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## Original Article

# The Effects of Using Artificial Insemination Techniques on Reproductive Performance in Ghezel Sheep

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## ABSTRACT

**Objective:** The aim of the present study was to evaluate artificial insemination techniques on reproductive performance in Ghezel ewes synchronized with CIDR during breeding season. **Methods:** All ewes were treated with controlled internal drug release device (CIDR) inserted into the vagina of the ewes for 14 days. All ewes were treated a single intramuscular (IM) dose of PMSG was injected. In this experiment 120 head ewes divided into four subgroups randomly and experimental groups consist of: control 1, ram mating, n=30; control 2, ram mating plus 550 IU PMSG, n=30; group 1, laparoscopic intrauterine insemination plus 550 IU PMSG, n= 30; group 2, cervical insemination plus 550 IU PMSG, n= 30. **Results:** In this experiment estrus responses were similar in all groups (control 1, 76.7%; control 2, 93%; group 1, 96%; group 2, 100%). There were no statistically significant differences ( $P>0.05$ ) between the treatment groups and the control groups for the estrus response. Pregnancy rates were 70%, 90.0%, 83.3% and 60% in control groups 1, 2 and AI groups 1, 2 respectively. Twinning rates (10% to 34.6%) and litter size (1.10 to 1.35) were significantly different in the treatment groups and the control group 1 ( $P<0.05$ ). As a result, conception rate in the laparoscopic intrauterine insemination was higher than cervical insemination.

## 1.INTRODUCTION

The primary goal of any artificial insemination (AI) program is to create better offspring. Laparoscopic AI is being used in the sheep industry around the world to extend the use of superior rams, and it offers the producer an opportunity to maximize the reproductive potential of his/her sheep. The primary economic benefit to the sheep producer is rapid infusion of valuable genetic traits into the flock. The development of artificial insemination (AI) and the consequent genetic

improvement of farm animals have led to a remarkable increase in the productivity of livestock. AI in sheep has been poorly implemented and is carried out mainly with chilled semen because of the low fertility results obtained when using frozen-thawed semen (Salamon et al., 2000). This is due to the high structural complexity of the ewe cervix (Kaabi, 2002; Anel et al., 2005), which prevents deep AI and reduces the efficiency of the technique. Nowadays, laparoscopic insemination is an alternative method for AI using frozen-thawed semen (Anel et al., 2005).

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Several factors affect the result of AI. Among them, we can highlight ewe handling, farm system, environmental elements, health, physiological status of the ewes, etc. (Paulenzetal et al., 2002; Anel et al., 2005). It is important to control these factors, taking into account their influence on the outcome of AI. Laparoscope was used in artificial insemination in ewes by direct manipulation of semen into the uterine horn as a means of genetic improvement. Routine intra cervical insemination could not be use in ewes because of some difficulties due to the anatomical structure of the cervix which consist of 5 cartilaginous rings that have an irregular opening between each of them thereby making the introduction of inseminating introduction of inseminating gun very difficult. In addition to the vaginal PH, estrus period tend to be more acidic due to the effect of estrogen hormone; therefore, the deposition of semen in the vagina will kill a large number of spermatozoa, so laparoscopic device is a favorable method for artificial insemination in ewes with no difficulties (Abdalbari et al., 2012).

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.

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 (Najafi et al., 2014). Therefore, the objective of this study was to determine the effects of using artificial insemination techniques with fresh semen on some reproductive performance of Ghezel ewes.

## 2. MATERIALS AND METHODS

### 2.1. Location

This experiment was carried out at breeding station of Ghezel sheep in Miyandoab in West Azarbayjan 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.

### 2.2. Animals and treatments and experimental designs

One hundred and twenty non-lactating fat-tailed (Ghezel breed), 3-4 years old ewes were used in breeding season. Animals were housed in flock barn accessed to a feeding lot and were fed with grass hay as ad libitum basis. Ewes were randomly divided in to four groups. Rams were separated one month before the initiation of the experiment.

Group 1 (laparoscopic intrauterine insemination plus 550 IU PMSG, n= 30): CIDR were inserted into the vagina for a 14 d period. Following this, 550 IU PMSG was injected intramuscularly (IM) after intravaginal devices had been removed.

Group 2 (cervical insemination plus 550 IU PMSG, n= 30): CIDR were inserted into the vagina for a 14 d period. Following this, 550 IU PMSG was injected intramuscularly (IM) after intravaginal devices had been removed.

Control 1 (ram mating, n=30): In the control 1 ewes were injected with 1ml normal saline solution at sponge removal to act as untreated control.

Control 2 (ram mating plus 550 IU PMSG, n=30): CIDR were inserted into the vagina for a 14 d period. Following this, 550 IU PMSG was injected intramuscularly (IM) after intravaginal devices had been removed.

### 2.3. Mating, artificial insemination, estrus and pregnancy detection

In the control groups three fertile Ghezel rams were introduced to each group (6 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 matings were recorded.

In the experimental groups 1 and 2 Ghezel rams (n=6) with probed fertility were utilized in the artificial insemination program. The semen was collected by an artificial vagina (AV); the AV was partially filled with hot water at 60°C to provide suitable environmental temperature of 40 to 45°C. Sunflower oil was used to lubricate the first third of AV to facilitate the entrance of male penis. Training was done 2 weeks before operation in order to get semen with natural ejaculated characters; also, provide a good diet to the male 12 h before collection to make sure a good semen quality was gotten (Donovan et al., 2001).

Having determined seminal characteristics, it was diluted with egg yolk-tris-fructose extender. Ewes that had exhibited estrus were allocated at random to one of the three rams for artificial insemination and they were inseminated with fresh diluted semen containing a dose of 300 million total sperm. Laparoscopic and cervical

artificial insemination was performed 48 to 54 hours after estrus detection.

One month after the natural and artificial Insemination, 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, Netherlands). 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 and Birth weight .

#### 2.4. Statistical Analysis

Continuous data (gestation period, birth weight) were analyzed by the PROC GLM procedure and discrete variables by the LOGISTIC procedure of SAS package software (Chapman & Hall 2002). Probability values of less than 0.05 were considered significant. Continuous data are expressed as Mean  $\pm$  SE and discrete data are expressed as mean. Pregnancy rates and Estrus response of the groups and litter size were compared by chi-square analysis.

### 3. RESULT AND DISCUSSION

Fertility in sheep is increased by hormone application to 20-50% (Yavuzer, 2005). For example, the use of PMSG after progestagens treatment, increases ovarian response, conception rate and percentage of multiple births from the induced ovulations (Boscos *et al.*, 2002). Estrus response and pregnancy rate (%), lambing and twinning rate (%), litter size, Lamb birth weight (kg) and Gestation period (day), in each subgroup of this trail are shown in Tables 1.

Estrus response in PMSG treatment groups with injection dose of 550 IU was the higher than control 1 that act as untreated control. Estrus response in groups 1 and 2 and controls 1 and 2 were 96%, 100%, 76.6% and 93%, respectively. In this experiment, there was no difference in estrus response between all groups. 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 (Koyuncu *et al.*, 2008). Estrus response ranging between 76.6% and 100% has been obtained in other experiments performed during breeding season by progestagens treatment and PMSG in Akkaraman cross-bred ewes

(Ataman *et al.*, 2006; Akoz *et al.*, 2006), Dorper ewes (Zelege *et al.*, 2005), Hamadani ewes (Timorkan and Yildize, 2005) and Karakul ewes (Hashemi *et al.*, 2006). The percentage of ewes exhibiting estrus in this trail was comparable to the value reported in the above literatures.

In this experiment, there was no difference in pregnancy rate between all groups. Various factors are effective in pregnancy rate, such as nutrition before and under mating season, mating system, age, natural or artificial insemination, type of insemination (Simonetti *et al.*, 2002), the time of PMSG administration before or after removal of sponge or CIDR (Zelege *et al.*, 2005) and PMSG dose (Wildeus, 1999). Previous studies demonstrated that pregnancy rates vary (20-80%) after different progestagens treatment following timed AI using fresh diluted semen than natural mating (Ustuner *et al.*, 2007). The pregnancy rate in group 2 this study is comparable with the results obtained by Simonetti *et al.* (2002) in Merino ewes in 400 IU PMSG (60%). In the study of Timurkan and Yildis (2005), there was no significant difference between 500 and 600 IU PMSG group for pregnancy rate in breeding season which is similar to the result of present study. In the present study, pregnancy rates were 70% and 90% in the control groups 1 and 2 and 83.3% and 60% in the experimental groups 1 and 2, respectively. Pregnancy rates of 70 - 80% have been reported following insemination with freshly diluted semen (Ehling *et al.*, 2003) which is similar to the present study. Cervical insemination using fresh semen gives a higher pregnancy rate than cervical insemination using frozen-thawed semen (Donovan *et al.*, 2004); 76% compared with 46% (Irish breed) and 36% (Norwegian breed). Pregnancy rates in excess of 60% can be achieved with a single artificial insemination of fresh semen deposited at the external cervical opening , corresponding rates for frozen-thawed semen occasionally exceed 45%, with values less than 17% not rare (O'Meara *et al.*, 2005). Milczewski (2000) investigated the effect of different types of extenders and reported that Citrate-yolk extended semen resulted with higher pregnancy rate (85.7%) in intrauterine insemination with chilled semen for 8 h at 5°C. Milczewski *et al.* (2000) recommended that higher pregnancy rates (69.56%) can be obtained with at least 250 million spermatozoa per dose of 0.4 ml suspension in intrauterine inseminations of ewes.

Fertility following AI in sheep depends on many factors, intrinsic and extrinsic to the inseminated female. These factors must be evaluated in order to improve AI results on farms. AI techniques have been considered in many previous studies. According to several authors LAI ensures significantly higher parturition rates than CAI, despite the fact that relatively lower numbers of frozen-thawed spermatozoa are used. This difference in fertility can be explained by the fact that the sheep cervix has a very high structural complexity, preventing deep CAI. LAI allows this barrier to be bypassed, improving fertility

even with lower quality frozen-thawed spermatozoa (Anel *et al.*, 2005).

In our current study, there was no difference in lambing rate between artificial insemination groups and the control groups. Lambing rates were 66.7%, 86.6%, 76.6% and 60% in the controls 1, 2 and AI groups 1, 2, respectively. A previous study performed by Smith *et al.* (1981) reported 66.3% lambing rate after cervical AI following a progestagen (MAP)-PMSG (375 IU) treatment in lowland ewes. In the study of Simonetti *et al.* (2002), working with other breed (Merino) a 54.32% lambing percentage was obtained when ewes were treated with the same gonadotropin dose and a higher lambing percentage (76.47%) was obtained when 400 IU PMSG were injected. Maxwell and Hewitt (1986) carried out studies on AI of mature Merino ewes during spring. In their experiments females were synchronized with sponges impregnated with fluorogestone acetate (FGA) and 400 IU PMSG were given at the end of treatment. After cervical AI with fresh diluted semen, they achieved a 47.5% lambing and 60% pregnancy rate, respectively. For AI in sheep, intrauterine semen deposition (LAI) is a necessity for obtaining high lambing rates. Considering the potentially lower quality of AI semen, intrauterine deposition eliminates the adverse effects on sperm transport or survivability that are imposed by the distal segment of the ewe's reproductive tract. It is possible to obtain lambing rates of 50-80% with frozen-thawed semen deposited directly in the uterine horn (Ivanka *et al.*, 2011). In our experiment, lambing rate in the Laparoscopic AI group (76.6%) was comparable to the value reported in the above literature.

In the presented study, twinning rate and litter size percentages in the PMSG treatment groups (control 2 and groups 1, 2) 30.94% and 1.31%, respectively, was higher than those in control 1 (10% and 1.10%, respectively) ( $P < 0.05$ ).

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). Ince and Karaca (2009) reported 1.33 and 1.39 litter sizes for 400 and 500 IU PMSG in Chio×Kivircik ewes which is similar to result our study. In Timorkan and Yildize (2005) trail on Hamadani ewes, the litter size was 1.06 and 1.25 for groups that were treated with 300 and 400 IU PMSG, respectively. Their results were lower compared to present study. Also, there are number of experiments

which reported a higher level of litter size than this research such as Simonetti *et al.* (2002), who reported an average litter size of 1.45 in Merino ewes treated with 400 IU PMSG. The observed variation depends on various factors such as breed, age, time and dose of PMSG administration (Dogan and Nur, 2006). It was pointed out that administration of PMSG increased the number of follicles and therefore raised the twinning and triplet rates (Timorkan and Yildize, 2005). Twining rate in experiment of Özbey and Tatli (2001) that synchronized the Awassi ewes for 14 d with sponges containing 40 mg of FGA and superovulated by 500 IU of PMSG injection were 46% that is higher than the result of current study obtained by using 550 IU PMSG. Multiple birth in experiment of Akoz *et al.* (2006) synchronizing of estrus by vaginal sponge with 40 mg FGA for 7 days in combination with 500 IU PMSG injection after removal sponge on Akkaraman cross-bred ewes in non-breeding season was 36.4% that is similar to the present result in 550 IU PMSG groups. Karagiannidis *et al.* (2001) reported that responses to the different PMSG doses among various breeds were different.

In the present experiment there was significant ( $P < 0.05$ ) effect of the hormonal treatment on the birth weight of lambs averaging  $4.9 \pm 0.54$ ,  $4.3 \pm 0.66$ ,  $4.2 \pm 0.59$  and  $4.4 \pm 0.64$  kg for control groups 1, 2, and AI groups 1, 2, respectively. The PMSG-treated groups had a significantly ( $P < 0.05$ ) higher twinning rate than untreated group (Table 1). As well the treatment had significant ( $P < 0.05$ ) effect on the duration of pregnancy, which averaged  $157 \pm 1.52$ ,  $150 \pm 1.70$ ,  $151 \pm 2.03$  and  $151 \pm 2.02$  days for control groups 1, 2 and AI groups 1, 2, respectively. It was seen that significant difference ( $P < 0.05$ ) between PMSG treated groups and untreated group at increasing litter size and twinning rate related to 550 IU of PMSG may result in shortened gestation periods and low birth weight, as reported by previous researches (Horoz *et al.* 2003; Safranski *et al.* 1992). These results are in accordance with those reported by some researchers (zeleke *et al.*, 2005; Timorkan and Yildize, 2005) with regard to effect of administered PMSG on litter size and gestation periods. 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  $163.8 \pm 4.9$  and  $157.2 \pm 2.8$  d, respectively.

**Table 1.**

Effect of artificial insemination techniques on reproductive performance of Ghezel ewes\*

|                        | Control 1             | Control 2             | Group 1                   | Group 2                   |
|------------------------|-----------------------|-----------------------|---------------------------|---------------------------|
| Groups                 | (Ram - Normal S.)     | (Ram - PMSG)          | (LAI <sup>1</sup> - PMSG) | (CAI <sup>2</sup> - PMSG) |
| <b>Parameters</b>      |                       |                       |                           |                           |
| Number of ewes         | 30                    | 30                    | 30                        | 30                        |
| Estrus response (%)    | 76.7 <sup>a</sup>     | 93 <sup>a</sup>       | 96 <sup>a</sup>           | 100 <sup>a</sup>          |
| Pregnancy rate (%)     | 70 <sup>a</sup>       | 90 <sup>a</sup>       | 83.3 <sup>a</sup>         | 60 <sup>a</sup>           |
| Lambing rate (%)       | 66.7 <sup>a</sup>     | 86.6 <sup>a</sup>     | 76.6 <sup>a</sup>         | 60 <sup>a</sup>           |
| Twinning rate (%)      | 10 <sup>b</sup>       | 34.6 <sup>a</sup>     | 30.4 <sup>a</sup>         | 27.8 <sup>a</sup>         |
| Litter Size (%)        | 1.10 <sup>b</sup>     | 1.35 <sup>a</sup>     | 1.30 <sup>a</sup>         | 1.28 <sup>a</sup>         |
| Lamb birth weight (kg) | 4.9±0.54 <sup>a</sup> | 4.3±0.66 <sup>b</sup> | 4.2±0.59 <sup>b</sup>     | 4.4±0.64 <sup>b</sup>     |
| Gestation period (day) | 157±1.52 <sup>a</sup> | 150±1.70 <sup>b</sup> | 151±2.03 <sup>b</sup>     | 151±2.02 <sup>b</sup>     |

\*The means in each row that have at least one common letter do not have significant difference (P&gt;0.05).

<sup>1</sup>Laparoscopical intrauterine artificial insemination, <sup>2</sup>Cervical artificial insemination

#### 4. CONCLUSION

Results of the present study indicated that using laparoscopical intrauterine artificial insemination technique improved conception rate and reproductive performance in Ghezel ewes during the breeding seasons, and also a single injection of PMSG after CIDR removal increases efficiency of fertility rate and may shorten the breeding period, which in turn may result in increased profit through better animal productivity.

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