

ORIGINAL ARTICLE

Comparison of Progestagen Sponges and Progesterone CIDRs Short-Term Treatments in Terms of Estrous Synchronization and Reproductive Performance during the Non-Breeding Season of Sanjabi Ewes

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Abstract

A more precise understanding of the commencement of behavioral estrus and pre ovulatory luteinizing hormone (LH) peak and the progesterone (P4) hormonal profile in sheep treated with either FGA-impregnated sponges or progestagen-loaded CIDR in short-term protocols would increase conception rate of fixed timed artificial insemination (FTAI). The present study aim was to investigate the efficiency of different treatments of progestogen with eCG in Sanjabi breed ewes out of breeding season. Fifty Sanjabi ewes were divided equally into sponge (SP) and CIDR groups. At the moment of CIDR or sponge removal (Day 6), the animals were injected with 400IU eCG. Estrous characteristics and reproductive performance were measured in each of the treated groups. The P4 concentration and the characteristics of LH surge were determined. There were no significant differences among the groups CIDR and SP for estrous response, onset, and duration, P4 concentration at device removal, on estrous day, on the 11th and 30th days after device removal, LH surge onset and concentration. Also, there were no significant differences in the conception, lambing, fertility, prolificacy and twinning rates in ewes synchronized with either SP or CIDR. The results of this study showed that short-term (6-days) protocols with FGA and/or CIDRs are equally effective for estrous induction and synchronization and no differences between the two treatments were apparent regarding estrus response, P4 and LH profiles, ovulation, fertility and reproductive performance. Thus, short-term progestagen treatment can be recommended as an alternative to traditional treatment (12-14 days).

Keywords

eCG, Heat onset, LH surge, Ovulation time, Sheep

1. Introduction

The commonly applied progestagen treatment for estrous synchronization in ewes lasted 12-14 days (Lombardo et al., 2020). Fertility after progestogens, however, had been reported to be lower than after natural estrus (Martinez-Ros et al., 2019). Possible causes have been related to the long-term duration of the treatment (Martinez-Ros et al., 2019). Maintenance of intravaginal progestagens for such a long period as 14 days was also related to the development of vaginitis and problems with lack of sponge retention (Martins et al., 2009). Shorten of progestagen administration to 5-6 days in combination with injection of eCG at the moment of insert removal can avoid these problems (Viñoles et al., 2001).

The use of progestogens in short-term treatments for improving fertility raises concerns, apart from secondary effects in long-term applications, about their efficiency. Short-term protocols are more and more frequently used for sheep artificial insemination under field conditions but this progestogen treatment regimen usage is lower than that of the classical long-term treatments. It has been reported that short-term treatment (5-6 days) with progestagen during the non-breeding season was as effective as long-term treatment to induce estrus and the subsequent fertility (Ungerfeld and Rubianes, 1999). Menchaca et al., (2017) comparing the short-term protocol (6 days) versus the traditional long-term protocol (14 days), reported a significantly greater pregnancy

rate in short-term protocol than the long traditional protocol (43.5% vs. 37.8% respectively; $p < 0.05$).

A more precise understanding of the commencement of behavioral estrus and pre ovulatory luteinizing hormone (LH) peak and the progesterone (P4) hormonal profile in sheep treated with either FGA sponge or CIDR in short-term protocols (6 days) would increase conception rate of fixed timed artificial insemination (FTAI). Such data may give substantial information for adapting protocols of artificial insemination and reaching maximal fertility. Hence, the goals of this study were to compare possible differences among these two protocols.

2. Materials and Methods

2.1. Animals Management and Study Location

Fifty, 3-5 years old Sanjabi ewes, weighting 65-70kg with a body condition score of 3.4 ± 0.25 (scale 1-5), and six healthy Sanjabi rams aged 3-4 years old, weighting 90-100kg with a body condition score of 3.5 ± 0.5 (scale 1-5), were used for the experiment. The study was accomplished on a private farm near Kermanshah city (Kermanshah province, Iran; Latitude $34^{\circ} 23' N$; Longitude $47^{\circ} 00' E$; 1360 m). All ewes had previously lambed and weaned their last lamb. The animals were kept indoor at night and had access to natural grazing area for most of the day. Each experimental ewes were received concentrate (300g/day) having 2700 Kcal ME (Metabolizable energy) and 8% crude protein throughout the experimental period. They accessed water and mineral licks *ad libitum*.

2.2. Experimental Design

Ewes were randomly assigned into two equal treatment groups ($n=25$) during the non-breeding season and were synchronized using one of the following hormonal treatments: Group I ewes (SP group) received intravaginal-sponges impregnated with fluorogestone acetate with a 40mg dose (Chronogest, grey sponges, Intervet, Netherlands). Group II ewes (CIDR group) received intravaginal drug-releasing device containing 0.3g progesterone (InterAg, Hamilton, New Zealand). The devices were maintained for 6 days and, at their removal, all the ewes in both groups received, one IM injection of 400IU of eCG- (Folligon, Intervet, Netherlands). At the day of device withdrawal, 6 teaser rams were allowed to run with the treated ewes. Behavioral estrus symptoms were monitored every 4h from 12-60h after device withdrawal by the use of trained vasectomized rams. Time from device withdrawal to first mounting acceptance was assumed as estrus onset. The interval between mating acceptance and mating rejection was considered the estrous length or duration. The end of estrus was considered to be the time when the ewe did not accept the ram. Estrous response, conception rate, lambing rate, fertility rate, prolificacy rate, and twinning rate were measured in each of the treated groups (Akoz et al., 2006). The number of CL was determined by transrectal ultrasonography on the 11th day after treatment removal and pregnancy was diagnosed based upon sustained P4 levels of >2.5 ng/mL and

via abdominal ultrasonography on the 30th day after treatment removal and confirmed by lambing.

2.3. Blood Sample and Hormone Assay

The serum samples collected at different days of treatment were used to determine the P4 concentration. Preovulatory LH surge were measured by collecting blood samples every 4h from 32-80h after device withdrawal. The volume of a blood sample taken into each heparinized tubes (5IU/mL) was 5ml. Each blood sample was centrifuged within 30min of collection at $3000 \times g$, for 15min at $4^{\circ}C$. Plasma was pipetted into 12×75 mm glass tubes using sterilized plastic disposable Pasteur pipettes and then stored at $-20^{\circ}C$. Plasma LH was measured using a commercial kit (LH Detect®; INRA, Tours, France), with sensitivity of 0.01ng/mL and inter- and intra-assay variation coefficients of 7.4% and 8.5% respectively. Plasma P4 was measured using a commercial radioimmunoassay kit (BioSource, Nivelles, Belgium), with sensitivity of 0.1ng/mL and inter- and intra-assay variation coefficients of 6.8% and 9.1%, respectively. Progesterone below 0.4ng/mL in plasma was considered as an indication of absence of CL (Megahed, et al., 2023).

2.4. Statistical Analysis

All data were analyzed using SPSS, software package statistical analyses. The repeated measure ANOVA test was performed to examine the differences among treatments using the Greenhouse significance level and Student-Newman-Keuls and Tukey post hoc tests to contrast the differences within the groups. Binomial dependent variable (response to treatment) was analyzed using chi square test. All the results were expressed as mean \pm standard deviation and the statistical significance was accepted from $P < 0.05$.

3. Results

3.1. Intravaginal-Device Retention Rate

In the present study, spontaneous expulsion of the sponge or CIDR was not observed in any of the treated animals and 100% retention rate was observed. In this study, none of ewe showed vaginal discharge or vaginitis.

3.2. Synchronization and Characteristics of Estrus

Estrous activity in treatment groups have been given in Table (1). All ewes (100%) in each treatment group exhibited estrus signs. No significant differences in percentage of estrous response, time to onset of estrus and duration of induced estrus were found between the CIDR and SP groups ($P > 0.05$).

3.3. Progesterone Concentration

Mean progesterone concentration in the two treatment groups is presented in Table (1). One day before intravaginal device insertion, the serum P4 concentration was similar between the CIDR and SP groups ($P > 0.05$). Mean progesterone concentrations at device removal, at estrus, and on the 11th and 30th days after treatment removal in treatment groups were similar.

3.4. LH Surge and Ovulation

Number of CL on the 11th day after treatment removal, LH surge onset (h), and LH surge concentration (ng/mL) in treatment groups are set out in **Table (1)**. Estrus behavior was accomplished with preovulatory LH surge. Analysis of LH concentrations showed device removal to LH peak (h) was similar between the CIDR and SP groups (P>0.05). The time interval from CIDR and/or sponge removal to LH peak was not significantly different (P>0.05) between the

treatment groups. The differences between groups in the mean timing for the onset of the preovulatory surge LH release after onset of estrous behavior were not significant (P>0.05). Concomitantly, there were no differences (P>0.05) in mean plasma LH peak concentrations at the preovulatory surge among the treatment groups. All the ewes expressing estrous behavior had ovulated regardless of the treatment, without significant differences between treatments in the number of corpora lutea.

Table 1. Effect of treatments on synchronization and characteristics of estrus in ewes of the experimental groups during the non- breeding season.

Variables	Experimental Groups	
	CIDR	SP
Estrus response (%)	100	100
Estrus Onset (h)	37± 0.33	38 ± 0.63
Estrous Duration (h)	38 ± 0.83	36.7 ± 0.40
P4 one day before device insertion (ng/mL)	0.28± 0.02	0.33 ± 0.13
P4 at device removal (ng/mL)	3.19 ± 0.01	3.97 ± 0.01
P4 on estrus day (ng/mL)	0.26 ± 0.03	0.35 ± 0.23
Onset of estrus to LH peak (h)	6.00 ± 0.02	6.80 ± 0.42
Device removal to LH peak (h)	44.20 ± 0.35	42.90 ± 0.65
LH peak concentration (ng/mL)	33.7 ± 0.76	32.1 ± 0.41
Number of CL on the 11 th day after device removal	3.6 ± 0.57	3.2 ± 0.08
P4 on the 11 th day after device removal (ng/mL)	4.8 ± 0.94	4.4 ± 0.88
P4 on 30 th day after device removal (ng/mL)	7.83 ± 0.77	7.03 ± 0.25

3.5. Reproductive Performance

Reproductive performance of ewes in the experimental groups are described in **Table (2)**. Conception rate, lambing rate and fertility rate were 100% in the all experimental ewes.

There were no statistically significant differences (P>0.05) between the CIDR and SP groups for conception rate, lambing rate and fertility rate. The mean prolificacy and twinning rates were similar between treatment groups.

Table 2. Effect of treatments on reproductive performance of ewes in the experimental groups during the non-breeding season.

Treatment Groups	N	Conception rate %	Lambing rate %	Fertility rate %	Prolificacy rate %	Twinning rate %
CIDR	25	100	100	100	176	66
SP	25	100	100	100	160	60

N: Number of treated ewes.

4. Discussion

The present study results showed that short-term (6-days) protocols with FGA and/or CIDRs are equally effective for estrous induction and synchronization and no differences between the two treatments were apparent regarding estrous response and fertility. Therefore, the present study results support the suitability of short-term progestagen treatment for managing reproduction in sheep.

In this study, none of ewe showed vaginal discharge or vaginitis and a 100% retention rate was observed. Similar values, ranging from 97.3 to 100%, were reported in earlier study (**Swelum et al., 2019**) using the CIDR device.

In this study, 100% estrous response was observed. Previous studies have reported 100% estrous response after sponge (**Almadaly et al., 2016**) or CIDR (**Dhami et al., 2015; Hashemi et al., 2017**) removal with the similar interval between devices removal to estrous induction. Contrary to the findings in the present study other researchers have reported lower estrous induction rates ranging from 46% to 96% after sponge or CIDR removal (**Naderipour et al., 2012**) and longer interval between devices removal to estrous

induction (**Khalilavi et al., 2016**). The short interval between progestagen devices removal and behavioral estrous may be related to the effect of eCG on follicular growth and estradiol secretion.

The present study results are similar with **Martinez-Ros et al., (2019)**, who reported that eCG administration reduced the intervals from progestagen devices withdrawal to estrous induction.

A 100% estrous induction following sponge or CIDR removal, show the effectiveness of sponges or CIDR treatments for 6 in conjunction with eCG in modifying ovarian activity assuring acceptable estrous expression rate out of season. In addition, there was no significant difference for the onset of estrous and estrous duration between the two types of intravaginal P4 devices which discloses no benefit of CIDR over FGA at least for estrous synchronization in sheep farm during the non-breeding season. The results obtained with the short-term treatments are in consistent with the findings of previous studies that reported short-term treatment of ewes with progestagen before ram introduction was adequate to induce fertile estrous (**Almadaly et al., 2016; Khalilavi et al., 2016**)

and no difference in estrous response was observed when anestrus ewes are primed for 6 or 14 days, with intravaginal sponge treatments (Martinez et al., 2015).

One day prior to device insertion, plasma P4 concentration was below basal levels (0.28 ± 0.02 and 0.33 ± 0.13 ng/mL for CIDR and SP groups respectively) in all experimental ewes, suggesting the absence of an active CL (Ferreira-Silva et al., 2017; Arsoy and Sağmanlıgil, 2018). Mean P4 concentrations at device removal in groups CIDR and SP was similar ($P < 0.05$). Mean plasma P4 concentration at the onset of estrus was at basal levels in ewes of all experimental groups which is consistent with data obtained by Mohammed et al., (2007). The high progesterone concentrations (>1 ng/mL) determined 11 days after insert removal in all ewes clearly demonstrated that both treatments reliably induced ovulation. P4 remained elevated through day 30 in ewes of all experimental groups, as result of pregnancy incidence. The increase of P4 levels at day 30 of pregnancy in ewes treated with eCG was similar to the previous findings of Wei et al., (2016). P4 concentrations 30 days after device removal were similar between CIDR and SP groups. Undoubtedly, P4 is essential for maintaining pregnancy and one of the important functions of the blastocyst is to ensure that uterine luteolytic mechanism is counteracted. Therefore, increasing P4 level during early pregnancy reduced embryonic losses and increased pregnancy rate and fertility.

Information regarding the occurring time of the LH peak could allow better prediction ovulation time. This estimation of ovulation time could be of great assistance in timing AI or mating for maximum conception in sheep breeding programs. In this study LH surge was reached 44.20 ± 0.35 and 42.90 ± 0.65 hrs post removal of CIDR or sponge, respectively. The intervals between removal of exogenous progesterone and the pre-ovulatory LH surge for ewes treated with CIDR or FGA sponge are longer than the range reported in previous studies (Kohno et al., 2005). These differences may be attributed to the different pessary, hormone administration and breed used. A direct relationship between dose of progesterone and the onset of the pre-ovulatory surge has been postulated. A previous study (He et al., 2017) reported that higher progesterone levels at the time of withdrawal of an exogenous progesterone source delays the onset of the pre-ovulatory surge. Another study (Van Cleef et al., 1998) found that treatment with two CIDR devices extended the interval from removal to gonadotropin surge, compared to treatment with one device. The time of ovulation could be more accurately estimated from estrus onset to LH peak compared to the commonly used interval from pessary withdrawal to LH peak because the latter shows higher variation due to the different progestagen treatments. In agreement with other researchers (Ozyurtlu et al., 2010; Martinez-Ros et al., 2018a) all the animals in both groups responded to the treatments displaying estrus behavior and ovulation, without significant differences in the mean timing of the onset of estrus behavior and preovulatory LH discharge, indicating that different mechanisms might

control the estrus and ovulation brain centers, respectively (Domanski et al., 1972). In this study, there were no differences in the mean timing of the onset of estrus behavior and preovulatory LH discharge and in mean plasma LH concentrations at the pre-ovulatory surge which are consistent with data obtained by Martinez-Ros et al. (2018a). However, in a study reported by Kohno et al. (2005) the mean time for LH surge in Suffolk ewes synchronized with CIDR occurred before estrous onset.

The reproductive achievement of ewes in the experimental groups indicated higher conception, lambing and fertility rates (100%) in all experimental ewes. The mean prolificacy and twinning rates were similar among groups CIDR and SP. The type of intervaginal device had no significant effect on the reproductive performance in Sanjabi ewes.

Conception in small ruminants subjected to estrous synchronization protocols is a major concern in production. Conception rate in this study was 100% for CIDR and SP groups and no significant difference was observed in the study groups which agrees with the findings of Martinez et al., (2015) and Jackson et al., (2014). The findings of the current study with regard to conception rate (100%) do not support previous research of Wani et al., (2017) that reported lower conception rate (10% to 55%). Likewise higher conception rates ranging from 71.42% to 100% were reported by Akoz et al., (2006). Ustuner et al., (2007) reported that the differences in terms of conception rate between the short-term and the long-term treatments were not significant. It seems short-term CIDR program has not negative effect on fertility during the non-breeding period. Moreover, the highest conception rate (100%) recorded in ewes of all experimental groups, was associated with the highest percent of estrus (100%). Results for the conception rate may vary depending on breed, nutrition, season and overall conditions of animal care.

Lambing rate in this study was 100% for CIDR and SP groups and no significant difference was observed in the study groups which agrees with the findings of Ozyurtlu et al., (2010) and Jackson et al., (2014). In contradiction to our results, a lower lambing rate of 46% to 87% was reported (Akoz et al., 2006; Naderipour et al., 2012). The highest lambing rate (100%) recorded in ewes of all experimental groups may reflect higher ovulation rate.

Fertility rate was significantly higher in the short-term (7 days) progestagen treated ewes (87.3%) compared to long-term (12 days) treated ewes (71.6%), during breeding season (Ozyurtlu et al., 2010). Conversely, in report of Martinez-Ros et al., (2018 b) there was no difference between short-term (7 days) and long-term (12 days) progestagen treatments for fertility parameters. Fertility rates in the CIDR and SP group were 100.0%. These percentages are higher than the fertility rate previously reported by Martinez et al., (2015, 59.6-67.4%), Ustuner et al., (2007, 20-55.6%), Ozyurtlu et al., (2010, 89.6 %). However, comparing fertility results of the different studies is difficult, because various breeds with

differing prolificacy were used in the experiments carried out in season or out-of-season using nulli- and pluriparous ewes.

Based on the improved conception, lambing and fertility rates of ewes that had sponge or CIDR for 6 day and eCG administration at sponge or CIDR removal may be related to the adding eCG into the synchronization programs, because the two FGA and CIDR regimens produced similar reproductive performance. Therefore the application of FGA-sponge or CIDR regimen during non-breeding season must be accompanied by eCG treatment and 6 days progestagen priming is effective to improve conception, lambing and fertility rates.

Prolificacy and twinning rates were similar among CIDR and SP groups. Prolificacy rate in CIDR (176%) and SP (160%) groups was higher than those reported by [Moradikor et al., \(2012\)](#), [Jackson et al \(2014\)](#) and [Almadaly et al., \(2016\)](#) and lower than those reported by [Lombardo et al., \(2020\)](#) and [Murali Mohan \(2017\)](#).

Variable results were reported across the literature. It has been reported that synchronization by sponge improved the prolificacy rate than CIDR ([Moradikor et al., 2012](#)), whereas [Mustafa et al., \(2007\)](#) found that the prolificacy rate in the CIDR group was higher than that in the SP group. On the other hand, many authors reported no significant differences in prolificacy rates between the ewes treated with CIDR and those treated with FGA sponges ([Martinez-Ros et al., 2018b](#); [Mirshamsollahi, 2016](#)).

In this study, twinning rates of CIDR and SP groups were 66% and 60% which were lower than 68.8% and 73.3% reported in ewes receiving long-term progesterone treatment and eCG injection at the removal of sponges and hCG (250IU) or GnRH (4.2µg) two days after sponge withdrawal as well ([Ahmadi and Mirzaei, 2016](#)). A lower twinning rate was reported by [Algan et al., \(2017\)](#), 36.5-44.4% in comparison to those obtained in SP (60%) and CIDR (66%) groups. This increase in the prolificacy and twinning rates can be attributed to the use of eCG in the synchronization regime, because using eCG increased ovulation rate, births and number of lambs born per ewe lambled as reported by [Akoz et al., \(2006\)](#). In breeds characterized by low litter size, administration of eCG can increase pregnancy and twinning rates ([Boscós et al., 2002](#)). One of the most important applications of eCG in ovine is to increase the prolificacy rates by modifying the ovulatory rate which depended on the used dose ([Boscós et al., 2002](#)).

5. Conclusion

The effect of short term treatments with CIDR or FGA sponges for estrous induction were equally effective and producing reproductive responses in anestrus ewes. Thus, short-term progestagen treatment for induction of estrus can be a good alternative to traditional treatment (12-14 days). Short-term priming has the advantage of allowing more flexibility the treatment protocols under field conditions. However, confirmation of this claim requires more large scale field study. Also, results obtained regarding to induced

estrous, LH surge concentration and the moment of the LH peak onset are value in timing AI or mating for maximum conception in sheep breeding programs when CIDR or sponges were used with 400IU eCG.

6. Conflict of Interest

Authors declare no conflict of interest.

7. References

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