



Original Article

The Effects of short and long-term progesterone treatment combined with PMSG on the fertility rate of Ghezel breed ewes during the breeding season

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ABSTRACT

The objective of this study was to compare the efficacy of short-term and long-term progesterone treatments on the fertility rate of Ghezel breed ewes during breeding season. Forty ewes were distributed in a randomized block experimental design, where the treatments consisted of two protocols for estrus synchronization (short, 7 days and long, 12 days) with twenty animals each. The progesterone treatment consisted of a vaginal sponge containing 30 mg fluorogestone acetate (FGA) inserted into the vagina of the ewes for 7 or 12 days. Afterwards, on the 5th and 10th days of the protocols, 0.5 ml Clo Pg@ and 400 IU of PMSG was intramuscularly administered to all the ewes. Percentage of estrous, pregnancy and lambing rates were 80%, 75% and 56.25% in the short term treated group and 95%, 84.21% and 73.68% in the long term treated group respectively. The litter size did not differ between the short term and the long term treated groups (1.44 vs. 1.50). There were no significant differences in fertility responses between protocols ($P>0.05$). The short-term progesterone treatment was effective to synchronize estrus in Ghezel breed ewes during breeding season. However, comparing the protocols, the long-term protocol presented better results of positive estrus percentage, pregnancy rate and lambing rate in Ghezel breed ewes.

اثرات کوتاه و بلند مدت پروژسترون درمانی ترکیبی با PMSG بر روی میزان باروری میش های نژاد قزل در فصل تولید مثل

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چکیده

هدف این تحقیق مقایسه کارایی درمان پروژسترونی کوتاه مدت و بلند مدت بر روی میزان باروری میش های قزل در فصل تولید مثل بود. ۴۰ میش در قالب طرح آزمایشی بلوک های تصادفی در دو پروتکل هم زمان سازی فحلی (کوتاه مدت ۷ روزه و بلند مدت ۱۲ روزه) و هر پروتکل با ۲۰ میش مورد استفاده قرار گرفت. درمان پروژسترونی شامل یک اسفنج واژنی محتوی ۳۰ میلی گرم فلوئوروجستون استات (FGA) بود که به مدت ۷ روز و ۱۲ روز در واژن میش ها قرار داده شد. پس از آن در روز های ۵ و ۱۰ پروتکل ها ۰/۵ میلی لیتر Clo Pg و ۴۰۰ واحد بین المللی PMSG به شیوه عضلانی به همه میش ها تزریق شد. درصد استروس، نرخ آبستنی و نرخ بره زایی در گروه درمانی کوتاه مدت به ترتیب ۸۰٪، ۷۵٪ و ۵۶/۲۵٪ و در گروه درمانی بلند مدت به ترتیب ۹۵٪، ۸۴/۲۱٪ و ۷۳/۶۸٪ حاصل گردید. نرخ دوقلو زایی در پروتکل های درمانی کوتاه مدت و طولانی مدت تفاوتی نداشتند (۱/۴۴ در برابر ۱/۵۰). تفاوت معنی داری در پاسخ های باروری در بین پروتکل ها مشاهده نگردید ($P>0/05$). پروژسترون درمانی کوتاه مدت در هم زمان سازی فحلی میش های قزل در فصل تولید مثل موثر بود. لیکن در مقایسه پروتکل ها، پروتکل طولانی مدت نتایج بهتری را در درصد استروس، نرخ آبستنی و نرخ زایش در میش های قزل نشان داد.

واژه های کلیدی: میش قزل، هم زمان سازی فحلی، هورمون، تولید مثل

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INTRODUCTION

Applications of exogenous hormones for increased reproductive performance in domestic ewes usually focused on estrous synchronization. Estrous synchronization in goats and sheep is achieved by control of the luteal phase of the estrous cycle, either by providing exogenous P4 or by inducing premature luteolysis. Progestagens, one of the types of hormone used for synchronization in ewes, can be given by oral administration, subcutaneous, or intravaginal insertion [1]. Progestagen-based protocols are commonly used worldwide by various methods, routes and doses [2]. The most common route of application in ewe is intravaginal sponges impregnated either with fluorogestone acetate (FGA), or methylacetoxo progesterone (MAP) or with a Controlled Internal Drug Releasing (CIDR) device containing 0.3 g of progesterone P4 [3]. According to the role of PGF₂" in luteolysis of Corpus Luteum double PGF₂" injections is common for estrus synchronization in ewes. Since efficacy of PGF₂" is limited to the breeding season where active CL, different protocols using combinations of P4 and gonadotropin Releasing Hormone (GnRH) or human Chorionic Gonadotropin (hCG) have been recommended for estrus synchronization outside the breeding season [4]. Few studies have used GnRH in ovulation synchronization of ewes. The GnRH-based protocols used during the non-breeding season aimed at providing a source of P4 for inducing ovulation or luteinization of follicles, moreover, the time required to accomplish this protocol is shorter than other methods [5]. Administration of intravaginal progestagens such as FGA or CIDR for 10-16 days followed by intramuscular (IM) injection of equine Chorionic Gonadotropin (eCG) appear to be the most practical method for estrus synchronization in ewes [6]. However, short-term protocols possibly allow for facilitating the managerial tasks, minimizing the vaginal

discharge and infection risks and thus increasing the fertility rates. Indeed, short-term sponge treatments (5-7 days) have been successful in sheep regardless of breeding season [7]. Long time progesterone applications have had suppressive effects on fertility and thus short period progesterone applications have been suggested as an alternative [8, 9]. Short-term sponge applications, such as for 5-7 days, have produced good results in establishing oestrus for ewes both in and out of the mating season [10]. The optimal fertility can be achieved with lower doses of progestagen by halving the intravaginal sponges. On the other hand, postulate that a high level of progestagen followed by its rapid withdrawal is a necessary prerequisite for acceptable fertility [11]. The Ghezel sheep is a high weight Iranian breed which is raised in the western north of Iran. This animal has a good compatibility in cold condition and has a good capability for grazing and walking. Meat is the main source of income for farmers [12]. Ghezel sheep numbering about 2 million are raised in North Western of Iran. This breed is native, fat-tailed and large-sized (38.2 to 41.7 kg at yearling in female and male respectively). They are well adapted to mountainous and cold conditions (-22.8 to -38.3°C). They are raised primarily for meat, with milk and wool being of secondary importance. Ways to increase meat production in sheep, in any system, are likely to be by producing more lambs per ewe and increasing growth performance of the lambs. The first objective can be achieved by increasing ewe productivity, including lambing rate and frequency, whereas the second objective requires enhancement of the growth potential and survival of lambs [13]. Recently, Kaşıkçı *et al.* have concluded that the sponges containing 20 mg FGA can be halved for a more economical estrus synchronization of ewe at the farm level, resulting in higher pregnancy rates [14].

Therefore, the objective of this study was to compare the efficacy of a 7-days and a 12-days treatment with progesterone sponges in combination with CLO PG® and PMSG to induce fertile estrus in Ghezel breed ewes during the breeding season.

MATERIALS AND METHODS

Location, animals and treatments

This experiment was carried out at suburbs of Miandoab in West Azarbaijan province in Iran in breeding season, from September to October. The site is located at 46°6'E latitude, 36°58'N longitude and 1314m from the sea level in the center of the plain areas which ends at south front of Lake Urmia. The annual rainfall in this region ranges from 250 to 300 mm. A total of 40 Ghezel breed ewes 2-4 years-old and weighing 45-55 kg, were used in this study. All the ewes were examined and clinically considered as healthy. The animals were kept indoors at night and had access to natural postures outdoors most of the day. Indoors ewes were offered diets of barley, wheat bran, and wheat straw supplemented with vitamins, Water and mineral licks were available ad libitum. The experimental design was completely randomized, where the treatments consisted of two protocols for estrus synchronization (short and long) with twenty animals each. Forty ewes were randomly allocated to 2 groups of 20 ewes which received short term or long term progesterone treatments, respectively. During the breeding season, a vaginal sponge containing 30 mg fluorogestene acetate (FGA, Chronogest, Intervet, Natherlands) was inserted into the vagina of the ewes for 7-days in the short-term protocol and for 12-days in the long-term protocol.

Ewes subjected to the short-term protocol were identified with green collars, On the 5th day of the protocol, 0.5 ml CLO PG® and 400 IU PMSG was intramuscularly injected in each ewe. On the 7th day, the sponge was removed to finish the protocol. Ewes subjected to the long-term protocol were identified with red collars, On the 10th day of the protocol, 0.5 ml CLO PG® and 400 IU PMSG was intramuscularly injected in each ewe. On the 12th day, the sponge was removed to finish the protocol.

Mating, estrus and pregnancy detection

After removing the sponges, in both groups, the ewes were organized according to the treatments and exposed to rams in a 1:5 ratio. Rams were greased in the pectoral region using oily mixture and pigment to detect covered ewes and a possible estrus. Estrus occurrence were grouped into three distribution periods (<30, 30 to 54, and 55 to 72 hours after sponge removal) using the vasectomized rams. After estrus confirmation, ewes were exposed to rams with proven fertility. The efficiency of the tested protocols was determined from positive estrus rate (%), pregnancy rate (%), and lambing rate (%), and Litter size (the number of total lambs/the number of lambing ewes). Data on the occurrence of estrus were described for each protocol. One month after the natural insemination by rams, conception rates of animals all groups were checked by transabdominal ultrasonography, using B-mode diagnostic ultrasound scanner (100 Falco, Pie Medical Application Manual, Equipment B.V., Maastricht, Netherland). The numbers of lambs born per ewe were recorded daily during lambing. Fertility was monitored in terms of Estrus rate, pregnancy rate and

lambing rate and litter size. The following parameters were recorded:

a) Estrus Rate: Number of ewes showing estrus/Total treated ewes in each protocol x100. b) Pregnancy Rate: Number of pregnant ewes/Number of inseminated ewes in each protocol x100. c) Lambing Rate: Number of ewes lambing/ Number of inseminated ewes in each protocol x100. d) Litter size: the number of total lambs/the number of lambing ewes.

Statistics

Data were analyzed with chi-square analysis to compare the estrous, pregnancy and lambing rates among the groups. Results were considered significant at $p < 0.05$.

RESULTS

Occurrence of estrus in ewes subjected to short-term and long-term protocols along 72 hours were presented in tables 1 and 2 respectively. The effect of short-term and long-term protocols of estrus synchronization on reproductive performance of Ghezel breed ewes has been presented in tables 3. During the breeding season, estrus, pregnancy, lambing rates and litter sizes were 80%, 75%, 56.25% and 1.44 in the short-term protocol and 95%, 84.21%, 73/68% and 1.50 in the

long-term protocol, respectively. There were no statistically significant differences ($p > 0.05$) between the short-term and long-term protocols for these reproductive parameters.

DISCUSSION

Reproduction of small ruminants can be controlled by several methods developed in recent decades. Some of these involve administration of hormones that modify the physiological chain of events involved in the sexual cycle [2]. Methods which utilize progesterone or its analogues are based on their effects in the luteal phase of estrous cycle, simulating the action of natural P4 produced by the corpus luteum after ovulation, which is responsible for controlling LH secretion from the pituitary gland. Although the current methods of estrus synchronization are very effective, they are applied over a relatively long period of time. However, it is possible to reduce the duration of treatment by combining progestagen with PMSG and PGF treatments [15]. Results obtained in our study indicate that a short term FGA treatment has an efficacy comparable to that of a long-term FGA treatment during the breeding season. The estrus response to short-term FGA treatment is comparable to that previously reported by others [16]. The short-term protocol presented an interesting successful

Table 1. Occurrence of estrus in ewes subjected to short-term protocol along 72 hours.

| periods | Ewes (n) | (%) |
|---------------------|----------|-------|
| < 30 hours | 3 | 15 |
| 30 to 54 hours | 8 | 40 |
| 55 to 72 hours | 5 | 25 |
| Without estrus | 4 | 20 |
| Total occurrence | Ewes (n) | (%) |
| Ewes with estrus | 16 | 80 |
| Ewes without estrus | 4 | 20 |

rate, where above 80% of ewes tested had estrus. The results obtained with the short-term treatment indicate that this method gives a high level of estrus synchronization and fertility. This result is in agreement with Öztürkler *et al.* who obtained a synchronization rate of 93.3% using a treatment associating short term progesterone treatment and PMSG [16].

been successful in sheep regardless of breeding season [19]. The percentage of ewes exhibiting estrus in this trial was comparable to values reported in the literature [20]. Ustuner *et al.* stated that as result of the intravaginal application of sponges for 6 and 12 days in Awassi ewes, they observed an estrus response of 77.1% in the short-term group and 100% in the long-term group [21].

Table 2. Occurrence of estrus in ewes subjected to Long-term protocol along 72 hours

| periods | Ewes (n) | (%) |
|---------------------|----------|-------|
| < 30 hours | 7 | 35 |
| 30 to 54 hours | 11 | 55 |
| 55 to 72 hours | 1 | 5 |
| Without estrus | 1 | 5 |
| Total occurrence | Ewes (n) | (%) |
| Ewes with estrus | 19 | 95 |
| Ewes without estrus | 1 | 5 |

Table 3. Effect of different protocols on reproductive performance of Ghezel breed ewes

| Parameter | Short-term Protocol (n=20) | Long-term Protocol (n=20) |
|----------------------|-------------------------------|------------------------------|
| Estrus rate (%) | 80 | 95 |
| Pregnancy rate (%) | 75 | 84.21 |
| Lambing rate (%) | 56.25 | 73.68 |
| Litter Size | 1.44 | 1.50 |

Although, administration of intravaginal progestagens such as FGA or CIDR for 10-16 days followed by intramuscular (IM) injection of equine Chorionic Gonadotropin (eCG) appear to be the most practical method for estrus synchronization in ewes [17]. But a prolonged time of administration could result in low conception rates [18]. Meanwhile, short-term protocols possibly allow for facilitating the managerial tasks, minimizing the vaginal discharge and infection risks and thus increasing the fertility rates. Indeed, short-term sponge treatments (5-7 days) have

Ataman *et al.*, (2006) reported that with 12 and 7 day intravaginal FGA sponge applications in ewes in their breeding season, a 100% estrous rate was obtained in both groups [22]. In sheep, the administration of progesterone is performed by intravaginal route by using silicone devices (like CIDR) or polyurethane devices (sponges) historically placed during 12-14 days associated with a dose of eCG at the end of the treatment. The technique is based on the inhibitory role of progestogens on the hypothalamus-pituitary axis, acting like an exogenous corpus luteum. However, the current information published in the last years

about follicular dynamics, endocrinology and control of the corpus luteum opens new questions about the use of these traditional long treatments. In sheep and goats, the insertion of an intravaginal device containing 0.3 g of progesterone results in a rapid increase in blood concentrations of this hormone (>5 ng/mL) during approximately 4-5 days of treatment, similar to those levels observed during medium-late luteal phase. However, after 6 or 7 days of treatment, blood progesterone concentrations decrease to subluteal levels (<2 ng/mL), enough for blocking ovulation but predisposing to a persistent growth of the dominant follicle. If the intravaginal device is maintained during 12 or 14 days, the detrimental conditions of low progesterone levels described above are present during an excessive period, and then fertility is affected. In order to avoid this situation, some years ago we put into practice the "Short-term protocols" for Fixed-time artificial insemination, which consist of only 5-7 days of exposure to progesterone. This treatment is associated with one dose of 200 to 400 IU of eCG to induce ovulation and one luteolytic dose of PGF 2α at device removal [23]. Additionally the long-term protocol in our study presented a high successful rate, where above 95% ewes tested had estrus (Table 2). Long term progestagen estrus synchronization protocols can affect follicular dynamics and fertility of ewes. Initially, a supraluteal effect is expected, which means that an increase in follicular renewal may occur. In the end, however, a subluteal effect may happen and decrease the speed of follicular renewal [24]. Progesterone blocks FSH and LH secretion by suppressing the hypothalamus and also indirectly the pituitary anterior lobe and temporarily stops follicular development. This suppression disappears with the removal of the sponges, and estrous behaviours are observed along with follicular

development. To obtain a high rate of estrus and ovulation, PMSG has to be applied together with progestogen impregnated sponges. Ovulation takes place in the 48-72 hours after the injection of PMSG, which is administered when the sponge is removed [26]. Methods which utilize progesterone or its analogues are based on their effects in the luteal phase of the cycle, simulating the action of natural progesterone produced by the corpus luteum after ovulation, which is responsible for controlling LH secretion from the pituitary. On the other hand, when compared both protocols (Table 3), the long-term protocol compared with short-term protocol presented better results regarding positive estrus and pregnancy rates of ewes. Previous studies reported that in short term treated ewes eCG provoked the development of follicular cysts. Therefore the reduction in fertility observed in short-term groups could be associated with the development of follicular cysts. The long progesterone treatment results in subluteal progesterone level. This phenomenon leads to increasing the LH pulses frequency, but the LH surge does not occur, and result in persisting largest follicle [27].

Husein *et al.* identified an estrus-exhibiting rate of 100% for ewes in a group treated with an FGA sponge + PMSG for 12 days and as 83% for the ewes in a group treated with an FGA sponge + PMSG for 4 days [28]. Although the short-term application of intravaginal sponges offers some advantages, it has a disadvantage in that the possibility of becoming pregnant decreases because the progestogen used for estrous synchronisation is at a low level. The reason for this decrease is explained by the prolonged life of the ovulatory follicle when progestogen is applied at a low level [29].

In this study the overall lambing rate and litter size recorded following different lengths of progestogen treatments (short-term and long-term) were 56.25, 1.44 and 73.68, 1.50, respectively. Ustuner et al. reported that after short and long-term sponge applications in ewes of the Awassi breed, the induction of estrus took place at a rate of 77% and 88%, respectively [30]. Ozyurtlu et al. noted that 7-day long progesterone applications in Awassi ewes were as effective as 14-day long progesterone applications. They also reported pregnancy rates of 66.7% and 58.3% and lambing rates of 83.3% and 66.7% for applications of 7 and 14 days, respectively [31]. However, in this study, close results were obtained with the long-term applications in terms of estrous response and lambing rates, whereas the results of short-term applications were found to be a bit lower. Differences among reports in the literature may be due to differences in synchronisation procedures, the amount of PMSG applied, and breed differences in the ewes studied. The conception rate of ewes in this study was comparable to that previously observed during the breeding season [16, 21]. Lambing rates in the short-term and long-term protocols were 56.25% and 73.68% respectively. These percentages are close to 80% reported by Khalilavi et al., in ewes treated with intravaginal sponges for 12-days followed by eCG administration and higher than 60% reported by Öztürkler et al., in ewes treated with intravaginal sponges for 5 days followed by PMSG injection in the breeding season [16, 32]. The litter size obtained in our study (1.44-1.50) was comparable with that (1.1-1.9) reported in earlier studies [1, 33].

The progestogens that are most commonly used for the purpose of inducing synchronisation of estrus during the breeding season are synthetic progesterone impregnated

vaginal sponges such as fluorogestone acetate (FGA) or medroxyprogesterone acetate (MAP) [25].

CONSLUSION

This study showed that the use of short-term progesterone treatment is effective to synchronize estrus in breeding Ghezel breed ewes. This protocol has some advantages. The duration of synchronization procedure is shorter than that required for other method such as long-term progesterone treatments, progesterone implant or double doses of PGF2 α . This protocol could be used as an alternative for the synchronization of estrus in Ghezel breed ewes.

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ETHICS

All procedures of the current research have been performed based on the ethical standards.

CONFLICT OF INTEREST

There is no conflict of interest.

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