



Contents lists available at BioMedSciDirect Publications

International Journal of Biological & Medical Research

Journal homepage: www.biomedscidirect.com



Original Article

Comparison reproductive Performance in Kermani ewes Treated with two synchronization methods and Subsequent eCG treatment out of the breeding season

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ARTICLE INFO

Keywords:

Estrus synchronization
CIDR
Flourogestone acetate
Kermani ewes
eCG

ABSTRACT

A completely randomized design experiment was carried out using seventy eight multiparous kermani ewes during out of season breeding to compare flourogestone acetate (FGA) sponges and controlled internal drug release (CIDR) dispensers to synchronize estrous. Intravaginal progestagen was administered for a 13-day period. Upon progestagen withdrawal, doses received 350, 500 and 650 IU equine chorionic gonadotrophin (eCG) intramuscularly. Teaser rams (1/10 ewes) were introduced 1 d after treatment removal to detect estrus. Ewes were subjected to fertile rams 48-60 h after FGA and CIDR removal. Twenty one days after mating, blood samples were taken from jugular vein in 10-ml vacuum tubes (venoject) for pregnancy diagnosis. The results of this experiment showed that both types of pessaries were effective when used with eCG. Although two progestagen treatments had significant effect on estrus response (92.89 in FGA vs 82.45 in SIDR), time to the onset of estrus did not differ significantly (32.65 vs 31.1h). Enhancing the level of eCG administration in sponge treated ewes led to a decrease in percentage of ewes in estrus, however non significant changes were observed in SIDR treated groups. Results indicated that the use of FGA and CIDR intravaginal progestagen treatments could be efficient in synchronization of estrus in sheep under warm arid area. The CIDR device is comparable to the FGA sponge for estrus synchrony during the out-of- season breeding and reasonable response can be achieved with the use of eCG.

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1.Introduction

Estrus synchronization or the induction of estrus is a valuable management tool for increasing the pregnancy rate in ewes [1]. Modern sheep husbandry has improved the efficiency of extensive production and controlled the reproductive process for intensive production [2]. The most economically important trait in sheep production is reproduction and it can be manipulated using hormonal treatments [3]. There are several methods for reproduction improving in ewes, often aim to increase the proportion of ewes having multiple ovulations, and thereby increase lambing rate [4]. Several techniques have been developed to induce out-of-season estrus in sheep, allowing farmers to raise and provide the market lambs year round.

Intravaginal devices containing different types of progestogens, maintained in situ during 12-14 days, associated with gonadotrophin administration is the most widely were used [5]. Sponges impregnated with progesterone provide estrus synchronization by extending the luteal phase during the treatment period in ewes [6]. Intravaginal sponges containing progesterone are one of the most commonly applied treatments for estrus synchronization in small ruminants during the breeding and non-breeding seasons. Sponges are used together with PMSG, particularly out of season, at the time of sponge withdrawal or 48 h prior to sponge removal. It has been reported that PMSG can increase pregnancy and twinning rates in breeds characterized by low litter size [7]. However, there are many factors influencing the effect of PMSG, including the dose and administration time of PMSG and season [8]. Fertility following a synchronized estrus is often depressed partly due to the poor synchronization of this induced estrus and ovulation [9]. Of the most important factors leading to this depressed fertility following the use of synthetic progestagens, is

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the dose level and method of preparation or impregnation. There are suggestions that optimal fertility, following synchronization with progesterone, can be achieved with lower doses of progestagen, by halving the intravaginal sponges [10]. Treatment with intravaginal sponge impregnated with FGA for a period of 10-16 days and intramuscular injection of PMSG at intravaginal device removal, have been successfully used to improve the reproductive performance in ewes [11]. 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 [12]. Injection of eCG after progesterone treatment, increase estrus response, conception rate and percentage of multiple births from the induced ovulation. In Iranian fat-tailed ewes, injection of eCG, especially in high dosage (500 vs. 350 IU at the time of CIDR removal) increases twinning and lambing rates [13, 14]. Hormonal treatments, particularly eCG (equine chorionic gonadotropin, formerly named PMSG, pregnant mare serum gonadotropin), have been largely used in animal production. In ovine and caprine production, this molecule is routinely used to induce and synchronize estrus and ovulation and is consequently a prerequisite to insemination [15]. The hormone eCG has been extensively applied in sheep and goats to encourage ovulation. Nevertheless, the results of eCG and Follicle Stimulating Hormone (FSH) on stimulation of ovaries are inconsistent in practice, although it may have a positive impact on follicular development [16]. Iran has more than fifty million sheep and in this regard ranks fifth in the world. The sheep population in Iran is composed mainly of fat-tailed native breeds. The Kermani is a fat-tail breed in southern Iran which has a dry and hot climate. Coat color is white with pigmented head and legs. It also presents a good carcass conformation and stands out as being very fertile. Therefore, main objective of this conducted experiment was determine the effects of two different intravaginal devices and injection of eCG on the reproductive performance of kermani ewes during the non-breeding season.

2. Materials and Methods

The experiment was conducted at farm animal science of Rezvan Junior College in kerman provinces (latitude 25° 55' N, longitude 53° 26' E, altitude 1755m) during the non-breeding season from May to August 2009. The average ambient temperature during experiment was 30 to 35°C. The annual rainfall in this region ranges from 140 to 155 mm, with an erratic distribution throughout the year. The animals were submitted to examination for general clinical condition, sanitary and reproductive health. Seventy eight kermani multiparous ewes weighing 45 ± 0.5 kg with a body condition score (3.8 ± 0.1) were allocated into two groups. Ewes received intravaginal sponges containing FGA (30 mg, Chronogest, Intervet, The Netherlands) or CIDR (0.3 g of progesterone, InterAg, Hamilton, New-Zealand). All sponges were injected with 10 mg of oxytetracyclinum to prevent vaginitis. All intravaginal devices remained in situ for 13 days. The ewes in groups 1 and 2 were injected intramuscularly with 350, 500 and 600 IU eCG (Intervet, The Netherlands) at the time of

progestagen removal from the vagina on day 14. Estrous activity was assessed by exposing all ewes to vasectomized rams, (1 ram per 10 ewes), 1 day after treatment removal. After estrus detection ewes were subjected to fertile rams for mating. Twenty one days after mating, blood samples were taken from jugular vein in 10-ml vacuum tubes (venoject) for pregnancy diagnosis. Serum was recovered by centrifugation (10 minutes at 2000 rpm) and stored at -20°C until assayed for Serum progesterone concentrations using commercially available ELISA kit (Demeditec Diagnostics GmbH, Kiel, Germany). Serum progesterone level greater than 1.4 ng per ml was taken as an indication of pregnancy.

2.1. Statistical analysis

The following traits were evaluated for each group: Oestrus response: number of ewes showing oestrus/total ewes treated in each group $\times 100$ [4]. Conception rate: number of pregnant ewes/number of ewes showing oestrus and mated in each group $\times 100$ [17]. fecundity rate: the number of lambs born/ewes mated. The onset of estrus and estrus response were statistically analyzed using analysis of variance and reproductive performance were analyzed using the chi-square test. The mean progesterone concentrations between groups were analyzed by ANOVA-repeated measures (GLM procedure of SPSS, Version 10.0). Fecundity rate were assessed by Chi-squared analysis. The significant differences among groups were carried out using Multiple Range Test of Duncan, [18]. All significant differences were set at $P < 0.05$.

3. Results and Discussion

The results of estrous response, onset of estrus, the conception rates and prolificacy in CIDR and FGA groups and Comparison between two groups are set out in Table 1, 2 and 3. The frequency of Onset to estrus and conception rates with FGA or CIDR were similar in the experiment. The same intravaginal devices also provided similar results when compared for estrous synchronization in cyclic ewes [19]. Our findings are in agreement with the results of Ataman et al, [20] obtained after long term priming's in anestrous ewes.

The Estrus rate in current study between CIDR and FGA groups was significantly different ($P < 0.05$). The Estrus response was longer (92.89) in the FGA group compared to CIDR group (82.45). The percentage of ewes exhibiting estrus in this trial was comparable to values reported in the literature [21, 12]. While Dogan, (12) reported 88.9% estrus response by using 60 mg of MAP and 500 IU of PMSG during non-breeding season, 80.87% estrus response have been obtained by Simonetti et al, [21] who used 60 mg MAP. Higher estrus responses (100%) have been reported by Hashemi et al, [22] in studies involving the use 60 mg of MAP and 500 PMSG in Karakul ewes, outside the breeding season. The slight differences between the results obtained in the current study and those of the proceed researchers may be due to differences in the breed of sheep used and the seasons in which the studies were executed. Oestrus synchronization success ranging between 85% and 100% has been obtained in other experiments performed during breeding season by progestagens treatment and PMSG in Akkaraman cross-bred

ewes [20, 4], Dorper ewes [17], Hamadani ewes [8] and Karakul ewes [22]. The percentage of ewes exhibiting oestrus in this trail were comparable to the value reported in the above literatures.

Table 1. Effect of CIDR and different dosage of eCG on reproductive performance of adult kermani ewes.

Groups	eCG (IU)	Ewes	Estrus rate (%)	Onset to estrus (h.) \pm S.D	Conception Rate (%) ¹	Fecundity rate (%) ²
CIDR	350	13	83.82	34.56 \pm 2	79.78	127.50 ^a
	500	13	83.82	30.11 \pm 1.9	68.67	119.60 ^b
	600	13	79.73	28.63 \pm 1.1	76.00	133.30 ^a

The means in each columns that have at least one common letter, do not have significant difference ($P > 0.05$). ¹: Number of ewes lambing/all ewes mated ²: Number of lambs born/ number of ewes lambing

Table 2. Effect of FGA and different dosage of eCG on reproductive performance of adult kermani ewes.

Groups	eCG (IU)	Ewes	Estrus rate (%)	Onset to estrus (h.) \pm S.D	Conception Rate (%) ¹	Fecundity rate (%) ²
FGA	350	13	99.94 ^a	37.91 ^a \pm 2.3	73.73	147.80 ^b
	500	13	92.91 ^b	31.60 ^{ab} \pm 1.8	62.00	130.50 ^c
	600	13	85.82 ^c	28.44 ^b \pm 1.3	79.78	169.00 ^a

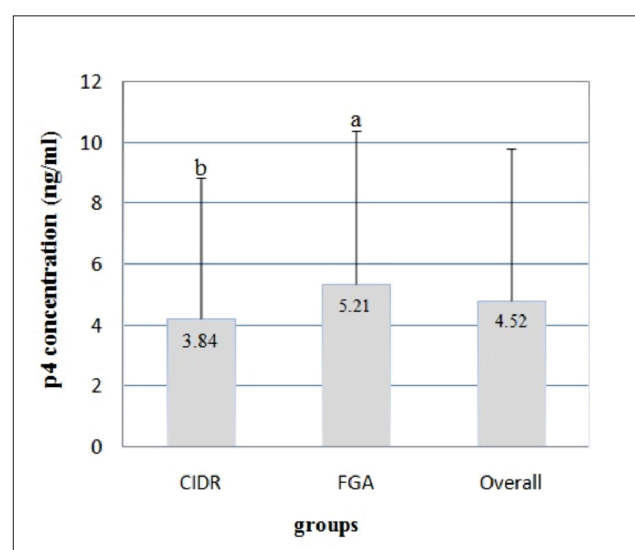
The means in each columns that have at least one common letter, do not have significant difference ($P > 0.05$).

Table 3. Comparison reproductive performance between two synchronization methods of kermani ewes.

Groups	Ewes	Estrus rate (%)	Onset to estrus (h.) \pm S.D	Conception Rate (%)	Fecundity rate (%)
CIDR	39	82.45 ^b	31.1 \pm 1.6	74.81	126.8 ^b
FGA	39	92.89 ^a	32.65 \pm 1.8	71.83	149.1 ^a

The means in each columns that have at least one common letter, do not have significant difference ($P > 0.05$).

Figure1: Mean P4 concentration in treatments group



The time from sponge withdrawal to estrous and the duration of the induced estrus period following the two methods of sponge treatment for the three different eCG doses are shown in Table 1, 2. The time from sponge withdrawal to the onset of estrus was not significantly different between the two progesterone application methods (Table 3). The mean intervals to onset of estrus were 31.1 ± 1.6 and 32.65 ± 1.8 in CIDR and FGA sponge respectively. The mean onset of estrus in FGA group was higher than CIDR group but this difference was not statistically significant (Table 3). In our study, we have shown that the use of progesterone sponges in combination with different doses of eCG decrease onset of estrus that in FGA group was higher than CIDR group. The time of onset of estrus was shorter than that reported by Ataman et al, [20] and longer than that reported by Dogan and Nur, [12]. Onset of estrus in FGA group with injection 600 IU eCG was higher observation that compare with 350 and 500 IU injection eCG hormone. There were statistically significant differences ($P < 0.05$), but onset of estrus in CIDR group with injection eCG hormone has not significant differences. The time of

estrus onset results (31.1 – 32.65) of the current trial are in agreement with the previous findings of Greyling and Van der Nest, [23] the time of onset (50.7±26.3) and with Dogan, [12] 30 and 60 hour. To the contrary, Amer and Hazzaa, [24] noted the time from sponge withdrawal to the onset of estrus to be later in ewes treated with FGA for 12 days. Simonetti et al, [21] recorded estrus to occur 55.94, 56.74 and 57.7 hours after using sponges impregnated with 40,50,60 mg progesterone respectively. Comparing the results of this study to the results obtained by Akoz et al, [4] who used different concentrations of progesterone in sponges, it is cleared that there is no significant differences between the using of two sponges or different progesterone levels in the sponge. Conception rate and Fecundity in the subgroups F600 and C350 were significantly higher than in the other subgroups ($P<0.05$). The type of intervaginal device had no significant effect on reproductive performance in kermani ewes. The results of this study are in agreement with Moeini et al, [14] in Lori ewes, Zonturlu et al, [25] in Awassi ewes and Bitaraf et al, [26] in goats. In this experiment, there was no difference in conception rate between treatment. Various factors are effective in conception rate, such as nutrition before and under mating season, mating system, age, natural or artificial insemination, type of insemination [21], the time of PMSG administration before or after removal of sponge or CIDR [17] and PMSG dose [27]. In this experiment with increasing in eCG dosage the fecundity rate was increased. Previous studies demonstrated that conception rates vary (20-80%) after different progestagens treatment following natural mating [28]. The conception rate in this study is comparable with the results obtained by Simonetti et al, [21] in Merino ewes in 400 IU PMSG (60%). In the study of Timurkan and Yildiz, [8] there was no significant difference between 500 and 600 IU PMSG group for conception rate in breeding season which is similar to the result of present study. However, the present results were lower than those obtained by Zeleke et al, [17], Akoz et al, [4] and Ince and Karaca, [29]. Fertility in sheep is increased by hormone application to 20-50% [30]. For example, the use of PMSG after progestagens treatment, increases ovarian response, conception rate and percentage of multiple births from the induced ovulations [7]. The mean serum P4 profiles of the ewes in the experiment treated with FGA, CIDR and eCG hormone are depicted in Fig. 1. The eCG treatment increased p4 concentration in FGA group That compare CIDR group, 5.21 and 3.84 respectively. There was a significant difference between experimental groups ($p<0.05$). The eCG administration has been reported to increase the number of CL [31] and plasma progesterone concentration [32]. The results of this study showed that synchronization and injection of eCG hormone in kermani ewes, improved the conception and Fecundity rate. These results are agreement with finding [33], they reported that synchronization estrus with PGF2 α and eCG injection increased the pregnancy rate and litter size in raieni goats.

4. Conclusion

It was concluded that CIDR and FGA sponges were equally effective for estrous induction in anestrus ewes. However, FGA sponges are as effective as those commercially available and the application of 350 IU of eCG was rather more effective than the administration of 500 and 600 IU of eCG in kermani ewes in the none breeding season. Based on the results of the experiments, it can be concluded that administration of 350 IU eCG increased the progesterone concentration and could improve the reproductive performance of kermani ewes out of the breeding season.

Acknowledgments

The authors would like to thanks staffs of Rezvan junior college animal science farm for with their assisting data collection and caring the ewes and also I would like to have a special thank to dear sister Ms, Nahid Mohseni Takaloo for criticism reading and editing of the manuscript.

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