Effect of Insertion Periods of Controlled Internal Drug Release Device (CIDR®) on Conception Rate by Fixed-Time Intrauterine Insemination with Frozen Semen in Seasonally Anestrous Ewes

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Abstract. Conception rates and plasma hormonal patterns were investigated after treating ewes with CIDR® (controlled internal drug release device) for different lengths of time during the non-breeding season. In Experiment 1, 101 Suffolk ewes were treated with CIDR® for 6, 9, 12 days; pregnant mare's serum gonadotropin (PMSG) was injected the day before CIDR® removal. At 42 to 50 h after treatment, ewes were inseminated with frozen-thawed semen using a laparoscope. Lambing rates were not significantly different among the groups (66.7, 76.5 and 85.3% for 6, 9, and 12 days, respectively). Seven days after insemination, the re-insertion of CIDR® for 14 days (n=51) did not improve the pregnancy and lambing rates or prolificacy compared to the controls (n=50). In Experiment 2, the mean plasma progesterone concentration on the day of CIDR® removal in Booroola-Dorset ewes treated for 12 days was significantly ($P<0.01$) lower than those in ewes treated with CIDR® for 6 or 9 days. However, the onset of estrus after treatment was similar in ewes treated for different periods with CIDR®. These results indicate that a satisfactory lambing rate (67 to 85%) can be obtained with frozen semen in ewes treated with CIDR®, regardless of the insertion period (6 to 12 days) during the non-breeding season.

Key words: CIDR®, Non-breeding season, Intrauterine insemination, Frozen semen, Sheep.

(J. Reprod. Dev. 40: 221–226, 1994)

Artificial insemination (AI) of ewes on a fixed-time basis following estrous synchronization during the breeding season and induction during the non-breeding season has a major advantage in time and labor when compared to performing estrus detection on non-synchronized ewes [1–7]. To synchronize or induce estrus throughout the year, a progesterone- or progestogen-impregnated intravaginal sponge [4–10] or a controlled internal drug release device (CIDR®) [6, 7, 11–13] are used in ewes with a variation of 7 to 14 days. However, there is evidence in sheep [14, 15] and cattle [16–

18] that long term (12 to 14 days) progestogen treatment adversely affects conception rate at the subsequently synchronized estrus in comparison to shorter term (7 to 10 days) treatment. In sheep, Fitzgerald *et al.* [10] showed that a 7-day insertion of medroxyprogesterone acetate (MAP) sponges combined with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) injection on day 6 effectively controls the estrous cycle during the breeding season. In our previous studies of the non-breeding season [4, 6, 7, 19], a 9-day insertion of MAP sponge or CIDR® device combined with 600 to 750 IU pregnant mare's serum gonadotropin (PMSG) was used to induce estrus, and a fixed-time insemination with frozen semen was possible.

Treating ewes with synthetic progestogen sponges for a prolonged period adversely affected secretion of cervical mucus, sperm transport, and conception rate at the first synchronized estrus [14, 20, 21]. It is not known whether long term insertion of a CIDR® device would have similar effects. The present study was conducted to compare the effect of different durations of CIDR® treatment (6, 9 or 12 days) on conception rate (Experiment 1) and hormone profiles (progesterone: P_4 and estradiol-17 β : E_2) in peripheral blood during and following CIDR® treatment (Experiment 2) in ewes treated during the non-breeding season.

Materials and Methods

Experiments were conducted at the Tawa Field Station (Farm T: Experiment 1), Shibecha-cho in Hokkaido, Japan, during April and the Obihiro University of Agriculture and Veterinary Medicine (Farm O: Experiment 2) during May and June.

Animals

In Experiment 1, 102 mature (2 to 3 years old) Suffolk ewes were used, and frozen semen from 5 mature (3 to 6 years old) Suffolk rams was used for AI. Fourteen mature (5 to 9 years old) Booroola-Merino $\times$ Polled Dorset ewes were used in Experiment 2.

Treatment

In Experiment 1, ewes were treated with CIDR®, an intravaginal device constructed of a progesterone (0.3 g)-impregnated silicon elastomer, (Eazi-breed, type G; Carter Holt Harvey Plastic Products Ltd., New Zealand) for 6 (Group 1: $n=33$), 9 (Group 2: $n=34$) or 12 (Group 3: $n=35$) days. Ewes were injected intramuscularly with 600 IU PMSG (Serotopin®: Teikoku-Zoki Co., Japan) the day before CIDR® removal. In Experiment 2, ewes were treated with CIDR® for 6 (Group 1: $n=5$), 9 (Group 2: $n=4$) or 12 (Group 3: $n=5$) days combined with the PMSG injection the day before CIDR® removal. Two mature rams fitted with a marking harness and an abdominal apron were penned with the ewes for estrous detection for 4 days after CIDR® removal.

Insemination (Experiment 1)

Semen for AI was collected from 5 rams and

frozen in pellet form. Freezing and thawing procedures and diluent followed the method of Salamon and Visser [22] and Fukui [23]. Out of 102 treated ewes, one ewe lost the CIDR® during the 12-day insertion period (Group 3), and, therefore, the ewe was excluded from the present study. A total of 101 ewes were assigned to an insemination group on a fixed-time basis (42 to 50 h) after removal of CIDR®, regardless of estrous occurrence. For each insemination, 0.1 ml of frozen-thawed semen containing 40 to 50 $\times 10^6$ spermatozoa was deposited in each uterine horn (0.2 ml per head) using the Cassou insemination pipette (Z. M. V., France) with the aid of a laparoscope (Storz, Germany). The method for intrauterine AI was as reported by Maxwell *et al.* [1]. Seven days after AI, about half of the ewes in each group (total $n=51$) were treated again with CIDR® for 14 days to examine the effect of post-AI CIDR® on conception rate. The remaining ewes in each group served as controls (total $n=50$).

Hormonal profiles (Experiment 2)

Jugular blood samples (5 ml) were collected into a heparinized vacuum container from all ewes of Groups 1, 2, and 3 every day following the CIDR® insertion until the day of AI. Plasma was separated immediately after collection by centrifugation at 1630 g for 15 min and stored at -20°C until measurement of P_4 and E_2 concentrations. Both steroid hormones were measured after ether extraction with double antibody enzyme immunoassays [24–26]. The sensitivity of the assays was 1 pg/ml for P_4 and 0.16 pg/ml for E_2 . The intra- and inter-assay coefficient of variations for P_4 were 9.4 and 15.6% and for E_2 were 6.3 and 11.3%, respectively.

Conception rate

In Experiment 1, pregnancy was first diagnosed by plasma P_4 determination (> 2 ng/ml) on Day 21 after AI and again by using an ultrasonic scanning instrument (Supereye, SSD 5000. Fujihira Co., Japan) on Day 61 after AI. Pregnancy was confirmed by lambing. Prolificacy (number of new-born lambs/ number of lambing ewes) was also compared among Groups 1, 2 and 3. In Experiment 2, AI was not performed.

Statistical analysis

Data on pregnancy (days 21 and 61) and lamb-

ing rates in Experiment 1 were analyzed by analysis of variance using the general linear models (GLM) procedure of Statistical Analysis System. Prolificacy in Experiment 1 and mean plasma P_4 and E_2 concentrations in Experiment 2 were analyzed by Student's *t*-test using GLM procedure.

Results

The pregnancy and lambing rates and prolificacy of ewes treated for three different insertion periods with CIDR® and inseminated with frozen-thawed semen (Experiment 1) are shown in Table 1. Pregnancy rates determined by P_4 levels on Day 21 were 78.8, 85.3 and 91.2% in the three groups of ewes treated with CIDR® for 6, 9, and 12 days, respectively, and were not significantly different among the groups. Pregnancy rates obtained by ultrasonic scanning on Day 61 in the three groups were 66.7, 76.5 and 85.3%, respectively, and decreased by 5.9 to 12.1% from Day 21 of pregnancy. There were no significant differences among the groups in the pregnancy rates on Day 61. No fur-

ther decrease in the pregnancy rate was observed from Day 61 to term in all groups.

Effects of CIDR® insertion after insemination (Day 7 to 21 after AI) on pregnancy and lambing rates and prolificacy are shown in Table 2. No significant differences in pregnancy and lambing rates were observed between the CIDR®-treated and untreated groups.

In Experiment 2, 13 out of 14 ewes showed estrus 18 to 24 h after CIDR® removal; one ewe in Group 2 (9 day insertion period) failed to show estrus. The patterns of plasma P_4 and E_2 concentrations in ewes treated for different insertion periods are shown in Fig. 1. At the day of CIDR® insertion, P_4 levels varied among the three groups but were within a range of 3 to 6 ng/ml and were not significantly different. P_4 levels rapidly decreased after CIDR® removal in all groups, and on the day of CIDR® removal, the P_4 concentration in Group 3 (12 days; 1.7 ng/ml) ewes was significantly ($P < 0.01$) lower than those in Group 1 (6 days; 3.7 ng/ml) and Group 2 (9 days; 3.7 ng/ml). On the day after CIDR® removal, P_4 levels were not significantly different among the three treat-

Table 1. Conception rate and prolificacy of ewes inseminated with frozen-thawed semen at a fixed-time after treatment for three different insertion periods with CIDR® device and PMSG during the non-breeding season (Experiment 1)

Insertion period of CIDR® (days)	No. of ewes inseminated	No. (%) of pregnant ewes			Prolificacy*
		Day 21	Day 61	Term	
6	33	26 (78.8)	22 (66.7)	22 (66.7)	1.41
9	34	29 (85.3)	26 (76.5)	26 (76.5)	1.42
12	34	31 (91.2)	29 (85.3)	29 (85.3)	1.65
Total	101	86 (85.1)	77 (76.2)	77 (76.2)	1.51

*Calculated from number of new born lambs/number of lambed ewes.

Table 2. Effect of reinserting CIDR® 7 days after insemination for 2 weeks on conception rate and prolificacy in ewes (Experiment 1)

CIDR® insertion*	No. of ewes inseminated	No. (%) of pregnant ewes			Prolificacy
		Day 21	Day 61	Term	
+	51	44 (86.2)	39 (76.5)	39 (76.5)	1.56
—	50	42 (84.0)	38 (76.0)	38 (76.0)	1.45
Total	101	86 (85.1)	77 (76.2)	77 (76.2)	1.51

*CIDR® was inserted (+) in ewes 7 days after insemination for 2 weeks (Day 7 to 21), and ewes not receiving CIDR® were controls (—).

Fig. 1. Patterns of plasma progesterone and estradiol-17 β concentrations (mean $\pm$ SEM) in ewes treated for different insertion periods with a CIDR® device during the non-breeding season (Day 0: the day of CIDR® removal).

ment groups. In contrast with P_4 levels, plasma E_2 levels increased with a peak on the day after CIDR® removal, and the levels before and after CIDR® removal were not significantly different among the three groups.

Discussion

The present results show that the insertion period of CIDR® (6–12 days) did not significantly affect conception rate and prolificacy of inseminated ewes during the non-breeding season. A longer insertion period (12 to 14 days) was used by many workers [2, 5, 8, 11, 14, 15, 20, 21] for synchronizing or inducing estrus and ovulation in ewes with progestogen-impregnated vaginal sponges or CIDR®s. However, the longer term progestogen treatment was reported to adversely

affect conception rate at the first synchronized estrus in ewes [14, 15] due to abnormal secretion of cervical mucus, sperm transport and impaired fertilization [20, 21]. Therefore, a shorter period (9 days) of treatment with intravaginal sponges impregnated with MAP or progesterone and CIDR® device was attempted to improve the conception rate in our laboratory for non-seasonal breeding of sheep in Japan [4, 6, 7, 19]. Seven days of treatment with a MAP-impregnated vaginal sponge was also effective in synchronization of estrus in ewes in combination with PGF $_{2\alpha}$ injection at the cessation of progestogen treatment [10]. Such short term treatment with progestogens and pre-treatment with estrogen was successful in estrous synchronization in cattle [16–18]. In sheep, however, Smith and Parr [12] reported that a short term treatment (10 days) with CIDR® was not as effective as 14 days in estrous synchronizing trials. They claimed that 10 days CIDR® insertion with estradiol treatment at the time of CIDR® insertion delayed the onset of estrus and also resulted in a high proportion of ewes not detected in estrus and a lower conception rate (53.5%) as compared with the longer treatment of CIDR® (62.3%). It has been considered that a short term (10 days) CIDR® treatment produced higher blood levels of P_4 at the time of removal and, thus, resulted in a later onset of estrus because an increased dose of progestogen in the sponge was shown to delay the onset of estrus [27]. In the present study, the plasma P_4 level in ewes treated for 12 days was significantly lower at the day of CIDR® removal than in ewes treated for 6 or 9 days. However, the onset of estrus after CIDR® removal was similar, and pregnancy and lambing rates were not significantly different among the three different treatments. Estrogen treatment at the time of CIDR® insertion was not conducted in the present study, but the use of PMSG injection the day before CIDR® removal likely allowed the short term (6 days) CIDR® treatment to produce a similar conception rate as the longer treatments (9 and 12 days).

Lambing rates obtained in the present study are considered satisfactory for the non-seasonal breeding when compared with our previous trials by fixed-time insemination with fresh [4] or frozen-thawed semen [3, 6, 7] as well as other reports [2, 5, 28–31]. One reason for the acceptable lambing rate may be that an improved intrauterine AI techniques with a finer laparoscope (6.5 mm in diam-

eter, 35.5 cm in length, Storz) caused less impairment to the ewes than the Olympus laparoscope (10 mm in diameter, 22.5 cm in length) used in our previous studies [3, 6, 7].

Reinserting CIDR[®] following AI did not effectively improve conception rates. In New Zealand, CIDR[®] has routinely been used for 3 to 7 days after embryo transfer to improve fertility in ewes [32] and cows [33]. Kittok *et al.* [34] showed that pregnancy rates were higher (58 vs 29%) when ewes were treated with successive injections of 100 IU human chorionic gonadotropin (hCG) on Days 11–13 after mating. However, our previous study [35] that examined effects of successive injections of hCG on Days 10–12 after natural mating as well as the present study using CIDR[®] for 2 weeks after AI did not show any improvement in conception rates for ewes treated during the non-breeding season. The reason is not clear at present, but the type of hormones (hCG, progesterone, or others),

duration of treatment, seasonality, and physiological status of ewes may affect maintenance of the luteal function in treated ewes.

In conclusion, when combined with PMSG injection, a short term (6 days) CIDR[®] treatment resulted in similar conception rates as the longer term (9 or 12 days) treatment in Suffolk ewes during the non-breeding season.

Acknowledgments

The authors thank the staff of the Tawa Field Station for the use of animals and facilities. We also thank the Carter Holt Harvey Plastic Products, Ltd., New Zealand and the Surge-Miyawaki Co., Ltd., Japan for the supply of CIDR[®]. Dr. J. F. Smith, AgResearch, New Zealand is also thanked for reading the manuscript and providing valuable comments.

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