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Short Communication

Effect of intravaginal device type and treatment length on estrus synchronization and reproductive performance of Farahani ewes out of breeding season

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Abstract

This study was conducted to evaluate reproductive performance of Farahani ewes subjected to four estrus synchronization programs. Twenty four ewes were synchronized with flurogestone acetate sponges (FGA) or controlled internal drug release (CIDR) devices for 6 (S6 and C6 groups) or 12 (S12 and C12 groups) days and an equine chorionic gonadotropin (eCG) on device removal. The mean prolificacy and twinning rates were higher in C6, C12 and S12 than in S6 group. Mean progesterone (P4) concentration at device withdrawal was higher in C6 and S6 than in C12 and S12 groups. Mean P4 concentration at estrus was higher in S12 than in S6 group. Mean P4 concentrations thirty days after device withdrawal were higher in C12 and S12 than in C6 and S6 groups. In conclusion, a short-term CIDR treatment has an efficacy comparable to that of a long-term FGA or CIDR treatment during the physiological anestrum of ewes.

Keywords: FGA sponges, CIDR, out of season, Farahani ewe, estrus synchronization

1. Introduction

Ovarian follicular growth in ewes occurs in a wave-like pattern, both during the breeding season (Evans, Duffy, Hynes, & Boland, 2000) and the non-breeding season (Evans, Duffy, Quinn, Knight, & Boland, 2001). Ovarian response of sheep to estrus synchronization varies according to the type of intravaginal device, kind of progestagen, nutritional status, stress, environmental aspects, and male effect (Kleemann & Walker, 2005). Long term progestagen estrus synchronization protocols can affect follicular dynamics and fertility of ewes (Diskin, Austin, & Roche, 2002). Circulating levels of P4 and its release during the 12 day period in which pessaries are in place decline over time. Initially, P4 peaks within two days of pessary insertion and a supra-luteal effect of the treatment causes an increase in the follicular turnover (Husein & Kridli, 2002). Supra-luteal concentrations of P4 decrease the growth of the dominant follicle and promote follicular turnover (Noel, Bister, Pierquin, & Paquay, 1994). Then, P4 concentration decreases gradually with time during the remaining period (Husein & Kridli, 2002), and a sub-luteal effect of the treatment slows down the follicular turnover (Hamra, Massri, Marcek, & Wheaton, 1986). Sub-luteal concentrations of P4 prolong the lifespan of large antral follicles in cyclic ewes (Vinoles, Meikle, Forsberg, & Rubianes, 1999) and delay the

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re-emergence of the following wave (Vifloles *et al.*, 1999). In addition, the use of P4 pessaries for long period is laborious and needs the time to follow-up on pessary retention, a treatment that has also been associated with accumulation of offensive vaginal mucus and bad smell upon pessary removal (Wheaton, Carlson, Windels, & Johnston, 1993) and vaginal contamination following and during the process of pessary insertion (Gatti, Zunino, & Ungerfeld, 2011).

Acknowledging that P4 treatment for an extended time (>12 days) may lead to decreased fertility, the use of progestagen for a short time would be an alternative solution, because it could improve estrus synchronization in ewes (Husein, Ababneh, & Abu-Ruman, 2007) reduce vaginal health risk, and make the reproduction management easier. Therefore the objective of this study was to evaluate the effects of a short time of estrus synchronization using CIDR and /or intravaginal sponge treatment, with an injection of eCG at the time of their removal, on reproductive performance of Farahani ewes during the non-breeding season.

2. Materials and Methods

2.1 Animals and experimental design

Twenty-four 3 to 4 years old pluriparous Farahani ewes, weighing 41.24 ± 1.6 kg with a body condition score of 3 to 4 (scale = 0-5) and four Farahani rams, aged 3-4 years, weighing 56.64 ± 5.6 kg with a body condition score of 3.5 to 4, were used for the experiment. The experiment was carried out on a private farm near Khomein city (Central province, Iran; 33° 38’ N; 50° 50’ E; latitude 1880 m) during mid to late spring (outside the natural breeding season). The ewes were given 300 g/day/ewe concentrate having 2700 Kcal ME (metabolizable energy) and 8% crude protein throughout the experimental period. Grass hay and water were supplied *ad libitum*. Ewes were randomly assigned in equal numbers to four treatment groups. The estrous cycles of the ewes in each group were synchronized using one of the following hormonal treatments:

C6 and C12 groups had insertion of a controlled intravaginal drug releasing device (CIDR) containing 0.3 g P4 (0.3 g of progesterone, InterAg, Hamilton, New Zealand) for 6 and 12 days, respectively. S6 and S12 groups had insertion of FGA sponges containing 40 mg intravaginal fluorogestone acetate (FGA, 40 mg, chronogest, grey sponges, Intervet, Netherlands) for 6 and 12 days, respectively. All ewes received 400 IU eCG (400 IU Folligon, Intervet, Netherlands) on device removal. Four harnessed Farahani teaser rams, which had been isolated from ewes, were joined upon sponge or CIDR removal. All animals were run together in a single pen and ewes were checked for breeding marks at 6 h intervals for 4 days. A schematic diagram of the treatments administered in this experiment is shown in Figures 1 and 2.

Behavioral observations were conducted by two trained observers every day from 7:00 to 9:00 am, from 13:00 to 15:00 pm, from 19:00 to 21:00 pm and from 1:00 to 2:00 am for four days following device removals. The observers took their place at least 15 min before the beginning of each observation period. To eliminate the effects of observer, they changed places after each observation period. After estrus detection, ewes were introduced to fertile rams for mating. Time from device removal to first mounting acceptance was assumed as estrus onset. The interval between mating acceptance and mating rejection was considered the estrous length or duration (Ergul Ekiz & Ozcan, 2006). Pregnancy was diagnosed based upon sustained P4 levels of >2.5 ng/mL on the 30th day after treatment removal and confirmed by lambing. The following traits were evaluated for each group (Akoz, Bulbul, Ataman, & Dere, 2006):

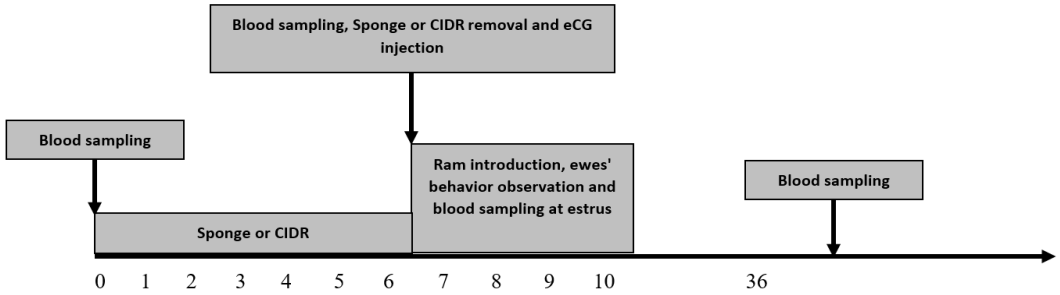

Figure 1. The experimental schedule in C6 and S6 groups.

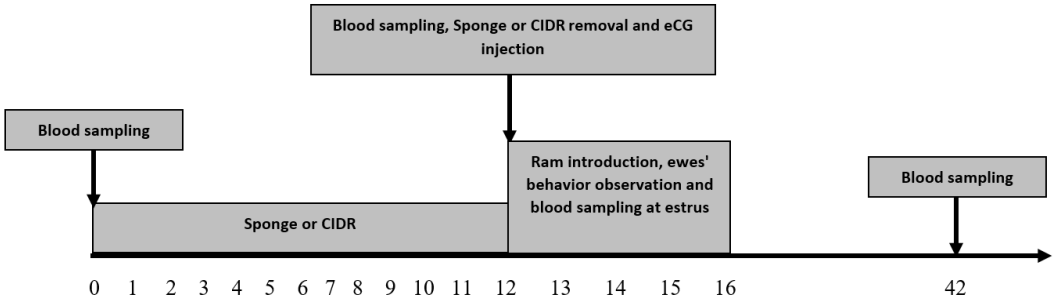

Figure 2. The experimental schedule in C12 and S12 groups.

1) Estrous response (number of ewes showing oestrus/ total ewes treated × 100).
2) Conception rate (number of pregnant ewes/number of mated ewes × 100).
3) Lambing rate (number of ewes lambing/number of pregnant ewes × 100).
4) Fertility rate (number of lambed ewes/number of ewes exposed to rams × 100).
5) Prolificacy rate (number of lambs born/number of lambed ewes × 100).
6) Twinning rate (number of twin lambing/total lambing in each group × 100).

2.2 Blood sampling and hormonal assay

P4 was measured one day before device insertion, at device removal, at estrus and 30 days after treatment**.** Blood samples were taken from all animals via jugular venipuncture to measure P4. Plasma P4 concentration 2.5 ng/mL on the 30th day after device removal was considered an indication of pregnancy (Boscos *et al.*, 2002). All blood samples (5 mL each) were drawn via jugular venipuncture into heparinized tubes (5 IU/mL). Blood samples were centrifuged within 30 min of collection at 3000 × g for 15 min. Plasma was pipetted into 12 × 75 mm glass tubes using sterilized plastic disposable Pasteur pipettes and then stored at –20 ºC until assayed for P4 using a commercial radioimmunoassay kit (BioSource, Nivelles, Belgium). The sensitivity of the assay was 0.1 ng/mL. The intra- and inter-assay coefficients of variation were 6.8 and 9.1%. P4 below 0.4 ng/mL in plasma was considered an indication of absence of corpus luteum (CL).

2.3 Statistical analyses

Data were analyzed by using Minitab statistical package (Minitab Inc., Pennsylvania, USA). The mean P4 concentrations, the onset of estrus and estrus duration were statistically analyzed using ANOVA. The significant differences among groups were determined by using the Multiple Range test of Duncan (Bewick, Cheek, & Ball, 2004). Reproductive performance was analyzed using the chi-square test. All significant differences were set at $P<0.05$.

3. Results and Discussion

The main objective of estrus synchronization is to obtain a uniform onset of estruses after the end of the synchronization treatment. In the present study, 100% estrus response was observed in all experimental ewes. Previous studies have reported 100% estrus response after sponge (Almadaly, Ashour, El-kon, Heleil, & Fattouh, 2016) or CIDR (Sirjani, Shahir, Kohram, & Shahneh, 2011) removal with similar durations from devices removal to estrus induction (Martemucci & D"Alessandro, 2010). However, other researchers have reported lower estrus induction rates ranging from 46% to 96% after sponge or CIDR removal (Naderipour, Yadi, Ghazikhani Shad, & Sirjani, 2012) and longer intervals between device removal and estrus induction (Knights, Maze, Bridges, Lewis, & Inskep, 2001). In the present study, the interval between progestagen device removal and the onset of estrus is shorter and less variable than that reported by a former study (Knights *et al.*, 2001). The short interval between progestagen device removal and the onset of estrus may be due to the action of eCG on follicular growth by mediating faster pituitary endocrine responses and estradiol secretion. Administration of eCG following 12 days of P4 treatment increased serum concentration of estradiol and resulted in synchronized estrus and ovulation in anoestrous ewes (Barrett, Bartlewski, Batista Arteaga, Symington, & Rawlings, 2004; Simonetti *et al.*, 2008). The results of our study are in agreement with Abdalla, Farrag, Hashem, Khalil, and Fattah (2014), who reported that eCG administration reduced the interval from progestagen device withdrawal to estrus induction and improved the efficiency of synchronization of estrus and ovulation during and outside the breeding season.

Estrus expression following FGA sponge or CIDR removal (100%) indicates the effectiveness of FGA sponges or CIDR administered for 6 or 12 days in conjunction with eCG in sensitizing ovarian activity, ensuring acceptable estrus expression rate out of season. In addition, there was no significant difference for the onset of estrus and estrus duration between the two types of intravaginal P4 devices and the two corresponding insertion periods of FGA sponges and CIDR devices, which discloses no benefit of CIDR over FGA and long-term (12 days) over short-term (6 days) priming, at least for estrus synchronization in sheep farm during the non-breeding season. Effect of the type of progestagen sponge on the time to estrus onset in ewes is not significant (Ustuner, Gunay, Nur, & Ustuner, 2007). An insignificant difference was observed in estrus response rates when anestrous ewes were primed for 7 or 12 days, with intravaginal sponge (FGA) treatments (Ataman, Akoz, & Akman, 2006). The results obtained with short-term treatments indicate that these methods give a high level of estrus synchronization and are as effective as the longer conventional treatments to induce fertile estrus in eCG treated ewes during the non-breeding season. This is consistent with the findings of previous studies reporting that short-term treatment of ewes with progestagen before ram introduction was adequate to induce fertile estrus (Knights *et al.*, 2001; Almadaly *et al.*, 2016) and no difference in estrus response was observed when anestrus ewes are primed for 6 or 14 days, with intravaginal sponge treatments (Ungerfeld & Rubianes, 2002). The present results extend these previous observations to seasonally anestrous ewes. It appears that the onset of estrus following device removal is unrelated to the type of intravaginal P4 device used and more dependent on the dose of P4 used in the device (Moakhar, Kohram, Shahneh, & Saberifar, 2012; Sirjani *et al.*, 2011). Further, the physiological status of the animal (Simonetti, Blanco, & Gardon, 2000), breed (Godfrey, Gray, & Collins, 1997) and the season (Fuentes, Sanchez, Rosiles, & Fuentes, 2001) may have some influence on the time taken for onset of estrus following intravaginal P4 device removal. The duration of estrus period is known to be influenced by the dose of P4 in the sponge as well as on the duration of P4 treatment (Gungor *et al.*, 2007) and on the breed (Gulyani *et al.*, 2009).

None of the ewes among the four treatment groups were cycling at the onset of the experiment. One day prior to device insertion, plasma P4 concentration was below basal levels (average 0.1 ± 0.02 ng/mL) in all experimental ewes, suggesting the absence of active luteal tissue (CL). Mean P4 concentrations at device removal in groups C6 and S6 were higher ($P<0.05$) than in groups C12 and S12, of which two the

group C12 was higher (P<0.05) than group S12; however, the difference between groups C6 and S6 was not significant (Table 1). It is worth noting that P4 concentrations at the time of intravaginal device removal were above basal levels in ewes of all experimental groups, reflecting exogenous treatment levels, which differed (P<0.05) significantly from those (average 0.1 ± 0.02 ng/mL) administered CIDR or FGA sponges. Short-term progestagen treatments (C6 and S6 groups) show higher P4 levels at the time of device removal. Short-term progestagen treatments can be a good alternative to traditional procedures, due to more flexibility under field conditions (Almadaly *et al.*, 2016). In addition to these advantages of short-term progestagen, treatments with low levels of progestagen for estrus synchronization reduce the subsequent fertility (Ungerfeld & Rubianes, 2002). This reduction was explained via an extension of the lifespan of the ovulatory follicle, as a consequence of low levels of P4 (Vinoles *et al.*, 1999). In cyclic ewes, the administration of eCG when progestagen treatment was terminated could compensate for the deleterious effect of long-term progestagen treatment on follicular dynamics by promoting the recruitment of new follicles (Noel *et al.*, 1994) and could overcome the problem of depressed fertility (Boland, Lemainqne, & Gordon, 1978). Mean P4 concentration at estrus was higher in S12 than in S6 group; however, there were no significant differences between C6, C12 and S12 groups and between C6, S6 and C12 groups (Table1). The mean plasma P4 concentration at the onset of estrus was at basal levels in ewes of all experimental groups (Table 1) which is consistent with data obtained by Mohammed, Lawrence, Clarence, and Joseph (2007) and Bonia, Baishya, Deka, and Barua (2008). 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 Najafi, Cedden, Mojtahedi, and Aliverdinasab (2014). In the present study, plasma P4 concentrations were different among pregnant ewes in the different experimental groups, but were almost at the normal P4 profile observed by Wallace *et al.* (1992). Mean P4 concentrations thirty days after device removal were similar between groups C12 and S12 and higher (P<0.05) than in C6 and S6 groups (Table 1). The difference between C6 and S6 groups was not significant (P>0.05; Table 1). 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 reduces embryonic losses and increases pregnancy rate and fertility (Ataman, Akoz, Sarıbay, Erdem, & Bucak, 2013).

The reproductive performances of ewes in the experimental groups are described in Table 2. Conception, lambing and fertility rates were 100% in all experimental ewes. The mean prolificacy rates were similar among groups C6, C12 and S12 and higher (P<0.05) than in S6 group (Table 2). The mean twinning rates were similar among groups C6, C12 and S12 and higher (P<0.05) than in S6 group (Table 2). The type of intervaginal device or insertion length had no significant effect on the reproductive performance in Farhani ewes. The findings of the current study with regard to conception rate (100%) do not support previous research of Mousavy, Sookhtehzari, and Vojgani, (2009) and Koyuncu & Altiçekiç (2010) that reported lower conception rates (43.75 to 86.2%). Vinoles, Forsberg, Banchero, and Rubianes (2001) obtained higher conception rate after short-term treatment compared to the traditional (12 days) treatment with eCG (formerly known as PMSG) at the time of vaginal sponge removal. Ustuner *et al.* (2007) reported that there were no significant differences in terms of conception rate between the short-term and the long-term treatments. Moreover, the highest conception rate (100%) recorded in ewes of all experimental groups, was associated with the highest percent of estrus (100%). The results of the present study with regard to lambing rate were in complete agreement with earlier results of Zonturlu, Ozyurtlu, and Kacar (2011), Taher (2014) and Almadaly, Ashour, El-Kon, Heleil, and Fattouh (2016) in which the lambing rate was at the maximum (100%). In

Table 1. Effect of treatments on synchronization of estrus and P4 concentration (ng/mL) in ewes of the experimental groups during the non-breeding season.

Treatment groups	N	Estrous onset (h)	Estrous duration (h)	P4 at device removal	P4 on estrous day	P4 (day 30 after device removal)
C6	6	30±0.33	19±0.83	3.69±0.01^{a}	0.22±0.01^{ab}	6.23±0.07^{b}
S6	6	29±0.33	18±0.80	3.67±0.01^{a}	0.19±0.01^{b}	6.03±0.05^{b}
C12	6	30±0.67	20±0.17	2.59±0.01^{b}	0.23±0.01^{ab}	6.86±0.02^{a}
S12	6	29±0.83	19±0.00	2.21±0.01^{c}	0.25±0.01^{a}	6.68±0.14^{a}

N: Number of treated ewes. a,b: Means in the same column with different superscripts differ significantly at P<0.05.

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

Treatment groups	N	Estrous response %	Conception rate %	Lambing rate %	Fertility rate %	Prolificacy rate %	Twinning rate %
C6	6	100	100	100	100	166^{a}	66^{a}
S6	6	100	100	100	100	117^{b}	16.7^{b}
C12	6	100	100	100	100	150^{a}	50^{a}
S12	6	100	100	100	100	150^{a}	50^{a}

N: Number of treated ewes. a,b: Means in the same column with different superscripts differ significantly at P<0.05.

contradiction to our results, a lower lambing rate of 67% (Titi, Kridli, & Alnimer, 2010) has been reported. The highest lambing rate (100%) recorded in ewes of all experimental groups may reflect a high ovulation rate. It has been reported that the fertility rate was significantly higher in the short-term (7 days) progestagen treated ewes (87.3%) than in the long-term (12 days) treated ewes (71.6%), at the onset of the breeding season (Karaca, Ataman, & Coyan, 2009). Conversely, Ataman *et al.* (2006) reported that no difference was observed between short-term (7 days) and long-term (12 days) progestagen treatments in the fertility parameters (conception rate, lambing rate and litter size) in Akkaraman crossbred ewes in the breeding season. Likewise, Ustuner *et al.* (2007) informed that short-term (6 days) and long-term (12 days) progestagen treatments resulted in similar pregnancy rates in Awassi ewes during the breeding season. The maximum (100%) fertility rate obtained in the present study is in agreement with the report of Koyuncu & Altiçekiç (2010), but higher than the fertility rate previously reported by Macías-Cruz *et al.* (2012; 95%), and by Karaca *et al.* (2009; 89.6 %). Koyuncu and Ozis Alticekic (2010) reported that eCG administration stimulated follicular development and increased ovulation rate in ewes. Also, Barrett *et al.* (2004) revealed that 500 IU of eCG given at the end of 12 days of treatment with progestagen-impregnated intravaginal sponges had limited effects on the dynamics of ovarian follicular waves in anoestrus ewes. Based on these finding the improved conception, lambing and fertility rates among ewes that had FGA sponge or CIDR for 6 or 12 days and eCG treatment at sponge or CIDR removal may be attributed to the incurporation of eCG into the synchronization protocols, since the two FGA and CIDR regimens and the two corresponding insertion periods of FGA sponges and CIDR devices produced similar reproductive performances. These observations imply that the application of FGA-sponge or CIDR regimen out of season must be accompanied by eCG treatment and 6 days progestagen priming is as effective as a longer traditional priming (12 days) to improve conception, lambing and fertility rates. Prolificacy and twinning rates were significantly ($P< 0.05$) higher in C6, C12 and S12 groups than in S6 group (Table 2). Meanwhile, no significant differences were detected among groups C6, C12 and S12. Prolificacy rate in C6, C12 and S12 was higher than those reported By Moradikor, Sadeghi, and Ziaei (2012) and Almadaly *et al.* (2016) and lower than those reported by Koyuncu, Uzun, and Şengül (2001). A higher and a lower twinning rate was reported by Nosrati, Tahmorespoor, Vatandoost, and Behgar (2011; 33.5%), in comparison to those obtained in S6 (16.7%) and C6 (66%), C12 (50%), and S12 (50%) groups, respectively. This increase in the prolificacy and twinning rates can be attributed to the use of eCG in the synchronization regime, because using eCG increases ovulation rate, multiple births and number of lambs born per ewe lambed as reported by Akoz *et al.* (2006). Boscos *et al.* (2002) observed that use of eCG after progestagen device treatment increases ovarian response, conception rate and percentage of multiple births from the induced ovulations. It has been reported that eCG can increase pregnancy and twinning rates in breeds characterized by low litter size (Boscos *et al.*, 2002). Gulyuz & Kozat (1995) pointed out that administration of eCG increased the number of follicles and therefore raised the twinning and triplet rates, which are of great value to sheep holders. One of the most important applications of eCG in ovine is to increase the prolificacy rate by modifying the ovulatory rate, which depends on the dose level (Boscos *et al.* 2002). In this study, the lowest prolificacy and twinning rates were observed in S6 group, and these were significantly lower in comparison to C6, C12 and S12 groups (Table 2). However, it is not clear to the authors why prolificacy and twinning rates were low in S6 group. However, there is a great variability in responses to eCG that may be associated with breed, individuals within a breed, female category, time of the year, general animal condition (Boscos *et al.*, 2002), and the dose as well as the administration time of eCG (Timurkan & Yildiz, 2005).

4. Conclusions

It was demonstrated that CIDR or FGA sponges were equally effective with 6 or 12 days of priming for estrous induction and in producing reproductive responses in anestrus ewes. Thus, a short-term progestagen treatment for the induction of estrus can be a good alternative to a traditional longer duration treatment (12-14 days). Short-term priming has the advantage of allowing more flexibility in the treatment protocol under field conditions. However, verification of this claim would require a large-scale field study. Also, the use of FGA for 6 days resulted in the lowest prolificacy and twinning rates. What is now needed is future work to find the reason for these low prolificacy and twinning rates.

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