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ESTRUS SYNCHRONIZATION AND TWINNING RATE OF GHEZEL EWES TREATED WITH CIDR AND PMSG DURING THE BREEDING SEASON

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ABSTRACT: The objective of this study was to investigate the efficacy of used controlled internal drug release devices (CIDR) and different doses of PMSG on estrus synchronization in Ghezel ewes. This investigation was conducted in 77 fat-tailed Ghezel ewes during the breeding season. All animals were divided randomly into four groups then a single intramuscular (IM) injection of PMSG (group 1, 350 IU, n=20; group 2, 450 IU, n=20; group 3, 550 IU, n= 20), group 4 (n=17) was made apart from 1 ml normal saline solution which was used as control group at time of CIDR removal . Estrus responses were similar in all groups (group 1, 100%; group 2, 90%; group 3, 95%; control group, 82.35%). There were no significant differences (P>0.05) between the treatment groups and the control group regarding the onset of estrus or estrus response. Pregnancy rates were 85%, 90%, 95% and 64.7% in groups 1, 2, 3 and the control group, respectively. Pregnancy rates were higher in groups 1, 2 and 3 than in control group (P<0.05). Lambing rates were obtained as 80%, 90%, 90% and 58.8% in groups 1, 2, 3 and in control group, respectively. Differences between the treated and the control animals in the Lambing rates were significant (P<0.05). Using PMSG at CIDR withdrawal increased twinning rate from 10% in control group to 33.3% in group 3, 550 IU. There were significant differences (P<0.05) between the treatment groups and the control group regarding the gestation period and the birth weight. Differences between the treated and the control animals in the Plasma P4 levels at day estrus after PMSG treatment and 30th day of pregnancy were significant (P<0.05). Plasma P4 levels at 30th day of pregnancy was 0.94ng/ml, 1.1ng/ml, 1.24ng/ml and 0.82ng/ml in groups 1, 2, 3 and the control group, respectively.

Keywords: Estrus synchronization, Ghezel Ewe, CIDR (controlled internal drug release devices), PMSG (pregnant mare serum gonadotropin), P4 (Progesterone)

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INTRODUCTION

Estrus synchronization or the induction of estrus is a valuable management tool for increasing the pregnancy rate in ewes. Modern sheep husbandry has improved the efficiency of extensive production and controlled the reproductive process for intensive production. The synchronization of estrus in ewes focuses on the manipulation of the estrus cycle (Zonturlu et al., 2011).

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 (Baneh, 2009). 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) (Figure 1). 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 (Baneh et al., 2010).

Figure 1. Ghezel sheep numbering about 2 million are raised in North Western of Iran

To have a better reproduction efficiency, it has been suggested to use new reproductive approaches such as controlling and synchronizing of estrus and using PMSG by applied AI to increase prolificacy that leads to gain practical and economical advantages. Recently, Progesterone or its analogues is generally used to synchronize estrous during the breeding and non-breeding season (Dogan et al., 2005). Administration of gonadotropins such as human menopausal gonadotropin (hMG)

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(Evans., 2003), PMSG (Lamrani et al., 2008), follicle stimulating hormones (FSH) and mixed gonadotropins preparations (Knights et al., 2003) after stopping progestogens treatment, causes in-creasing rate of ovulation.

Between all endocrine approaches to increase lambing rate, administration of PMSG is more usual than others. Ustuner et al. (2007) reported that injection of PMSG at the end of the progestogens treatment causes more precise synchronization of oestrus in small ruminants. Injecting PMSG after CIDR removal causes oestrus signs to begin earlier, become more pronounced and prolonged. A prolonged estrus probably results in elevation of circulating estrogen that causes luteinizing hormone (LH) peak (Yıldız et al., 2004). This might increase the rate of ovulation and enhances twining rate. It has been shown that an adequate dose of PMSG improves proliferation, but the use of high dose induces multiple gestations and thus, an increase in fetal or lamb mortality (Ataman *et al*., 2006). Hence, to avoid non desirable fetal or losses and large litter sizes, the dosage level of such gonadotropin has to be adjusted according to breed, season and the physiological status of the ewes (Nosrati et al., 2011).

Therefore, the objective of this study was to determine the influences of different PMSG doses on reproductive performance and twinning rate of Ghezel ewes that natural inseminated by rams.

MATERIALS AND METHODS

Location, animals and treatments

This experiment was carried out at breeding station of Ghezel sheep in 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 77 Ghezel ewes 2-4 years-old and weighing 45-55 kg, were used in this study. CIDR were inserted into vagina of the ewes for 14 days. In group 1 (n=20), group 2 (n=20) and group 3 (n=20) 350 IU, 450 IU and 550 IU of PMSG was administered, respectively, at the time of sponge withdrawal. In the control group (n=17), ewes were injected with 1ml normal saline solution at sponge removal to act as untreated controls.

Mating, estrus and pregnancy detection

Three fertile Ghezel rams were introduced to each group (12 rams totally) twice a day (0800 - 1100 and 1700 - 2000 h), starting about 24 h after CIDR withdrawal, and left with them for estrus detection and natural mating. Ewes were observed continuously during the 3 h when rams were introduced to them and their mating were recorded. One months 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 conception rate (percentage of pregnant ewes /ewes inseminated) and mean litter size (lambs born/ ewes inseminated). For prevention of pregnancy toxicity in late pregnancy, all ewes received additional 250 g/day/doe barley grain.

The following parameters were recorded:

- Percentage of Animals in Estrus*:* Number of ewes showing estrus/Total treated ewes in each group x100
- Pregnancy Rate: Number of pregnant ewes/Number of inseminated ewes in each group x100
- Lambing Rate: Number of ewes lambing/ Number of inseminated ewes in each group x100
- Duration of pregnancy
- Birth weight.

Blood samples

After the CIDR implantation (day 0), a series of blood samples was collected at days Estrus after PMSG treatment and 30th day of pregnancy. Blood samples were obtained from a jugular vein using vacutainer vials and centrifuged immediately after collection at 3000 rpm for 10 minutes at 4°C. The blood plasma was then stored at -20°C until assayed. Concentrations of progesterone were determined by ELISA kit (Monobind®; USA) with 0.1 ng/ml sensitivity.

Statistics

Estrus response and Pregnancy rates of the groups and reproductive performance were analyzed using the chi-square test. Statistical analyses on the concentration progesterone were performed on a microcomputer using Statistical Package for Social Science (SPSS) program (version 20.0). Data were analyzed through analysis of variance (ANOVA) with significant difference level of $P<0.05$.

RESULTS

Effects of CIDR (controlled internal drug release device) which used to synchronize estrus and different doses of PMSG on fertility parameters were presented in Table 1. The rates of estrus in groups 1, 2, and 3 which received different doses of PMSG and the control group were found as 100, 90, 95, and 82.35%, respectively. There was no significant difference between groups ($P>0.05$).

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Pregnancy rates were 85, 90, 95 and 64.70% in groups 1, 2, 3 and the control group, respectively. There were significant differences between the treated groups and the control group (P<0.05).

The lambing rates for groups were 80, 90, 90 and 58.82%, respectively. The mean lambing rate in groups 3 and 2 were higher than in groups 1 and 4 (P<0.05). Using PMSG at CIDR withdrawal increased twinning rate from 10% in control group to 33.3% in group 3, 550 IU (P<0.05).

Gestation periods of the animals in groups 1, 2, 3, and 4 were found to be 153±0.21, 148±0.12, 148±0.16 and 158±0.27 d, respectively. There were significant differences between the treated groups and also between the treated groups and the control group (P<0.05). There was significant (P<0.05) effect of the hormonal treatments on the birth weight of lambs averaging 4.9±0.09, 4.0±0.10, 3.9±0.10 and 4.9±0.13 kg for groups 1, 2, 3 and 4 respectively.

Mean progesterone concentration at day estrus were 0.37±0.05, 0.40±0.12, 0.45±0.06 and 0.39±0.03 ng/ml in groups 1, 2, 3 and the control group, respectively. There was significant (P<0.05) effect of the hormonal treatments on the mean progesterone concentration at day estrus between groups.

Mean progesterone concentrations at 30th day of pregnancy were 0.94±0.21, 1.10±0.19, 1.24±0.18 and 0.82±0.27 ng/ml in groups 1, 2, 3 and 4, respectively. Mean progesterone concentration in group 4 with injection dose of 550 IU (1.24±0.18 ng/ml) was the highest value between all groups (P<0.05).

Table 1 - Some reproductive parameters in Ghezel ewes treated with CIDR with PMSG during the breeding season.

Groups	1	2	3	4
Estrus response (%)	100^{a}	90^{a}	95^{a}	82.35^{a}
Pregnancy rates (%)	85^{a}	90^{a}	95^{a}	64.7^{b}
Lambing rate (%)	80^{b}	90^{a}	90^{a}	58.8^{c}
Twinning rate (%)	12.5^{c}	22.2^{b}	33.3^{a}	10^{c}
Lamb birth weight (kg)	4.9±0.09^{a}	4.0±0.10^{b}	3.9±0.10^{b}	4.9±0.13^{a}
Gestation period (day)	153±0.21^{b}	148±0.24^{c}	148±0.16^{c}	158±0.27^{a}
P4 levels, Day of estrous (ng/mL)	0.37±0.05^{ab}	0.40±0.12^{ab}	0.45±0.06^{a}	0.39±0.03^{b}
P4 levels, 30th day of pregnancy (ng/mL)	0.94±0.21^{ab}	1.10±0.19^{ab}	1.24±0.18^{a}	0.82±0.27^{c}

^{a,b,c}: Means in the same row with different superscripts differ significantly (P< 0.05)

DISCUSSION

The breeding season of Ghezel ewes in West Azarbaijan province in Iran usually lasts from July to November. However, the majority of ewes are bred between July and early September in this zone. The present study was performed at the beginning of the breeding season, in mid-July.

In the present study Ghezel ewes have received different doses of PMSG following 13-day progesterone treatment. Progestogens and PGF 2α or their analogues were used in order to condense parturition and oestrus of the ewes in the breeding season. Hormones such as GnRH, PMSG, FSH, and LH may be used to increase pregnancy rate and numbers of lambs (Monika, 2001). Injection of 500 IU of PMSG following the treatment of ewes in the breeding season with vaginal sponges containing 30-40 mg of FGA resulted in 90% and 85% oestrus and conception rates, respectively (Miljkovic et al., 1989). Pregnancy rates in ewes receiving the same dose of PMSG and FGA were higher than in the controls (Dumitrescu et al., 1985).

Similarly, Karagiannidis et al. (2001) reported that responses to different PMSG doses varied among various breeds. The results on estrus rate for treated groups were consistent with that reported by some other researchers (Domingues et al., 1991).

The conception rate was comparable to those reported by Miljkovic et al. (1989). The different reproductive performance may be associated with animal use of different breeds, age and body condition, and also with nutritional factors, type of insemination or management systems. In this study, the percentages of estrus and pregnancy rates in group 3 received 550 IU of PMSG were determined as 95%. The rate of estrus response was similar to the previous findings of Krajinovic et al. (1985).

Moreover, there were significant differences regarding pregnancy and lambing rates between trial and control groups. Hence, Nosrati et al. (2011) observed no significant difference in pregnancy rates for different PMSG doses (300 IU, 400 IU, 500 IU and 600 IU).

Zeleke et al. (2005) recorded a pregnancy rate as 75% and a lambing rate as 94.6% in ewes treated with sponges and 300 IU of PMSG. Also, Zarkawi *et al*. (1999) reported a higher lambing rate (80%) in Awassi ewes which have received 600 IU of PMSG after 60 mg of medroxyprogesterone acetate (MAP) during out of breeding season. Al-Merestani et al. (1999) conducted a study in which Syrian Awassi sheep were treated with intravaginal sponges combined with 400 IU of PMSG. They have reported a lambing rate as 78%. It was thought that fertility parameters could be affected by different treatment seasons such as anestrus, breeding or transition season.

In the presented study, the percentages of lambing and twinning rates in group 3 given 550 IU of PMSG were determined as 90% and 33.3%, respectively. It was pointed out that administration of PMSG increased the number of follicles and therefore raised the twinning and triplet rates (Gulyuz et al., 1995).

PMSG injection increased twinning rate from 10% in CIDR-treated ewes without PMSG, to 33.3% in 550 IU PMSG-injected ewes. This increase is of great value to sheep holders, and is similar to that obtained by Zarkawi (2001) who reported that Awassi ewes in Syrian, treated with sponges plus PMSG, had a twinning rate of 50%

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compared with 20% for sponge-treated ewes without PMSG. Some papers reported that administration of 300 IU PMSG was not sufficient to stimulate additional follicular development or was weak for some breeds response (Koyuncu et al., 2008; Zonturlu et al., 2011). Twining rate in experiment of Nosrati et al. (2011) that synchronized the Kurdi ewes for 14 d with CIDR and superovulated by 500 IU of PMSG injection were 33.5% that was similar to the result of current study obtained by using 550 IU PMSG.

In the presented study, the percentages of lambing rate in groups 2 and 3,90% was higher than that in groups 1 and 4 , 80% and 58.8%, respectively (P<0.05). These results were in agreement with those reported by Zonturlu et al. (2011) and Timurkan et al. (2005). Koyuncu et al. (2001) reported that the administration of 700 IU of PMSG increased multiple-birth rates and lambing rates. Zeleke et al. (2005) recorded a lambing rate of 94.6% in ewes treated with sponges and 300 IU of PMSG. Also, Zarkawi et al. (1999) reported a higher lambing rate (80%) in Awassi ewes, outside the breeding season, which were administered 600 IU of PMSG after 60 mg of medroxyprogesterone acetate (MAP), compared to ewes in the control group. Al-Merestani et al. (1999) in a study in which Syrian Awassi sheep were treated with intravaginal sponges combined with 400 IU of PMSG, reported a lambing rate of 78%. The results obtained by Zarkawi et al. (1999) and Al-Merestani et al. (1999) for lambing rate were statistically important when PMSG injected groups were compared with animals that received no treatment. Safranski et al. (1992) reported that average gestation periods in control and trial groups received melengesterol acetate (MGA) + PG-600 (400 IU of PMSG+200 IU of HCG) were found as 163.8±4.9 and 157.2±2.8 d, respectively.

In our study, gestation period was 153±0.21, 148±0.12, 148±0.16, and 158±0.27 d in groups 1, 2, 3, and 4, respectively. It was seen that there were significant differences both within treated groups and as well as between trial and control groups (P<0.05). Because increased lambing rate related with increased PMSG dose may result in shortened gestation periods, as reported by previous researches (Safranski et al. 1992; Horoz et al. 2003).

There was significant (*P*-<0.05) effect of the hormonal treatments on the birth weight of lambs averaging 4.9 ±0.09, 4.0±0.10, 3.9.1±0.10 and 4.9±0.13 kg for groups 1,2,3 and 4 respectively. This result also can be attributed to increased twinning rate related in the increase of PMSG dose.

Mean progesterone concentration at day estrus were 0.37±0.05, 0.40±0.12, 0.45±0.06 and 0.39±0.03 ng/ml in groups 1, 2, 3 and the control group, respectively. These results were consistent with that reported by Cunningham et al. (1975). Cunningham et al. (1975) conducted a study in which Cheviot ewes were used to determine levels of progesterone in the plasma during the estrous cycle. In the study of Cunningham et al. (1975) Plasma progesterone levels increased progressively during the period 15 to 9 days before estrus to a mean level of about 2-5 ng/ml, and remained at this level for several days. By 2 days before estrus, the mean plasma progesterone concentration had fallen to 1.42 ng/ml, and on the following day it had dropped to <0.5 ng/ml. It remained at this low level until after Day 2 of the cycle, and then again showed a progressive rise.

The data presented above confirm that, in the cyclic ewe, plasma progesterone values fall to very low levels on the day before estrus, also PMSG, when injected immediately after the removal of CIDR increased the rate of ovulation hence, increasing multiple births and litter size (Akoz et al., 2006).

Mean progesterone concentration at 30th day of pregnancy was 0.94±0.21, 1.10±0.19, 1.24±0.18 and 0.82±0.27 ng/ml in groups 1, 2, 3 and 4, respectively. Mean progesterone concentration in group 4 with injection dose of 550 IU (1.24±0.18 ng/ml) was the highest value between all groups (P<0.05). This value in groups 3 (1.10±0.19 ng/ml) and 2 (0.94±0.21 ng/ml) were higher than group I (0.82±0.27 ng/ml) (P<0.05). Some papers reported that administration of 300 IU PMSG and less than was not sufficient to stimulate additional follicular development or was weak for some breeds response (Fallah et al., 2007; Nosrati et al., 2011; Oyedipe et al., 1989). In the present study we observed a PMSG-dose-dependent increase in progesterone levels between groups at 30th day of pregnancy. This result was similar to the previous findings of Oyedipe et al. (1989).

Oyedipe et al. (1989) conducted a study in which Yankasa ewes were used to determine the effect of dose of pregnant mare serum gonadotrophin on estrus parameters, ovulation rate and peripheral progesterone concentrations. Ovulation rates (based on number of corpora lutea) averaged 1.0±0.0, 1.3±0.3, 2.0±0.0, 5.5±0.5 and 7.0±1.2 for ewes treated with 0, 250, 500,750 and 1000 IU PMSG, respectively.

CONCLUSION

The increased prolificacy and twinning rate in treated Ghezel ewes indicate the relevance of using both treatments (CIDR + PMSG). Therefore, it seems beneficial to use CIDR for estrus synchronization in local Ghezel ewes during the breeding season, and to use PMSG as a tool to increase twinning rate.

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