CAANTE ANIMALE; FRANCE) was administered (IM) to half of each groups of ewes 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 ultra-sonography (Piemedical, Falco100; Holland, 8 MHz), at CIDR removal and 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 hydrosoluble 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 (> 2 to 3mm) and large (4 to ≥ 5 mm) 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 and middle pregnancy on days 30 and 75 from the jugular vein into EDTA tubes. Blood samples were centrifuged (at 3000 rpm for 15 min), and plasma was collected and stored at -20°C until progesterone assay. Concentrations of progesterone were determined by ELISA kit (Monobind®; USA) with 0.1 ng/ml sensitivity. To study the results of the protocol treatment, lambing rate and lambs births weight, were recorded.

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 PMSG treatment are shown in Table 1. The ultrasonography observation showed that the mean number of small follicles on the right, left and total (right + left) ovaries between the two period groups (Weeks 1 and 2) was not different in CIDR removal. The mean number of small follicles on the right ovary in week 1 was lower than in week 2 at estrus. On the other hand, the results show that the mean number of small follicles on total ovaries were not different between CIDR removal and estrus days.

No significant differences were observed in the mean number of medium follicles on the right, left and total ovaries between the two period groups as well as between the CIDR removal and at estrus (Table 1). Following PMSG treatment, the mean number of large follicles on total ovaries increased ($p<0.05$) from CIDR removal to estrus. Moreover, follicular responses on the right ovaries were better than those on the left ovaries at estrus (Table 1).

All ewes showed estrus behavior within 48 to 54 h after CIDR removal and in the same time two rams were introduced to each group. All the ewes were diagnosed pregnant by using ultrasonography 30 days after mating. The lambing rate in ewes that received GnRH (162.5%) was more ($p < 0.05$) than that in the no-received group (125%). The mean weight of lambs born in received and no-received groups was 5.5 ± 0.1 and 5.2 ± 0.1,

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

Parameter	CIDR removal (day 0)			Estrus (day 1)		
	Week 1	Week 2	Total	Week 1	Week 2	Total
Small follicles						
Right ovary	2.08 ± 0.53	1.12 ± 0.53	1.60 ± 0.50	0.33 ± 0.53^{ac}	2.95 ± 0.61^{bc}	1.45 ± 0.54
Left ovary	1.23 ± 0.50	1.28 ± 0.52	1.27 ± 0.33	1.48 ± 0.50^{d}	1.31 ± 0.59^{d}	1.39 ± 0.36
Total	3.10 ± 0.10	2.30 ± 1.01	2.88 ± 0.65	1.60 ± 1.03^{a}	4.13 ± 1.23^{b}	2.65 ± 0.75
Medium follicles						
Right ovary	1.55 ± 0.63	1.66 ± 0.63	1.61 ± 0.39	1.55 ± 0.63	1.29 ± 0.72	1.44 ± 0.42
Left ovary	1.50 ± 0.46	1.22 ± 0.47	1.33 ± 0.31	2.00 ± 0.46	1.01 ± 0.53	1.57 ± 0.34
Total	2.97 ± 0.50	3.20 ± 0.52	3.03 ± 0.47	3.47 ± 0.50	2.28 ± 0.74	2.92 ± 0.55
Large Follicles						
Right ovary	0.84 ± 0.73	0.75 ± 0.69	0.64 ± 0.40	2.09 ± 073^{c}	1.19 ± 0.77	1.54 ± 0.42
Left ovary	1.02 ± 0.53	0.67 ± 0.54	0.84 ± 0.36	1.02 ± 0.53^{d}	1.03 ± 0.61	1.03 ± 0.38
Total	1.74 ± 1.06	1.26 ± 1.12	1.48 ± 0.71^{e}	2.99 ± 1.06	2.47 ± 1.60	2.85 ± 0.82^{f}

Different superscripts $^{(a,b)}$ in the same row within week period indicate a significant difference ($P < 0.05$). Different superscripts $^{(c,d)}$ in the same column within different side ovaries indicate a significant difference ($P < 0.05$). Different superscripts $^{(e,f)}$ in the same row within total data indicate significant difference ($P < 0.05$).

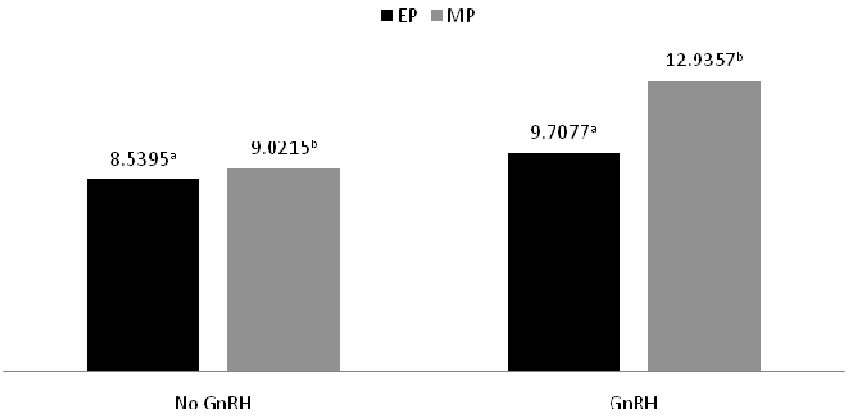

Figure 1. The mean ± SEM progesterone concentrations (ng/ml). at early pregnancy (EP) and middle pregnancy (MP) of the two groups. Different superscripts $^{(a,b)}$ in different columns within period indicate a significant difference ($P < 0.001$).

respectively but it was not significantly different.

Progesterone concentration of blood samples increased significantly ($P < 0.001$) in the two period groups from early to mid-pregnancy (Figure 1).

DISCUSSION

In this study, we were able to detect ovaries by rectal ultrasonography during the standing position; more comfortable for the ewes than dorsal recumbency (Riesenberg et al., 2001; Moakhar et al., 2010). The left and right ovaries after PMSG treatment were noticeable and clear. This finding could not be explained either by a higher number or by a smaller diameter of follicles, suggesting that the ovaries themselves were enlarged after PMSG stimulation (Riesenberg et al., 2001; Moakhar et al 2010).

The outcome of this study confirmed that PMSG administration had no effect on the number of small and

medium sized follicles at estrus (Samartzi, 1995; Ali 2007). It also showed that PMSG administration significantly increases the number of large follicles at estrus (Noel et al., 1994).There are several possible mechanisms by which eCG increases the number of large follicles. It may enhance the entry rate of small and medium follicles into larger sized follicles and it may also prevent the occurrence of natural follicular atresia (Bister et al., 1999; Mandiki et al., 2000).

The mean number of large follicles on the right ovary was more than that on the left ovary following PMSG treatment and it suggests that possibly, the right ovary responded to PMSG treatment better than the left ovary. These results are in agreement with the observations of the experiment carried out on ovaries after injection of different doses of PMSG (Casida et al., 1966; Scaramuzzi and Downing, 1997). Moreover, it was shown (Naqvi and Guliany, 1999; Moakhar et al., 2010) that the right ovary had a tendency to produce more CL than the left one in response to PMSG or FSH treatment. However, in another study by Ali (2007), it was suggested that ovulations were distributed equally between the right and left ovaries after injection of PMSG.

In this study, administration of PMSG in combination with GnRH increased ($P < 0.05$) lambing rate in comparison with PMSG alone (162.5 vs. 120%) in Afshari ewes. These results are in line with other previous reports (Jabbour et al., 1991; leyva et al., 1998). In another study (Turk et al., 2008), it was indicated that GnRH treatment following CIDR at estrus did not increase lambing rate. Therefore, it was confirmed that PMSG could increase not only the number and size of antral follicles but also raise ovulation rate (Ali, 2007). Other studies established that administration of GnRH at estrus can decrease variable time of LH surge and ovation rate and it improves reproductive performance in ewes (Walker et al., 1986; Eppleston, 1991; Jordan et al., 2009).

For progesterone concentrations, followed by advances in pregnancy and increase in the number of embryos, progesterone concentration increased (Figure 1), and these results are similar to other studies outcomes (Theodosiadou et al., 2004). Average weight of lambs born in the received GnRH ewes group (5.2 ± 0.1), was lower than that in the no-received GnRH ewes group (5.5 ± 0.1). It can be because of the increase of lambing rate in the received GnRH ewes group and this attributes to production of more lambs with lower weight mean (Alifakiotis, 1986).

In conclusion, using progesterone CIDR along with PMSG injection was effective for ewes estrus synchronization and as a result, the mean number of large follicles following PMSG treatment increased significantly ($P < 0.05$) at estrus but it was not showed in CIDR removal. Also, GnRH administration is effective for the increase of ovulation rate and consequently lambing rate.

ACKNOWLEDGEMENTS

The authors thank the manager of the Training-Research Field of Zanjan University (Mr.Aliyari) and Mr. N.Baradaran, Mr. A.A.Asadzadeh and Mrs. F. Zarei for their sincere assistance.

REFERENCES

Ali A (2007). Effect of time of eCG administration on follicular response and reproductive performance of FGA-treated *Ossimi ewes*. Small Rumin. Res. 72: 33-37.

Alifakiotis T (1986). Increasing ovulation rate and lambing percentage by active immunization against androstenedione in dairy sheep breeds. Theriogenology, 25: 681-688.

Barrett DM, Bartlewski PM, Batista-Arteaga M, Symington A, Rawlings NC (2004). Ultrasound and endocrine evaluation of the ovarian response to a single dose of 500 IU of eCG following a 12-day treatment with progestogen-releasing intravaginal sponges in the breeding and nonbreeding seasons in ewes. Theriogenology, 61: 311-327.

Boscos CM, Samartzi FC, Dellis S, Rogge A, Stefanakis A , Krambovitis E (2002). Use of progestagen-gonadotrophin treatments in estrus synchronization of sheep. Theriogenology, 58: 1261-1272.

Bowen JM, Dahl GE, Evans NP, Thrun LA, Wang Y, Brown MB, Karsch FJ (1998). Importance of the gonadotropin-releasing hormone (GnRH) surge for induction of the preovulatory luteinizing hormone surge of the ewe: dose-response relationship and excess of GnRH. Endocrinology, 139: 588-595.

Casida LE, Woody CO, Pope AL (1966). Inequality in function of the right and left ovaries and uterine horns of the ewe. J. Anim. Sci. 25: 1169-1171.

Eppleston J, Roberts EM (1991). Effect of time of PMSG and GnRH on the time of ovulation, LH secretion and reproductive performance after intrauterine insemination with frozen ram semen. Anim. Reprod. Sci. 26: 227-237.

Evans NP, Dahl GE, Caraty A, Padmanabhan V, Thrun LA, Karsch FJ (1996). How much of the gonadotropin-releasing hormone (GnRH) surge is required for generation of the luteinizing hormone surge in the ewe? Duration of the endogenous GnRH signal. Endocrinology, 137: 4730-4737.

Evans NP, Dahl GE, Mauger DT, Padmanabhan V, Thrun LA , Karsch FJ (1995). Does estradiol induce the preovulatory gonadotropin-releasing hormone (GnRH) surge in the ewe by inducing a progressive change in the mode of operation of the GnRH neurosecretory system. Endocrinology, 136: 5511-5519.

Jabbour HN, Ryan JP, Evans G, Maxwell WM (1991). Effects of season, GnRH administration and lupin supplementation on the ovarian and endocrine responses of merino ewes treated with PMSG and FSH-P to induce superovulation. Reprod. Fertil. Dev. 3: 699-707.

Kermani Moakhar H, Kohram H, Salehi R, Zare Shahneh A (2010). Follicular and ovulatory responses of the right versus left ovaries to eCG treatment in Shall Iranian ewes. Afr. J. Biotechnol. 9(10): 1523-1527.

Khan TH, Beck NF, 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. 102: 247-257.

Langford GA (1982). Influence of PMSG and time of artificial insemination on fertility of progestogen-treated sheep in confinement. J. Anim. Sci. 54: 1205-1211.

Leyva V, Buckrell BC, Walton JS (1998). Follicular activity and ovulation regulated by exogenous progestagen and PMSG in anestrous ewes. Theriogenology, 50: 377-393.

Lopez-Alonso C, Encinas T, Veiga-Lopez A, Garcia-Garcia RM, Cocero MJ, Ros JM, McNeilly AS , Gonzalez-Bulnes A (2005). Follicular growth, endocrine response and embryo yields in sheep superovulated with FSH after pretreatment with a single short-acting

dose of GnRH antagonist. Theriogenology, 64: 1833-1843.
Mandiki SN, Noel B, Bister JL, Peeters R, Beerlandt G, Decuypere E, Visscher A, Suess R, Kaulfuss KH , Paquay R (2000). Pre-ovulatory follicular characteristics and ovulation rates in different breed crosses, carriers or non-carriers of the Booroola or Cambridge fecundity gene. Anim. Reprod. Sci. 63: 77-88.
Menchaca A, Vilarino M, Pinczak A, Kmaid S, Saldana JM (2009). Progesterone treatment, FSH plus eCG, GnRH administration, and Day 0 Protocol for MOET programs in sheep. Theriogenology, 72: 477-483.
Naqvi SMK, Gulyani R (1999). Ovarian response and embryo recovery to different superovulatory regimens in Rambouillet ewes under semi-arid conditions. Small Rumin. Res. 34: 127-131.
Noel B, Bister JL, Pierquin B, Paquay R (1994). Effects of FGA and PMSG on follicular growth and LH secretion in Suffolk ewes. Theriogenology, 41:719-727.
Reyna J, Thomson PC, Evans G, Maxwell WM (2007). Synchrony of ovulation and follicular dynamics in merino ewes treated with GnRH in the breeding and non-breeding seasons. Reprod. Domest. Anim. 42: 410-417.
Riesenberg S, Meinecke-Tillmann S, Meinecke B (2001). Ultrasonic study of follicular dynamics following superovulation in German Merino ewes. Theriogenology, 55: 847-865.
Samartzi A.F Boscos C, Vaini E , Tsakalof P. (1995). Superovulatory response of Chios sheep to PMSG during spring and autumn. Anim. Reprod. Sci. 39: 215-222.
Scaramuzzi RJ, Downing JA (1997). The distribution of ovulations from the ovaries of merino and Border Leicester x merino ewes and its effect on the survival of their embryos. Anim. Reprod. Sci. 47: 327-336.
Theodosiadou E, Goulas P, Kouskoura T, Smokovitis A (2004). Oestrogen and progesterone concentrations in plasma and oviductal tissue of ewes exhibiting a natural or induced oestrus. Anim. Reprod. Sci. 80: 59-67.
Turk G, Gur S, Sonmez M, Bozkurt T, Aksu EH, Aksoy H (2008). Effect of exogenous GnRH at the time of artificial insemination on reproductive performance of Awassi ewes synchronized with progestagen-PMSG-PGF2alpha combination. Reprod. Domest. Anim. 43: 308-313.
Walker SK, Smith DH, Frensham A, Ashman RJ, Seamark RF (1989). The use of synthetic gonadotropin releasing hormone treatment in the collection of sheep embryos. Theriogenology, 31: 741-752.
Walker SK, Smith DH, Seamark RF (1986). Timing of multiple ovulations in the ewe after treatment with FSH or PMSG with and without GnRH. J. Reprod. Fertil. 77: 135-142.
Wildeus S (1999). Current concepts in synchronization of estrus : sheep and goats. Proc. Am. Soc. Anim. Sci. E38: 1-14.