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The Effects of Controlled Internal Drug Release for Different Days on the Reproductive Performance of Out of Mating Season Sheep

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ABSTRACT

This study was conducted to determine the effects on reproductive performances of CIDR implants in different periods (days) out of the mating season in a private sheep enterprise. For this purpose, 312 heads ewes of different ages were divided into groups 3 by ages and body conditions. After applying CIDR (include 330 mg of Progesterone), they have been taken on fifth day for group 1, on seventh day for group 2 and twelfth day for group 3 and after removing CIDR; PMSG (400 IU), PGF2α (100 μg) and GnRH (100 μg) injected (IM) to all. It has been determined that oestrus rate were found 86.53% for group 1 (5 days), 94.23% for group 2 (7 days) and 82.69% for group 3 (12 days), mating rates were 76.92%, 80.76% and 74.03%, the pregnancy rates were 58.65%, 56.73%, and 47.11%, litter size (prolificacy) by hundred lambed sheep were 146.42%, 128.57% and 136.17%, fecundity were 78.84%, 69.23% and 62.53%. Mortality rate of lambs at birth in group 1, 2 and 3 were found 7.32%, 4.17% and 1.54% respectively. Birth weights of single lambs have been significantly higher than multiple births weights of lambs ($p<0.05$). It can be concluded that the highest mating rate ($p<0.05$) has been obtained by the CIDR implementation to the sheep for 7 days, out of the mating season and a quite successful rate is provided when it is compared to external lambing in terms of pregnancy rates.

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INTRODUCTION

Reproduction is acknowledged as the physiological basis of all animal production. In sheep husbandry, reproduction influences milk and meat (lamb) production and has economic implications. Optimizing the reproductive potential of animals depends on improving fertility and increasing the number of lambing within a unit of time (e.g. two lambing per year or three lambing per 2 y) or enhancing twinning rates (Eliçin et al., 1986). The length of the mating season varies according to daylight, management and nutrition conditions, as well as by breed and age (Jainudeen and Ese, 1993;

Baştan, 1995; Özyurtlu and Macun, 2005). When sheep reach puberty, oestrus occurs several times in the mating season, and each oestrus interval varies between 14 and 19 d. The reproduction period of sheep consists of follicular and luteal phases. The follicular phase includes a 2–3 d period during which follicles in the ovary mature and ends with ovulation. The luteal phase occurs after ovulation and a corpus luteum is found in the ovary (Goodman, 1988). The oestrus period lasts on average about 24–36 h (Özyurtlu and Macun, 2005). There are two main objectives in sheep breeding: 1) To achieve high productivity without increasing costs or expenses and 2) to enhance reproductive performances as much as possible (Lindsay, 1991). To achieve these goals, the reproduction cycle of sheep can be managed, and their reproduction performances can be increased using natural methods and various hormones, in addition to technological innovations. These methods, including oestrus stimulation, can result in increased fecundity in sheep (Lindsay, 1991; Özyurtlu and Macun, 2005; Tarhan and Torun, 2011; Tajaddodchelik and Torun, 2012). The aim of the present study was to investigate the impact of controlled internal drug release (CIDR) outside of the mating season on the luteal phase in a mixed flock with different genotypes in a commercial lamb production enterprise.

MATERIAL and METHOD

This study was carried out in May 2015 at a private sheep enterprise located in a region in Southern Turkey with a Mediterranean climate in Hamidiye village of Adana-Ceyhan which has a typical Mediterranean climate. Winters are warm and summers are hot, dry and the average annual rainfall is 625 mm.

The study included 312 crossbred sheep (Tahirova, İvesi, Sakız, Akkaraman) aged between 2 and 3 years that had given birth to one or two lambs. Prior to inclusion in the experiment, the body condition of each animal was assessed. The sheep were then randomly divided into three equal sized groups. Forty days before mating, the sheep received a high-energy ration (flushing), and water was available ad libitum.

CIDR which contained 330 mg progesterone was administered for 5 d (Group 1), 7 d (Group 2) and 12 d (Group 3). Following the withdrawal of CIDR, PMSG (400 IU), PGF2α (100 μg) and GnRH (100 μg) injections were administered intramuscularly (IM). Following the withdrawal of CIDR, the ewes entered oestrus after 24 h. Rams were then introduced to the ewes for 3 d, with intervals of 12 h. After mating, the sheep and rams were separated, and their ear tags were recorded.

Forty-five days after mating, pregnancy was evaluated using an ultrasonography device (Esaote Falco 100, Pie Medical, Maastrich, Nederland). The first cycle was started 4 October 2015 and completed on 16 October 2015, depending on the mating

system of the flock. The lambing date, birth type, sex and ear tag details of the ewes and lambs were recorded.

Statistical Analysis

A variance analysis was carried out using a randomized block design. Duncan's test was performed for comparisons of averages. For differences, a value of $p < 0.05$ was accepted as statistically significant. The SPSS package program was used for data analysis (SPSS, 2011).

RESULTS and DISCUSSION

Data on the reproduction performances of the groups were given in Table 1. Ewes (169) were pregnant from 312 (54,16%) when CIDR was applied in the first cycle. One hundred fifty-nine sheep were lambed and 10 sheep had stillbirths. One hundred six sheep had a single lamb, 46 sheep had twins, and 7 sheep had triplets. Of these 209 lambs; 100 were males and 109 were females.

Table 1. Reproduction performances of the groups.

Parameters	1.Group	2. Group	3. Group	Total
Number of sheep	104	104	104	312
Number of sheep in oestrus	90	98	86	274
Number of mated sheep	80	84	77	244
Number of pregnant sheep	61	59	49	169
Number of lambed sheep	56	56	47	159
Single births	33	40	33	106
Twin births	20	16	10	46
Triplet births	3	0	4	7
Number of lambs born alive	82	72	65	219
Number of male lambs	39	26	35	100
Number of female lambs	37	43	29	109
Number of deat lambs	6	3	1	10
Mortality ratio (%)	7.32	4.17	1.54	4.34

Group1: CIDR was applied for 5 days.
Group2: CIDR was applied for 7 days.
Group3: CIDR was applied for 12 days.

A comparison of the reproduction performances of the three groups was presented in Table 2. The groups were compared with respect to various characteristics, such as the number of sheep in oestrus (oestrus rate), number of mated sheep (mating rate), number of pregnant sheep (pregnancy rate), number of lambed sheep (lambing rate), number of lambs (fecundity) and number of stillbirths. The numbers of mated ewes

were 80, 84 and 77 in Groups 1, 2 and 3, respectively. Oestrus was detected 90, 98 and 86 in Groups 1, 2 and 3, respectively.

Table 2. Comparison of the reproduction performances of the groups.

Parameters	1. Group n (%)	2. Group n (%)	3. Group n (%)	P1-2)	P(1-3)	P(2-3)	Total
Number of sheep	104	104	104	-	-	-	312
Number of sheep in oestrus (oestrus rate)	90(86.53)	98(94.23)	86(82.69)	0.0599	0.442	0.009	274
Number of mated sheep (mating rate)	80(76.92)	84(80.76)	77(74.03)	0.497	0.629	0.245	244
Number of pregnant sheep (pregnancy rate)	61(58.65)	59(56.73)	49(47.11)	0.778	0.095	0.165	169
Number of lambed sheep (lambing rate)	56(53.84)	56(53.84)	47(45.19)	1.000	1.000	0.212	159
Number of lambs (fertility)	82(78.84)	72(69.23)	65(62.53)	0.113	0.009	0.306	219
Mortality (mortality ratio)	6(7.32)	3(4.17)	1(1.54)	0.397	0.076	0.349	10

There was a statistically significant between-group difference in oestrus rates ($p < 0.05$). Although the recorded oestrus rates in Group 2 and Group 3 were statistically significant ($p < 0.05$), there was no statistically significant difference in the between-group comparison ($p>0.05$). The mating rate, pregnancy rate, lambing rate and stillbirth rate were statistically insignificant ($p>0.05$). The numbers of lambs in Groups 1, 2 and 3 were 82, 72 and 65, respectively. In total, 219 lambs were born. Fecundity was 78.84% in Group 1, 69.23% in Group 2 and 62.53% in Group 3. There was a statistically significant different in the number of lambs in Group 1 as compared with that in Group 3 ($p < 0.05$).

Table 3 provides information on the number of lambed ewes; number of single, twin and triplet lambed ewes; and number of male and female lambs in the different groups.

Table 3. Birth type and sex of the lambs in the different groups (%)

Parameters	1. Group n (%)	2. Group n (%)	3. Group n (%)	P (1-2)	P (1-3)	P (2-3)	Total
Number of lambed ewes	56	56	47				159
Single births (single ratio)	33(58.93)	40(71.43)	33(70.21)	0.161	0.228	0.893	106
Twin births (twin ratio)	20(35.71)	16(28.57)	10(21.28)	0.417	0.099	0.390	46
Triplet births (triplet ratio)	3(5.36)	0(0.00)	4(8.51)	0.075	0.533	0.037	7
Number of lamb	82	72	65				219
Number of male lamb (male lamb ratio)	39(47.56)	26(36.11)	35(53.85)	0.147	0.448	0.034	100
Number of female lamb (female lamb ratio)	37(45.12)	43(59.72)	29(44.61)	0.067	0.951	0.074	109

As can be seen in Table 3, the ratio of single births from the 159 lambed sheep in Groups 1, 2 and 3 was 58.93%, 71.43% and 70.21%, respectively. When the groups were compared in terms of single births, the differences between the groups were not

statistically significant ($p > 0.05$). The twinning rate in Group 1 was 35.71%, whereas it was 28.57% in Group 2 and 21.28% in Group 3. When the groups were compared with respect to twin lambing, there were no statistically significant between-group differences ($p > 0.05$). The ratio of triplet lambing was 5.36% in Group 1, 0.00% in Group 2 and 8.51% in Group 3. Between-group comparisons of the ratio of triplet births revealed a statistically significant difference ($p < 0.05$). However, there was no significant difference in this parameter in Group 1 versus Group 2 or Group 1 versus Group 3 ($p > 0.05$). There was a statistically significant difference in the ratio of triplet births in Group 2 versus Group 3 ($p < 0.05$). In terms of sex, there were 39 (47.56%) male lambs in Group 1, 26 (36.11%) male lambs in Group 2 and 35 (53.85%) male lambs in Group 3. One hundred male lambs were obtained from 159 lambed ewes. The results of the analysis of between-group differences between revealed a statistically significant difference in the number of male lambs ($p < 0.05$). However, there was no statistically significant difference in this parameter in Group 1 versus Group 2 or in Group 1 versus Group 3 ($p > 0.05$). There was also no statistically significant in the difference in the number of male lambs in Group 2 as compared with that in Group 3 ($p < 0.05$). In terms of sex, there were 37 (45.12%) female lambs in Group 1, 43 (59.72%) male lambs in Group 2 and 29 (44.61%) male lambs in Group 3. One hundred and nine lambs were obtained from 159 lambed ewes. In terms of the number of female lambs, the differences between the groups were not statistically significant ($p > 0.05$).

Table 4. Birth weights and standard deviations of the groups according to the birth type of the lambs

Parameters	1. Group			2. Group			3. Group			Total		
	$\bar{x}$	S	n	$\bar{x}$	S	N	$\bar{x}$	S	n	$\bar{x}$	S	n
Single lambs	4.89	1.121	32	4.96	0.777	39	4.76	0.966	32	4.87a	0.947	103
Twin lambs	4.13	1.105	38	4.17	0.973	30	4.33	1.334	20	4.19b	1.109	88
Triplet lambs	2.73	0.852	6	0	0	0	2.50	0.455	12	2.58c	0.600	18
Total	4.34	1.236	76	4.62	0.945	69	4.20	1.322	64	4.39	1.184	209

As shown in Table 4, the live weights of the lambs according to birth type were compared in the three groups. In Group 1, the mean live weight of single lambs was 4.89 kg, whereas it was 4.96 kg and 4.76 kg in Groups 2 and 3, respectively. The mean live weights of twin lambs in Groups 1, 2 and 3 were 4.13 kg, 4.17 kg and 4.33 kg, respectively. In Group 1 and Group 3, the mean live weights of triplet lambs were 2.73 kg and 2.50 kg, respectively. The mean live weight of the all 209 lambs was 4.39 kg, and there was no statistically significant association between the type of birth and lamb birth weight. However, the live weights of single lambs were higher than those of lambs from multiple births (twins and triplets).

Table 5 provides information on the lamb birth weights and between-group comparisons in this parameter according to the sex of the lambs.

Table 5. Birth weights and standard deviations of the groups according to the sex of the lambs.

Parameters	1. Group			2. Group			3. Group			Total		
	$\bar{x}$	S	n	$\bar{x}$	S	n	$\bar{x}$	S	n	$\bar{x}$	S	n
Male	4.46	1.223	39	4.88	0.801	26	4.12	1.261	35	4.45	1.170	100
Female	4.21	1.252	37	4.46	0.998	43	4.30	1.408	29	4.33	1.198	109
Total	4.34	1.236	76	4.62	0.945	69	4.20	1.322	64	4.39	1.183	209

In terms of the sex of the lambs, the mean live weight of the 39 male lambs in Group 1 was 4.46 kg, whereas that of the 26 male lambs in Group 2 was 4.88 kg. The mean live weight of the 35 male lambs in Group 3 was 4.12 kg. In terms of the live weights of the female lambs, the mean live weights of the female lambs in Group 1 (n = 37), Group 2 (n = 43) and Group 3 (n = 29) were 4.21 kg, 4.46 kg and 4.30 kg, respectively. The mean total live weight of the 100 male lambs was 4.45 kg, whereas that of the 109 female lambs was 4.33 kg. Among the groups, sex had no statistically significant effect on the birth weights of the lambs, although the live weights of the male lambs were higher than those of the female lambs.

The results of birth weight according to the sex and birth type of the lambs were given in Table 6.

Table 6. Birth weights of the lambs depending on birth type and sex

Features	Male			Female			Total		
	$\bar{x}$	S	n	$\bar{x}$	S	n	$\bar{x}$*	S	n
Single lambs	5.01	0.859	51	4.79	1.017	52	4.87[a]	0.947	103
Twin lambs	4.15	1.094	40	4.22	1.132	48	4.19[b]	1.109	88
Triplet lambs	2.62	0.533	9	2.53	0.691	9	2.58[c]	0.600	18
Total	4.45	1.170	100	4.33	1.199	109	4.39	1.184	209

In terms of the type of birth (single, twin or triplet) and live weight, the mean live weight of 51 single birth male lambs was 5.01 kg, and the average live weight of 52 single birth female lambs was 4.79 kg. The average weight of 103 single birth female and single birth male lambs was 4.87 kg. The average live weight of 40 twin male lambs was 4.15 kg, and the average live weight of 48 twin female lambs was 4.22 kg.

The average live weight of the twin female and male lambs (n = 88) was 4.19 kg. The average live weight of nine triplet male lambs was 2.62 kg, and the average live weight of nine triplet female lambs was 2.53 kg. The average live weight of the female and male lambs (n = 18) was 2.58 kg. The average live weight of the 100 male lambs was 4.45 kg, whereas that of the 109 female lambs was 4.33 kg. In the whole population (n = 209), the average live weight of male and female lambs was 4.39 kg.

DISCUSSION

This study was carried to investigate the effects of a CIDR device implanted for different periods on 312 sheep aged between 2 and 3 y outside of the breeding season on fertility characteristics. For this purpose, sheep in a private facility in Southern Turkey were separated into three test groups according to their age and body conditions. A vaginal CIDR implant containing 330 mg of progesterone was then left in situ for 5 d (Group 1), 7 d (Group 2) or 12 d (Group 3). Thereafter, PMSG (400 IU), PGF2α (100 μg) and GnRH (100 μg) injections were administered IM.

In a previous study, Jackson et al. (2014) examined the effects of oestrus synchronization protocols on sheep in which a vaginal CIDR device was left in situ for 5 d. Within the first 17 d of the mating season, they detected no statistically significant difference in the pregnancy rate, CIDR attachment, PGF2α injection or GnRH injection (37 ± 7.8%, 62 ± 7.5%, 56 ± 8.1% and 46 ± 7.7%) respectively. Within the first 17 d outside of the mating season, in terms of successful pregnancy outcomes, there were no significant differences in CIDR using PGF2α, GnRH, PGF2α and a control (42 ± 12.4%, 37 ± 11.3%, 35 ± 11.1% and 50 ± 10.9%. There were also no differences in lambing and fertility rates, irrespective of the type of application. The administration of $PGF_2\alpha$ and GnRH decreased the oestrus period in sheep in which CIDR was applied for 5 d as compared with that of sheep in which no treatment was applied. However, overall, it did not affect pregnancy, lambing or fertility rates.In the present study, pregnancy rates varied between 47 and 58%, and lambing rates varied between 45 and 53%. These findings are in accordance with those of Jackson et al. (2014). In study on in and out of season Kıvırcık sheep, Ekiz (2005) examined synchronization of oestrus in following the application of fluorogestone acetate (FGA) vaginal sponges. In the out of season group, oestrus synchronization occurred in 41.7% of sheep within 24 h of sponge withdrawal, whereas it occurred in 91.7% of sheep within 36 h of sponge removal in the season group. In another study, 48 h after removal of FGA sponges, Hashemi and Hasani (2005) reported that all sheep were in oestrus. In the same study, 93.3% of Karagul sheep entered oestrus following CIDR plus eCG administered out of season. In this study, CIDR consisted of 0.3 g of progesterone and was left in situ for 12 d. Moeini et al. (2007) applied CIDR plus 400 IU eCG for 13 d in out of season Sanjabi and Lori sheep and reported oestrus rates of

64.4% and 82.2%, respectively. In meat-type out of season sheep, Tarhan and Torun (2011) reported an oestrus rate of 90% and lambing, litter size and fecundity rates of 35.0%, 143% and 50%, respectively, following treatment with a CIDR implant including 0.3 g of progesterone. In the present study on out of breeding season sheep treated with CIDR for 5 d (Group 1), 7 d, (Group 2), or 12 d (Group 3) (n = 104 in each group), followed by PGF2α and GnRH, in addition to a 400 IU PMSG injection, the oestrus rates were 90%, 98% and 86% in Groups 1, 2 3, respectively. The pregnancy/fecundity rates in the three groups were 58.65%, 56.73% and 47.11%, respectively, and the lambing rates were 53.84%, 53.84% and 45.19%, respectively. Previous studies reported pregnancy rates of 20.0–93.3% and lambing rates of 42.0–63.0% following hand mating or free mating and stimulation of oestrus by various progesterone devices (vaginal sponge CIDR and ear implants) for different periods and a combination of different dosages of PMSG (Emrelli et al., 2003; Kaçar et al., 2008; Güngör et al., 2007; Yilmaz, 2008; Ataman et al., 2009). These studies were based on out of season sheep and transition period sheep (various breeds) in different geographical regions.

In out of season sheep, Güngör et al. (2007) administered CIDR-G via an intravaginal implant for 12 d in one group and CIDR-G (used CIDR [U-CIDR]) in a second group for 12 d. Following the removal of the implants, the sheep received an injection of 500 IU PMSG IM. They reported a pregnancy rate of 53.3% in the CIDR group and 60.0% in the U-CIDR group. Ocak et al., (2007) studied Çukurova meat-type sheep (n = 88) treated with vaginal sponges impregnated with 30 mg of FGA for 14 d in Turkey. Following the removal of the sponges after 14 d, 500 IU PMSG was administered IM. Sheep in which oestrus was detected were mated with suitable rams and became pregnant. The lambing rate in their study was 42.0%. Kaçar et al. (2008) treated out of breeding season Tuj sheep with vaginal sponges impregnated with 40 mg of FGA for 12 d. On the day of sponge removal, the sheep received an injection of 600 IU PMSG IM. After the treatment, oestrus was detected by using teaser rams, and impregnation was achieved by controlled natural mating. Kaçar et al. (2008) reported a pregnancy rate of 50.0%. In out of season anestrus Merino sheep (n = 10), Emrelli et al. (2003) studied the effect of progestagen plus PMSG applications on ovarian activities and reproduction performance parameters. The sheep were treated with vaginal sponges impregnated with progesterone (30 mg of FGA) for 14 d. On the day on which the sponges were removed, the sheep received an injection of 500 IU PMSG IM, and the sheep were ram mated. They reported an oestrus rate of 80%, pregnancy rate of 70% and twinning rate of 71.4%.

Yilmaz (2008) determined the effect of oestrus synchronization on the reproduction performances of sheep under farm conditions and oestrus cycles at two different times (1.5 months after the mating season and in the mating season). In their study, the sheep were treated with sponges impregnated with 30 mg of FGA for a 12–14 d

period. After the sponges were removed, 500 IU PMSG was injected IM. The sheep were mated with Sakiz rams using the natural mating method. In the study, the average pregnancy and lambing rate was 62.5% and 58.9% respectively.

In a study on sheep at the beginning of the breeding season, Ataman et al. (2009) investigated the effect of vaginal sponges impregnated with 30 mg of FGA (FGA-30) in one group and 40 mg of FGA (FGA-40) in a second group. The sponges were removed after 12 d. In a third group, an ear implant containing 3 mg of Norgestomet (Chronogest, Intervet, Turkey) was placed under the skin of the ear and removed after 9 d. Following the removal of the vaginal sponges and implants, the sheep received an injection of 600 IU PMSG IM, and the sheep were then mated by natural mating. The pregnancy and lambing rates in the FGA-30 group were 93.3% and 78.57%, respectively, whereas those in the FGA-40 group were 86.66% and 84.61%, respectively. In the ear implant group, these figures were 93.33% and 85.71%, respectively. In the present study, the pregnancy rate was 47.11% in Group 3 (n = 104) in which CIDR was applied for 12 d in out of mating season sheep, followed by the application of PGF2a and GnRH, in addition to an injection of 400 IU PMSG after removal of the CIDR device.

The results obtained in previous studies (Emrelli et al., 2003; Yilmaz, 2008; Ataman et al., 2009) detected differences in different sheep races. These differences may be explained by genetic factors specific to race or environmental factors, such as feeding, age, duration of CIDR application and method of application.

CONCLUSION

A decrease in the luteal phase to 7 d in out of mating season had no negative effects on reproduction performances in sheep. The present study illustrates the effect of the application of CIDR on the promotion of sexual cycles by mimicking the luteal phase for 5, 7 and 12 d out of the breeding season. The method used herein can be used on sheep farms, without any adverse effects on reproductive performance. Irrespective of the breeding season, the application of CIDR was associated with a high rate of oestrus and accordingly a high breeding rate. The mating season, temperature, number and quality of ovule eggs, nutrition and sperm characteristics of rams may reduce pregnancy rates. In out-of-season applications, particular attention should be paid to the temperature and nutritional requirements. Early embryonic deaths may occur in the presence of high temperatures and underfeeding during mating. Similar studies including different genotypes of sheep would be beneficial, in addition to studies performed under different environmental conditions.

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Conflict of Interest Statement

The authors have declared that that there are no competing interests.

Researchers' Contribution Rate Statement Summary

AT contributed to the project idea, design and execution of the study. OT and YE supervised the experiment and wrote the manuscript.

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