High twin lambing rate of synchronized ewes using progestagen combined with the gonadotropins injection in breeding season

E. AHMADI, A. MIRZAEI*

Department of Clinical Sciences, Faculty of Veterinary Medicine, Shiraz University, Shiraz, Iran

*Corresponding author: mirzaei@shirazu.ac.ir

SUMMARY

The effect of gonadotropins (hCG, GnRH) injection combined with the long-term progestagen treatment on the lambing rate especially twin lamb birth rate was investigated in 75 fat tailed ewes during natural breeding season. All animals were synchronized using intravaginal progestagen sponges left in situ for 14 days and treated with PMSG at sponge removal. Then, the ewes were randomly divided into three equal groups (n = 25 ewes per group) including hCG, GnRH, and untreated control ewes. Treatment and control groups were injected (im) using hCG, GnRH, and distilled water respectively two days after sponge withdrawal. Serum progesterone concentration was used to identify pregnant ewes on the 19th day after sponge removal and lambing data was recorded. Lambing birth rate was not significantly different among the groups (P > 0.05). The percentages of pregnancy rate or lambing rate (the number) of ewes following the induced oestrus for control, GnRH, and hCG treatment groups were 76% (19/25), 62.5% (15/24), and 69.6% (16/23) respectively. Twin lambing birth rate of GnRH (73.3%) and hCG (68.8%) treatment groups was significantly higher than control group (31.6%; P < 0.05). The litter size (Mean ± SD) of ewes following the induced oestrus for control, GnRH, and hCG treatment groups were 1.32 ± 0.48, 1.73 ± 0.46, and 1.69 ± 0.48 respectively. The mean number of lambs (litter size) was significantly greater (P < 0.05) for ewes treated with GnRH and hCG, in comparison with the control ewes. So, treatment with hCG and GnRH two days after long-term progestagen treatment during breeding season can result in enhanced twin lambing birth rate and consequently, litter size of treated ewes.

Keywords: Breeding season; Ewe; Lambing; hCG; GnRH; Litter size

 introduction

Exogenous GnRH treatment, immediately prior to a short-term (7d) progesterone treatment results in increasing fertility rate, multiple births, and litter size in ewes at the onset of the breeding season [10]. The high pregnancy rate obtained in anoestrous ewes treated with a progesterone-GnRH-PGF2α [8]. Beck et al. [5] reported the induction of high fertility during breeding season in ewes following the injection of GnRH 5 days prior to prostaglandin F2α (PGF2α) analogue treatment. Jordan et al. [9] compared the effects of pretreatment with a gonadotropin releasing hormone (GnRH) and P4 on the reproductive response of ewes introduced to rams during seasonal anoestrus. Mirzaei et al. [13] investigated the effect of a GnRH analogue (buserelin) combined with the ram effect on the ovulation of ewes at the onset of breeding season. The use of GnRH in combination

RESUME

Taux de gémellité à l'agnelage chez les brebis synchronisées par progestatif combiné avec injection de gonadotrophine pendant la saison de reproduction

L'effet des gonadotrophines (hCG, GnRH) en injection combinées à un traitement à la progesterone sur le taux d’agnelage en particulier de gémellité a été étudié chez 75 brebis pendant la saison de reproduction naturelle. Tous les animaux ont été synchronisés en utilisant des éponges de progesterone intravaginale pendant 14 jours puis on été traitées avec PMSG au retrait de l'éponge. Les brebis ont alors été réparties au hasard en trois groupes égaux (n = 25 brebis par groupe) recevant de l'hCG, de la GnRH, et des témoins non traitées. Les traitements ont été effectués par injection intra-musculaire deux jours après le retrait de l'éponge, les témoin recevant de l’eau distillée. La concentration sérique de la progestérone a été utilisée pour identifier les brebis gestantes le 19e jour après le retrait de l'éponge. Le taux d'agnelage n'a pas été significativement différent entre les groupes (P> 0.05). Les taux de gestation ou taux d'agnelage après l'oestrus induit étaient respectivement de 76% (19/25), 62,5% (15/24), et 69,6% (16/23), pour les groupes contrôle, GnRH, et hCG. Les taux de gémellité étaient significativement plus élevé dans les groupes GnRH (73,3%) et hCG (68,8%) que dans le groupe témoin (31,6%). Les tailles de portée (moyenne ± SD) étaient respectivement de 1,32 ± 0,48, 1,73 ± 0,46, et 1,69 ± 0,48 pour les groupes témoins, GnRH, et hCG. Ces résultats révèlent que le traitement par l'hCG et la GnRH deux jours après le traitement de progestatif à long terme au cours de la saison de reproduction peut entraîner une augmentation du pourcentage de gémellité et par conséquent de la taille des portées chez la brebis.

Mots-clés: brebis; agnelage; synchronization; hCG; GnRH; jumeaux; portée
with other hormones has been reported in several studies which increased the prolificacy of reproductive performance of ewe [10, 9, 22, 14]. The physiological basis for using GnRH and its analogues is that the injection of this hormone can result in ovulation by stimulating LH release [23]. Consequently, it may increase the fertility or lambing and multiple birth rate together with the high litter size in ewes.

Abnormally corpus luteum formation results in luteal deficiency which can cause embryonic mortality and reduce reproductive performance of ewes [16]. Preovulatory LH pulses and surge affects the follicular and oocyte maturation and ovulation. So, hCG with the LH like in function, enhances the induction of ovulation [21] and increases the luteal weight in order to increase progesterone production in ewes [17]. An injection of hCG at artificial insemination or breeding for improving the reproductive performance of ewes have been administrated [7, 15]. Zamiri and Hosseini [25] reported the increase of prolificacy in ewes which received hCG twenty-four hours after the second injection of PGF2α in comparison with the control group. Also it has been shown that the hCG treatment on the day of mating improves embryo viability and fertility in ewe lambs [12]. Mirzaei et al. [14] reported the higher lambing rate of treated ewes with hCG two days after progesterone withdrawal in comparison with the ewes of control group during seasonal anoestrus. Whereas most of previous researches in this field were done during anoestrous season, the objective of the present study was to examine the effect of an injection of hCG or GnRH two days after sponge withdrawal on the lambing and especially twin lambing birth rate of fat tailed ewes in a commercial flock during breeding season.

Materials and methods

ANIMALS

This study was conducted during breeding season (October to February) in Iran. A total of 75 fat tailed ewes were used. Body weight, age and BCS (1-5 points) were recorded (Table I). All animals were managed under the same conditions on the farm. They were kept under natural field conditions, having access to good quality grasses and water and maintained in good health. The ewe flock was kept away from the rams.

TREATMENTS

Oestrus was synchronized in all ewes using intravaginal progestagen sponges (Chronogest, Intervet, UK) left in situ for 14 days. All groups were treated with Pregnant Mare Serum Gonadotrophin (400 IU PMSG-Folligon, Intervet, Holland; i.m.) at the time of sponge removal. Afterward, the ewes were divided into three groups including hCG (n = 25), GnRH (n = 25) and untreated control ewes (n = 25). Twenty five of ewes received hCG (250 IU; Intramuscular Injection, Chorulon), twenty five of ewes were treated with GnRH (4.2 µg; Buserelin; im; Vetocept) two days after sponge withdrawal in the treatment groups, respectively. Twenty five of ewes were allocated as the control group (no treatment and injected with distilled water as placebo). Then, immediately after injections, the introduction of 8 intact fertile rams (for 51 days) occurred two days after progestagen removal. Blood samples were collected on the 19th day after sponge removal from all animals via jugular venipuncture to measure serum progesterone. Serum progesterone concentration on the 19th day after sponge removal was considered indicative pregnancy [6]. Serum was separated by centrifugation at 1500 × g for 15 minutes and stored at -21°C until P4 was assayed. Progesterone concentrations were determined using ELISA kit (DRG Instruments GmbH, Germany), which detects the concentrations as low as 0.05 ng/ml and has coefficients of variation of 5.8 and 9 % for intra- and inter-assays, respectively. The lambing performance of the ewes in all groups was assessed by lambing data including lambing rate following the induced oestrus and litter size (number of lambs born after mating at first service) which were recorded at lambing. The number of single and twin lambs was recorded for each treatment group. The ewes lambing during two periods, most of them conceived at the first estrous cycle (induced oestrus) following hormonal treatment, and lambed at the first period. Ewes which conceived at the second oestrous cycle (natural oestrus), lambed at the second period. We considered lambing data to compare the groups at the first period.

STATISTICAL ANALYSIS

Data of two ewes of hCG and those of one ewe of GnRH group was excluded before analysis, because of different reasons such as death and sale. The results were statistically analyzed using the SPSS statistical software (Version 15.0, SPSS Inc, Chicago, Illinois). Differences between the percentage of lambing rate, single and twin births rate of control, GnRH and hCG treatment groups were analyzed using the Chi-squared test. Mean numbers of lambs (litter size) in each of the treated groups were compared to that of the control group using the Independent Samples t-test. Data is presented as the percentage or mean (± SD) and values of P ≤ 0.05 were considered as significant data.

Results

There were no significant differences in the pregnancy rate or lambing rate of treated ewes in comparison with that of the control group (Table II). The percentages of pregnancy or lambing rate (number) of ewes after induced oestrus were 76 (19/25), 62.5 (15/24) and 69.6 (16/23) for control, GnRH, and hCG treatment groups, respectively.

The percentages of the single and twin lambing birth rate of ewes following the induced oestrus are shown in Table II. Twin lambing birth rate of GnRH (73.3%) and hCG (68.8%) treatment groups was significantly (P < 0.05) higher than control (31.6%) group in the breeding season. The overall lambing and twin lamb birth rate during the induced oestrus
for all combined treatment and control groups reached 69.4% and 56% in the present study.

Comparing the litter size (number of lambs) at birth in the studied groups was shown in Figure 1. The mean number of lambs was significantly (P < 0.05) greater for ewes treated with GnRH and hCG, in comparison with the control ewes in breeding season. The litter size (Mean ± SD) of ewes following the induced oestrus for control, GnRH and hCG treatment groups were 1.32 ± 0.48, 1.73 ± 0.46, and 1.69 ± 0.48, respectively.

**Discussion**

The effects of hCG or GnRH injection combined with the progestagen and PMSG on the lambing rate and also twin lambing birth rate of ewes were studied during breeding season. The results of the present study showed that hCG and GnRH caused the increase of twin lamb birth rate, however, the lambing rate was not affected by the above-mentioned hormones.

Lambing rate of the ewes treated with hCG was 69.6% following the induced oestrus after treatment in the present study. This is higher than 64.3% [1], 64.6% [2] and 67% [22] reported in ewes fitted only with intravaginal sponges followed by PMSG injection. So, an injection of hCG after progesterone treatment induced high lambing rate during breeding season in ewes. The lambing rate following induced oestrus was not significantly different in treated groups in comparison with control groups in breeding season.

The injection of 4 μg GnRH five days prior to a 100 μg of prostaglandin F2α (PGF2α) analogue injection induced high fertility (88.9 %) during breeding season in ewes [5]. The injection of GnRH five days prior to PGF2α treatment resulted in high oestrus response and fertility and induced a more compact synchronization rate than merely double PGF2α injection [3]. Karaca et al. [10] reported that the fertility rate was higher after exogenous GnRH treatment, immediately prior to a short-term (7d) progesterone treatment – in comparison with the long-term (12d) treatment with progesterone without GnRH treatment (89.6 % vs. 71.6 %) in ewes at the onset of the breeding season. In the study of Husein and Kridli [8], the obtained pregnancy rate was higher (75% vs. 62.5%) in anoestrous ewes treated with a progesterone-GnRH-PGF2α, in comparison with a GnRH-PGF2α treatment. The results of the present study in the hCG group are in agreement with the long-term progesterone treatment, without GnRH administration in the study of Karaca et al. [10]. They are also in accordance with the results of the study of Husein and Kridli [8]. A combination of GnRH with different hormones used in a range of protocols and dose and time treatment may result in these discrepancies of the results in the various studies. Combination of GnRH with the progesterone programs increased the prolificacy of reproductive performance of ewe [10, 9, 22].

In this study, twin lamb birth rates of hCG and GnRH groups were 68.8% and 73.3% which were higher than 30% [26] and 47.2% [10]; Zarkawi et al. [26] and Karaca et al. [10] reported in ewes receiving only long-term progesterone treatment and eCG injection at the removal of sponges.
Twin lamb birth rate of control group was 31.6% in this study which was similar to the result of Zarkawi et al. [26]. In the present study, twin lamb birth rate of treated groups was significantly higher in comparison with those in control group. The number of lambs born per lambed ewes (litter size) of GnRH and hCG groups in the present study (1.73 and 1.69, respectively) were higher than 1.31 [22]. The litter size of GnRH and hCG treatment groups were greater than control group (1.32) in this study. The study of Titi et al. [22] was done at the beginning of the breeding season, but the ewes used in the present study were cyclic ones during breeding season. Fertility and prolificacy of ewes were not affected by hCG at the time of insemination; and hCG treatment at the time of AI may be beneficial in increasing fertility of ewes from farms with low fertility rates [7]. Zamiri and Hosseini [25] reported increased prolificacy in ewes which received hCG twenty-four hours after the second injection of PGF2α in comparison with the control group. Treatment with GnRH or hCG on the 12th day postmating of ewes enhanced luteal weight, amniotic sac width and length, crown-rump length and embryo weight [11]. Thus, human chorionic gonadotropin with similar function with LH, can increase the fertility rate and improve the pregnancy rate [17, 21].

The formation of preovulatory follicle occurs at the end of oestrous cycle of ewes. The injection of eCG at the progestagen removal can increase the number of selected follicles in order to increase maturation of many preovulatory follicles. Therefore, twin lambing birth rate increased following the GnRH and hCG treatment two day after progestrogen removal in the breeding season. Injection of hCG or GnRH generally induces an increase in LH pulse frequency and provoke ovulation. Higher lambing rate of treated ewes with hCG (85.7%) two days after progesterone withdrawal in comparison with the control ewes during seasonal anoestrus was reported [14]. They also reported that there were no significant differences between treatment groups in comparison with control group in twin lambing rate. Treatment with hCG or GnRH was performed in order to induce ovulation and the formation of a corpus luteum in the ewes. Hence, in the present study, it was proposed that the number of ewes with ovulated follicles would increase in response to treatment - resulting in high lambing rate especially twin lamb birth rate. According to the results of the present study, treatment with hCG or GnRH two days after long-term progestagen treatment during breeding season could result in better twin lamb birth rate in cyclic ewes, however, it doesn't increase lambing rate of these ewes.

In conclusion, the effect of hCG or GnRH on the number of lambs at birth (litter size) maybe more efficient than in control group. It can be concluded that the combination of hCG or GnRH with progestagen maybe effective in increasing twin lambing birth rate in breeding season.


