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Efficacy of CIDR or FGA Sponges with hCG Treatments on the Conception Rate and Prolificacy in Lori Ewes Out of the Breeding Season

Research Article

M.M. Moeini[1*], F. Alipour[1] and M.R. Sanjabi[2]

[1] College of Agriculture, Razi University, Kermanshah, Iran
[2] Department of Animal Science, Iranian Research Organization Science and Technology, Tehran, Iran

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*Correspondence E-mail: mmoeini@razi.ac.ir

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ABSTRACT

Two experiments were performed to determine the effectiveness of different progestogens contained in intravaginal devices, different doses of eCG and subsequent hCG treatment on the reproductive performance of estrus-induced mature Lori ewes. In the first experiment, 88 ewes were allocated into two groups, and were treated with either fluorogestone acetate (FGA) sponges or Controlled Internal Drug Release devices (CIDR). The sponges were withdrawn 13 days after insertion and then ewes were treated with either 350 IU or 500 IU of eCG by intramuscular injection. There was no significant difference among treatments in the percentage of ewes in estrus or the interval to the onset of estrus. However, the conception rate and prolificacy of ewes treated with 350 IU eCG, in both the FGA and CIDR groups, was higher than ewes treated with 500 IU of eCG. In the second experiment 384 ewes were randomly divided into three groups and after synchronization with FGA sponges and 350 IU of eCG, 128 ewes in the first group (T1) were injected 250 IU hCG when artificially inseminated, 128 ewes in the second group (T2) were injected 250 IU hCG 12 days after AI and the 128 ewes in the third group (C) acted as the control group. Estrous was determined by monitoring 35 teaser rams to calculate estrous rate. Prolificacy and conception rate were assessed and serum progesterone (*P4*) concentrations were measured on days 12, 14 and 16 days after AI. Prolificacy was increased in the T1 group compared with control group ($P<0.05$) and conception rates were higher in hCG treatments ($P<0.05$). The weight of single lambs on the day of birth increased with the hCG injection on days 0 and 12 ($P<0.05$). The *P4* concentration was higher in the hCG-treated groups compared with the control ewes on day 16 ($P<0.05$). It is concluded that CIDR and FGA sponges were equally effective for estrous induction in anestrous Lori ewes and *P4* concentrations increased with 200 IU hCG given at the time of AI or 12 days after AI which could improve reproductive performance.

KEY WORDS conception, estrus synchronization, hCG , Lori ewes.

INTRODUCTION

The most economically important trait in sheep production is reproductive performance which can be manipulated using hormonal treatments (Atsan *et al.* 2007). Several techniques have been developed to induce out-of-season estrus in sheep, thus allowing farmers to supply lambs to market throughout the year. Intravaginal devices containing different types of progestogens, maintained in situ during 12-14 days, associated with intramuscular administration of gonadotrophin is the most commonly used method of inducing estrus. Low concentrations of progesterone (*P4*) may result in an extension of the lifespan of the ovulatory follicle and may be associated with a low viability of the ovu-

lated oocyte (Viñoles *et al*. 1999). The fertility of the ewe is affected in a dose-dependent manner by fluorogestone acetate (FGA) (Allison and Robinson, 1970) or progesterone in intravaginal devices (Ungerfeld and Rubianes, 1999). Treatment with intravaginal sponge impregnated with FGA for a period of 10-16 days and intramuscular injection of pregnant mare serum gonadotropin (PMSG) at intravaginal device removal, have been successfully used to improved the reproductive performance in ewes (Gomez *et al*. 2006).

It has been shown that the administration of gonadotropins such as equine chorionic gonadotropin (eCG) stimulates follicular growth and increases ovulation rate and fertility and induces a tighter synchrony of ovulation in both anestrous and cycling sheep (Dogan and Nur, 2006). Injection of eCG after progesterone treatment increases estrus response, conception rate and the percentage of multiple births from the induced ovulation.

In Iranian fat-tailed ewes, injection of eCG, especially at a high dose (500 vs. 350 IU at the time of CIDR removal) increases twinning and lambing rates (Zare Shahneh *et al*. 2006; Moeini *et al*. 2009). In sheep, 30-40% of fertilized eggs are lost during the first 3 weeks of pregnancy. One of the major causes of embryonic loss is likely to be inadequate luteal function (Ashworth *et al*. 1989). Human chorionic gonadotropin (hCG), which is similar to luteinizing hormone (LH) in function, has been shown to increase luteal weight and endogenous synthesis of progesterone from the corpus luteum (CL) in sheep (Nephew *et al*. 1994).

The increase in *P4* concentrations after hCG treatment suggests that hCG by its LH like activity may provide luteotrophic stimulation to CL. The beneficial effect of hCG administration on embryo survival may be through the stimulatory effect of hCG-induced progesterone on fetal growth as Kleemann *et al*. (1994) showed that *P4* supplementation increased subsequent fetal growth. At different times during the cycle, after AI or breeding, hCG has been administered in an attempt to reduce embryonic mortality and improve reproductive performance. The effectiveness of these treatments however has been inconsistent between studies and the timing of such hormonal treatments also appears to be an important factor. The administration of hCG on the day of mating, 4, 5 and 12 days post mating has been reported (Thatcher *et al*. 2001; Cam *et al*. 2002; Khan *et al*. 2003). Ishida *et al*. (1999) and Fukui *et al*. (2001) reported that the hCG) treatment given during the early luteal phase increased the plasma *P4* levels in the hCG treated ewes, but this was not reflected in the pregnancy and lambing rates of the inseminated ewes. In order to improve fertility, hCG would have to increase the fertilization rate and reduce the embryonic death rate or both.

The current study was conducted to determine the effects of two different intravaginal devices and an injection of eCG or hCG on the efficiency of estrus synchronization and subsequent conception rate and prolificacy in these breeds during the non-breeding season.

MATERIALS AND METHODS

The first experiment was conducted on a farm in the Lorestan provinces (latitude 35˚ 15' N, longitude 48˚ 30' E , altitude 1350 m) during the non-breeding season from May to August 2007 using 88 Lori multiparous ewes grazed on native pastures. Ewes with a body condition score (3.8±0.1) were allocated into two groups (Russel *et al*. 1969). Ewes received intravaginal sponges containing FGA (30 mg, Chronogest, Intervet, The Netherlands; Group FGA, n=44) or CIDR (0.3 g of progesterone, Inter Ag, Hamilton, New Zealand; group CIDR, n=44). All sponges were injected with 10 mg of oxytetracyclinum to prevent vaginitis. All intravaginal devices remained in situ for 13 days. Prior to withdrawal of the intravaginal device, each sheep was randomly assigned to one of the two further groups, F500 (FGA+500 IU eCG, n=22) and C500 (CIDR+500 IU eCG, n=22) were injected 500 IU eCG, (Folligon, Intervet, The Netherlands) and F350 (FGA+350 IU eCG, n=22) and C350 (CIDR+350 IU eCG, n=22) were injected 350 IU eCG intramuscularly.

Estrous activity was assessed by exposing all ewes to vasectomized rams, 1 ram per 10 ewes; rams were fitted with a marking harness at 12 h intervals, until artificial insemination. Semen was collected using an artificial vagina from Lori rams. It was diluted with milk extender containing 1000000 IU penicillin G potassium. Cervical artificial insemination was performed 12 h after estrous onset.

In the second experiment, a total of 384 Lori ewes (42±0.5 kg) during the non-breeding season were used. Estrus was induced by treating all ewes with an intravaginal sponge impregnated with synthetic progestagen (Fluorogestone acetate, 30 mg FGA) for 13 days and then injection of 350 IU eCG at the time of removal of sponges. At AI, ewes were randomly allocated into three treatment groups. Ewes were either left untreated (C group, n=128) or treated with 250 IU hCG (Vetecor®, Laboratórios Calier do Brasil Ltda, São Paulo, Brasil) at the time of AI (T1, n=128) or 12 days later (T2, n=128) intramuscularly. Blood samples were collected from the jugular vein from 30 ewes chosen at random from each group on days 12, 14 and 16 after treatment. Soon after collection, blood samples were centrifuged to separate serum that was stored at -20 ˚C until the time of hormone assay. The weight of each lamb was also recorded at lambing.

Statistical analysis

Frequencies of estrus and conception rates (% ewes lambing per ewes inseminated) were compared using the *Chi-squared*-test. The interval from device withdrawal to estrus onset was compared using a mathematical model that included a fixed effect due to intravaginal devices and residual error. The plasma progesterone was analyzed via NAME repeated measures test. Prolificacy (No. of lambs born alive per ewe lambing) were assessed using the *Chi-squared*-test.

RESULTS AND DISCUSSION

The results of estrous response, onset of estrus, the conception rates and prolificacy are set out in Table 1. In the first experiment, the frequency of ewes in estrus, conception rates and prolificacy rates with FGA or CIRD were similar. Similar results were reported for these devices when compared for estrous synchronization in cyclic ewes (Ungerfeld *et al*. 2000) or in short term priming for "ram effect" estrus induction (Ungerfeld *et al*. 1999). Our findings are in agreement with the results of Ataman *et al*. (2006) obtained after long term priming's in anestrous ewes. Conception rate and prolificacy in the subgroups F350 and C350 were significantly higher than their respective subgroups (P<0.05) (Table1).

The type of intravaginal device had no significant effect on reproductive performance in Lori ewes (P>0.05). The results of this study are in agreement with Moeini *et al*. (2009) in Lori ewes, Zonturlu *et al*. (2008) in Awassi ewes. In the second experiment, the data on reproductive performance of the estrus induced and cervical inseminated ewes in the hCG treatments and control group are set out in Table 2.

Ewes injected with hCG, either at AI or 12 days after AI had increased prolificacy and conception rates compared to control ewes.

The mean weight of singleton lambs born to ewes in T1 and T2 was greater than singleton lambs born to control ewes (P<0.05).

The results of this study showed that in synchronized and artificially inseminated Lori ewes, the injection of 250 IU hCG at the time of AI or 12 day after AI improved the conception rate and prolificacy. These results are similar to those previously reported by (Khan *et al*. 2003), in which hCG given on the day of mating increased the pregnancy rate and litter size. The mean serum *P4* profiles of the ewes in the hCG treated (T1 and T2) and control groups are shown in Figure 1.

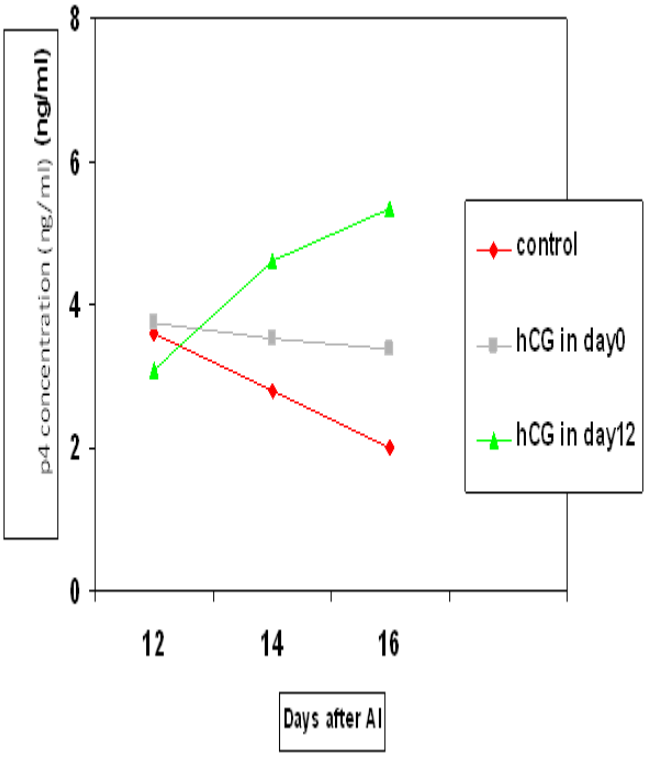

Figure 1 Mean *P4* concentration in different treatments group

Table 1 Effect of CIDR and FGA sponges and different dosage of eCG on estrus response, conception rate and prolificacy in multiparous Lori ewes

Group	eCG (IU)	No. ewes	No. ewes in estrus (%)	Onset to estrus (h) ±SD	Conception rate (%)	Prolificacy (%)
FGA	350	22	90	31.8±1.8	54.5[a]	133.3[a]
	500	22	95.4	27.3±1.3	38.8[b]	88.5[b]
CIDR	350	22	100	31.1±1.9	45.4[a]	130[a]
	500	22	90	28.6±2.1	32.8[b]	85.7[b]

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Table 2 The effect of administration of 250 IU hCG at AI (T1) or 12 days after AI (T2) on estrus response, conception rate, prolificacy and lamb birth weight

	T1	T2	C
No. of ewes in estrus	127	121	125
Conception rate (%)	50[a]	47.90[a]	35.20[b]
Prolificacy (%)	1.50[a]	1.29[a b]	1.18[b]
Mean birth weight (kg)			
Singleton	3.75±0.14[a]	3.70±0.12[a]	3.25±0.12[b]
Twin	2.25±0.08	2.32±0.10	2.22±0.16
Triplet	1.87±0.08	2.00±0.09	1.94±0.10

The hCG treatment increased *P4* concentration on day 16 in T1 and T2 groups compared to ewes in the control group. In addition, ewes treated with hCG on day 12 (T2) had higher *P4* concentrations than all other groups ($P<0.05$). The hCG administration has been reported to increase the number of CL (Beck *et al*. 1998) and plasma progesterone concentration (Nephew *et al*. 1994). Nephew *et al*. (1994) reported that hCG treatment in the middle of the luteal phase increased plasma *P4* concentrations in ewes and enhanced pregnancy and lambing rates. In addition, Ishida *et al*. (1999) and Fukui *et al*. (2001) reported that the hCG treatment given at the early luteal phase increased the plasma *P4* levels in hCG treated ewes. The effect of the hCG on pregnancy rate and fetal weights was attributed to its effects on progesterone production. This may result in a stronger signal for maternal recognition of pregnancy from embryos in the hormonal treatment groups which would otherwise degenerate. When evaluating changes in *P4* concentrations in the control group, it should be noted that the *CL* regressed between day's 12- 16 and therefore control group and plasma *P4* levels on days 12, 14 and 16 were higher in the hCG-treated ewes. It is noteworthy that the birth weights of singleton lambs born to ewes in the T1 group were significantly higher than singleton lambs born to control ewes. This finding is in agreement with the findings of Cam and Kuran (2003) for Karayaka ewes supplemented with hCG. Treatment with hCG resulted in an increase in the number of lambs born per ewe lambing due to an increase in the ovulation rate. Gomez *et al*. (2006) and Cam and Kuran (2003) hypothesized that these differences could be due to the differences in the protocols used. However, it is also probable that other factors such as breed, management systems, nutritional and physiological status could have affected the response of animals.

CONCLUSION

It was concluded that CIDR and FGA sponges were equally effective for estrous induction in anestrous ewes. In addition, the intramuscular injection of 350 IU of eCG was more effective in increasing conception rates and prolificacy than the administration of 500 IU of eCG in Lori ewes in the none breeding season. Based on the results of the experiments, it can be concluded that administration of 350 IU eCG and subsequent 250 IU hCG increased the progesterone concentration and could improve the reproductive performance of Lori ewes out of the breeding season.

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