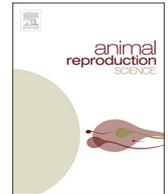




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Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Evaluation of estrous synchronization protocols on ewe reproductive efficiency and profitability

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ARTICLE INFO

Keywords:
Reproduction
Sheep
Estrous synchronization

ABSTRACT

A 2-year experiment was conducted to determine the effect of a single injection of prostaglandin after initiation of the breeding season on ewe estrous synchronization. Rambouillet ewes ($n = 101$; Year 1 = 52; Year 2 = 49) assigned to one of three treatments: untreated (CON); 12-d CIDR insert (CIDR); or 1 injection of prostaglandin at d 2.5 (1 PG) after rams were placed with ewes. Rams were placed with ewes at the time of CIDR removal (d 0) and remained with ewes during a 35-d breeding season. Both the CIDR- (94%) and 1 PG (73.5%) treatment groups had a larger number ($P \leq 0.01$) of ewes bred in the first 5 d of the breeding season compared to ewes of the control (33%) group. As expected, CIDR-treated ewes had a shorter time to mating, than 1 PG-treated ewes and ewes of the control group had a longer interval to mating than both CIDR- and 1 PG-treated ewes ($P \leq 0.01$). The number of lambs born per ewe and kg of lamb weaned per ewe was not different ($P \geq 0.31$) among treatment groups. Additionally, there was no difference ($P = 0.78$) in net profit per ewe among treatment groups. Based on these data, utilizing a single injection of PG 2.5 d after initiation of the breeding season resulted in similar pregnancy rates at d 5 of the breeding season when compared with CIDR-treated ewes indicating the potential utilization of the 1PG protocol in a confinement setting as a viable method for estrous synchronization.

1. Introduction

In animal production systems, synchronization of estrus is utilized to shorten the breeding season, create a more uniform group of offspring, and induce estrus in anestrus females (Patterson et al., 2003; Dixon et al., 2006; Holm et al., 2008; Fierro et al., 2013). Progesterone based estrous synchronization protocols utilizing an intravaginal controlled internal drug release (CIDR) device are the most widely utilized protocols for ewes. With this treatment, there is an attempt to control the behavioral and physiological aspects of the estrous cycle by extending the period of progesterone suppression of estrous onset and ovulation. Upon removal of the CIDR, progesterone (P4) concentrations in circulation decrease allowing for initiation of estrus and associated physiological processes (Wildeus, 2000). Imposing of prostaglandin F₂α (PG) based protocols lead to shortening of the luteal phase by lysing the corpus luteum (CL). Results of previous research indicate administration of PG between d 5 and 14 of the estrous cycle will induce luteolysis (Acritopoulou and Haresign, 1980). Advantages of PG-based compared with progestin-based protocols, include reduced labor inputs, ease of administration, and reduced fiscal costs (Fierro et al., 2013). A major disadvantage of PG-based protocols is the animal must

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<https://doi.org/10.1016/j.anireprosci.2019.106191>

Received 5 February 2019; Received in revised form 17 July 2019; Accepted 9 September 2019

Available online 10 September 2019

0378-4320/ © 2019 Published by Elsevier B.V.

have returned to estrus after parturition for PG administration to result in lysis of the CL, and for there to be subsequent synchronization of estrus.

A single injection of PG 4.5 d after initiation of the breeding season has been proposed as a viable estrous synchronization protocol in cattle (Whittier et al., 1991; Larson et al., 2009). The lack of CL responsiveness to PG for at least 96 h after ovulation is the reason that the protocol of administering PG 4.5 days after the initiation of breeding season is effective (Larson et al., 2009). The CL of ewes is refractory to treatment with PG for 2 d post-ovulation (Rubianes et al., 2003). We hypothesized that placement of ewes with rams 2.5 d before PG administration would result in estrus and mating while not affecting development of the CL after ewes were treated with PG, resulting in a similar lambing distribution and an increase in net profit compared with ewes estrous-synchronized using a CIDR protocol. The objective of this study was to compare estrous response and reproductive efficiency using the protocol of a single PG administration for estrous synchronization as compared with the use of a CIDR protocol.

2. Materials and methods

All animal procedures and facilities used to conduct the present study were approved by the New Mexico State University Institutional Animal Care and Use Committee.

2.1. Animals and treatments

Over a 2-year period, 101 (Year 1 = 52; Year 2 = 49) Rambouillet ewes were blocked by age and BW and randomly assigned to one of three treatments: untreated (CON; $n = 33$); 12-d CIDR insert (EAZI-BREED CIDR, Zoetis Animal Health, Florham Park, NJ; CIDR; $n = 33$; as described by Carlson et al., 1989; Iroz et al., 2009; Lankford et al., 2010); and one 10-mg injection of PGF₂α (5 mg/mL; Lutalyse, Zoetis Animal Health) at d 2.5 (1 PG; $n = 34$) after the initiation of the breeding season. Treatments were initiated in October of each year and all ewes were housed in the same pen. Before treatment initiation, ewes were isolated from rams. Rams were placed with ewes on d 0 at a ewe to ram ratio of approximately 17:1. Marking paint (Raddle powder, Premier 1, Washington, IA) was applied to rams every 3 d as needed. Ewes were placed with rams at the time of CIDR insert removal (d 0) and remained with rams during the 35-d breeding season. Ewes were observed twice daily to determine time of onset of behavioral estrus. Observation of breeding marks at the 0730 and 1700 h, starting on d 0, was used to determine time of onset of behavioral estrus.

2.2. Blood collection

Beginning on d -3 and continuing through d 6 following CIDR removal, blood samples were collected daily at the 0730 h before feeding. Blood samples were collected from a subset of ewes in Year 1 ($n = 24$; CON = 8; CIDR = 8; 1 PG = 8) and all ewes in Year 2 ($n = 49$; CON = 16; CIDR = 16; 1 PG = 17) via jugular venipuncture into serum separator vacuum tubes (Corvac, Kendall Healthcare, St. Louis, MO). Samples were allowed to clot at room temperature for 30 min then subjected to centrifugation ($1200 \times g$ for 20 min at 4 °C). Serum was decanted and stored at -20 °C until hormone assays were conducted. Serum progesterone concentrations were quantified using an RIA utilizing components of a solid phase kit (MP Biomedicals, LLC, Santa Ana, CA) and modified for use in ruminant serum as reported by Schneider and Hallford (1996). Intra-assay coefficient of variation in Year 1 was 10.1%. In Year 2, the intra-assay coefficient of variation was 11.6% and the inter-assay coefficient of variation was 3.8%.

2.3. Postpartum measurements

Following the end of the breeding season, ewes were maintained on a ration formulated to meet or exceed NRC (2007) requirements for each stage of production. In Year 1, pregnancy was confirmed using a blood sample collected by jugular venipuncture. Serum progesterone concentrations were quantified using an RIA as previously described and utilized to confirm pregnancy as reported by Camacho et al. (2008). In Year 2, pregnancy was confirmed using abdominal ultrasonography. Ewes were sheared approximately 2 months before lambing and vaccinated against *Clostridium perfringens* type C and D and *Clostridium tetani* (Bar Vac CD/T, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO). At birth, lambs were individually identified using ear tags. Lamb birth weight, sex, and type of birth was recorded. On d 2, tails were docked utilizing an electric tail docking device (L & H Electric Docker, L & H Manufacturing Co., Mandan, ND) and lambs received 1 mL intramuscular injections of BO-SE (Merck Animal Health, Intervet Inc., Madison, NJ), which contained 1 mg of selenium and 50 mg of vitamin E. Lambs and dams were kept in small pens for 1–2 wk before returning to the main flock. Corn-based creep feeding of lambs started at approximately 10 d of age and continued until weaning. At approximately 30 d of age, lambs were vaccinated for *Clostridium perfringens* type C and D and *Clostridium tetani* (Bar Vac CD/T). Male lambs were also castrated using the banding procedure at this time. Lambs were weaned and weighed at approximately 60 d of age.

2.4. Economic evaluation

Gross economic profit per ewe was based on kilograms of lamb weaned per ewe multiplied by the average lamb price. Average lamb prices were based on USDA Agricultural Marketing Service prices reported for San Angelo, TX for feeder lambs at weaning (USDA-AMS, 2015, 2016). Total cost of estrous synchronization was calculated from the cost of the materials and associated labor costs. The total cost of estrous synchronization was then subtracted from the gross profit per ewe to determine net profit per ewe.

Table 1
Effect of estrous synchronization protocol on reproduction in Rambouillet ewes.

Item	CON ¹	CIDR ²	1 PG ³	SEM	P-value
<i>n</i>	33	33	35	–	–
Age, year	2.7	2.6	2.7	0.3	0.98
Julian breeding date, d	293	287	290	4.6	< 0.01
Days to breeding, d	8.1	2.2	4.9	0.7	< 0.01
Bred first 5 days ⁴ , %	33(11/33) ^a	94(31/33) ^b	74(25/35) ^b	7.1	< 0.01
Bred first 4 days ⁴ , %	21(7/33) ^a	94(31/33) ^b	50(17/35) ^c	7.1	< 0.01
Bred first 3 days ⁴ , %	21(7/33) ^a	82(27/33) ^b	35(12/35) ^a	7.5	< 0.01
Rebred ⁵ , %	27.3	27.3	17.1	10.6	0.53
Final pregnancy rate, %	94	91	100	5.9	0.20

^{a-c}Means with different superscripts differ $P \leq 0.05$.

¹ CON = untreated ewes.

² CIDR = ewes treated with a 12 d CIDR insert.

³ 1 PG = ewes treated with a single injection of prostaglandin 2.5 d following initiation of the breeding season.

⁴ Corresponds to the number of days from initiation of the breeding season (day 0) to marking by the ram.

⁵ Proportion of ewes returning to estrus and marked by the rams during the breeding season ≥ 17 days from date first marking was detected.

2.5. Statistical analysis

Data were analyzed using the PROC MIXED and GLIMMIX procedures in SAS (SAS Inst., Inc., Cary, N.C.). For reproductive and lambing data, animal was considered the experimental unit with estrous synchronization treatment assigned as a fixed effect. Based on findings using the power analysis, sample size was appropriate to ensure that a type II error was avoided with confidence of 95%. The initial statistical analysis included year as a fixed effect and the treatment \times year interaction. The treatment \times year interaction was not significant and was removed from the model and year considered a random effect. Estrous and lambing distribution was determined using the PROC FREQ procedure of SAS. Serum progesterone changes in concentration, as a result of estrous synchronization treatment, were subjected to repeated measures using an ANOVA for a split-plot design with ewe considered as the experimental unit. Treatment was included in the main plot and treatment \times day interactions included in the sub plot. Analysis of P4 concentrations was conducted using the MIXED procedure of SAS. Gross profit and net profit per ewe were analyzed utilizing the MIXED procedure of SAS. A P -value ≤ 0.05 was considered significant.

3. Results and discussion

3.1. Animal performance

There was no trt \times year interaction for reproductive performance or time of lambing, therefore, the main effect of estrous synchronization treatment will be discussed. Ewe reproductive performance is reported in Table 1. Mating distribution data are depicted in Fig. 1. The results of the present study support the hypothesis that placement of ewes with rams 2.5 d before PG administration resulted in estrus and mating, while not affecting the development of the CL following ovulation.

A greater ($P < 0.01$) proportion of CIDR-treated ewes were bred in the first 3 d of the breeding season (82%; 27/33), compared

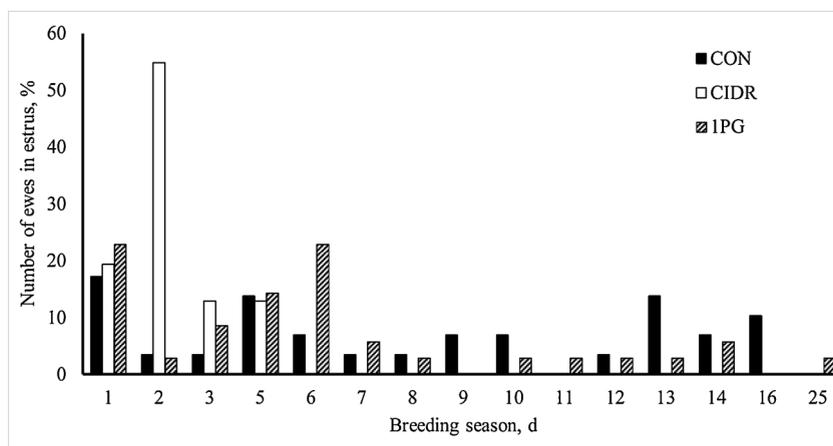
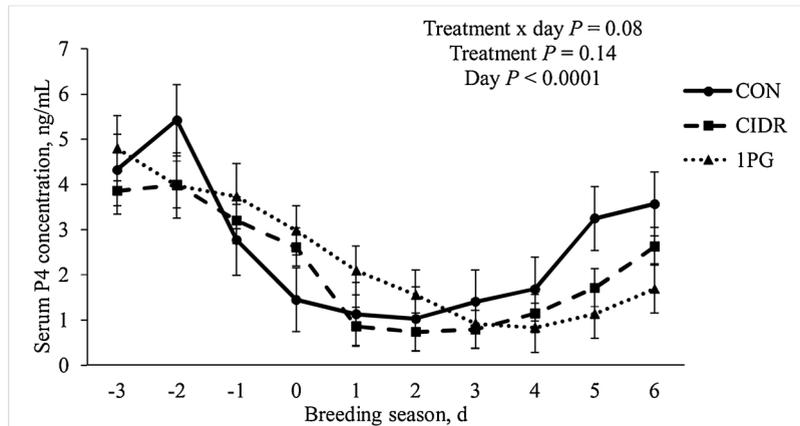


Fig. 1. Distribution of estrus in ewes estrous synchronized using a CIDR protocol, a single injection of PG at d 2.5 after initiation of the breeding season, or untreated control ewes; Note breeding season represents days after initiation of the breeding season (d 0).

A



B

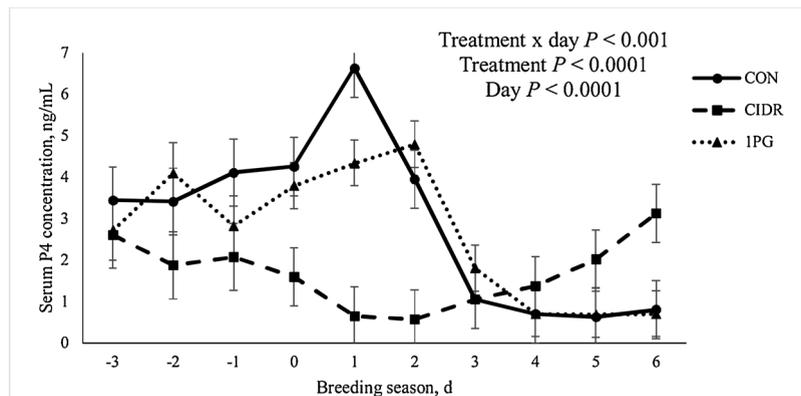


Fig. 2. Effect of estrous synchronization protocol on serum progesterone (P4) concentration in Rambouillet ewes before and after initiation of the breeding season (d 0); (A) Ewes expressing estrus before day 3; No significant treatment \times day interaction ($P = 0.08$); Serum P4 concentrations similar among treatment groups from d -3 to d 6; (B) Ewes expressing estrus from d 3 to d 5; Treatment \times day interaction ($P < 0.001$); Serum P4 concentrations for CIDR-treated less than 1PG-treated and CON ewes on d 1 ($P < 0.01$) of the breeding season; Progesterone concentrations remained greater in 1PG ewes through d 2; Treatments with PG imposed on d 2.5, resulting in reduced P4 concentrations on d 3 in 1PG-treated ewes.

with 1PG-treated (35%; 12/35) ewes or control (21%; 7/33) ewes. Additionally, there was a greater ($P < 0.01$) proportion of CIDR-treated ewes bred in the first 4 d of the breeding season (94%; 31/33) compared with 1PG-treated (50%; 17/35) or control (21%; 7/33) ewes. There was a greater ($P < 0.01$) proportion of ewes bred in the first 5 d of the breeding season in both the CIDR- (94%; 31/33) and 1PG- (74%; 25/35) treatment groups compared with the control group (33%; 11/33) ewes.

In the current study, there was a comparable estrous response of ewes that were estrous-synchronized using the CIDR and single injection of PG protocol indicating the effectiveness of the 1 PG treatment protocol for this purpose. Similarly, [Gonzalez-Bulnes et al. \(2005\)](#) reported there were no differences in estrous response in ewes that were estrous-synchronized with use of either a prostaglandin- or progestin-based protocol (81.5% and 72.4% respectively). As expected, the number of days from initiation of the breeding season to detection of behavioral estrus was fewer ($P < 0.01$) for CIDR- (2.2 d) than 1PG- (4.9 d) treated or control (8.1 d) ewes.

Control ewes had a later ($P < 0.01$) Julian breeding date (293 d) compared with 1PG- (290 d) or CIDR- (287 d) treated ewes, but overall pregnancy rates did not differ among treatment groups ($P = 0.20$). The percentage of ewes rebreeding during the breeding season was not different ($P = 0.53$) among treatment groups.

Serum P4 concentrations are depicted in [Fig. 2](#). There was a significant treatment \times day interaction for serum P4 concentrations for ewes expressing estrus from d 3 to 5 ([Fig. 2b](#)). Serum P4 concentrations were similar among CON and 1 PG treatment groups from d -3 to 0 ($P > 0.17$). As expected, P4 concentrations for CIDR-treated ewes were less than both 1PG-treated and CON ewes on d1 ($P < 0.01$) of the breeding season. The lesser P4 concentrations can be attributed to the removal of the exogenous progestin source on d 0 with results of the present study being similar to those in previous studies ([Dixon et al., 2006](#); [Benavidez et al., 2007](#)) where there were lesser P4 concentrations after than before CIDR removal. Serum P4 concentrations remained greater in 1 PG-treated ewes through d2.

Treatment with PG occurred at d 2.5, resulting in a decrease in P4 concentrations on d 3. Blood serum P4 concentrations in the

Table 2
Effect of estrous synchronization protocol on lamb production in Rambouillet ewes.

Item	CON ¹	CIDR ²	1 PG ³	SEM	P-value
<i>n</i>	33	33	35	–	–
Lambing date, J	80	78	79	5.8	0.40
Number of lambs per ewe	1.4	1.3	1.5	0.1	0.31
Lambled first 17 d, %	82.0	70.8	82.4	9.5	0.44
Adjusted kg weaned per ewe ⁴ , kg	20.0	17.6	18.0	1.8	0.59
Days to lambing ⁵ , d	159.2	157.8	158.9	1.4	0.67

¹ CON = untreated ewes.

² CIDR = ewes treated with a 12 d CIDR insert.

³ 1 PG = ewes treated with a single injection of prostaglandin 2.5 d following initiation of the breeding season.

⁴ Adjustment based on factors from (Scott, 1977).

⁵ Days to lambing was calculated from the day rams were first placed with ewes until lambing.

1 PG-treated ewes on d 3 were similar ($P = 0.79$) to that of CIDR-treated ewes on d 0, indicating there was lysis of the CL in ewes not previously mated. In ewes expressing estrus before day 3 (Fig. 2a) there was no significant treatment \times day interaction ($P = 0.08$). Serum P4 concentrations were similar among treatment groups from d -3 to 6. Effectiveness of PG treatment was verified as a result of approximately 60% of 1 PG-treated ewes responding to the synchronization treatment within 72 h after PG administration. These data are consistent with those reported by Acritopoulou and Haresign (1980) where 50% of ewes treated on d 3 of the estrous cycle responded to PGF₂ α , utilizing a similar dose of PG (10 mg) to that used in the current study. In ewes treated with a single injection of PG on day 2.5 of the breeding season, 75% (18/24) had progesterone concentrations of less than 1/ng/mL between day 3 and 6.

3.2. Postpartum measurements

Lamb production data are summarized in Table 2. Lambing distribution is depicted in Fig. 3. There was no difference ($P = 0.40$) in the Julian lambing date between the three treatment groups even though the CIDR-treated ewes mated 2.7 and 5.9 d earlier than 1PG-treated and control ewes, respectively. Additionally, there was no difference ($P = 0.67$) in days to lambing among treatment groups. The number of lambs born per ewe and kilograms of lamb weaned per ewe were similar ($P > 0.31$) among all treatment groups.

Data for economic implications of the estrous synchronization protocol are reported in Table 3. Gross profit per ewe, estimated as kg lambs marketed per ewe, was similar ($P = 0.95$), regardless of treatment group. In addition, net profit per ewe was similar ($P = 0.78$) among treatment groups. As expected for CON ewes, there was the greatest net return per ewe (\$99.33) due to the lesser costs for labor and no expense for the labor and materials needed for estrous synchronization.

The 1 PG estrous synchronization protocol has advantages compared with a CIDR-based protocol because of ease of administration, less labor input, and decreased cost for the synchronization of estrus. Ewes treated with a single injection of PG had similar estrous response rates at d 5 of the breeding season, compared with CIDR-treated ewes, as well as similar overall pregnancy rates. Furthermore, administration of PG on d 2.5 resulted in a decrease in progesterone concentrations on d 3 in ewes not previously

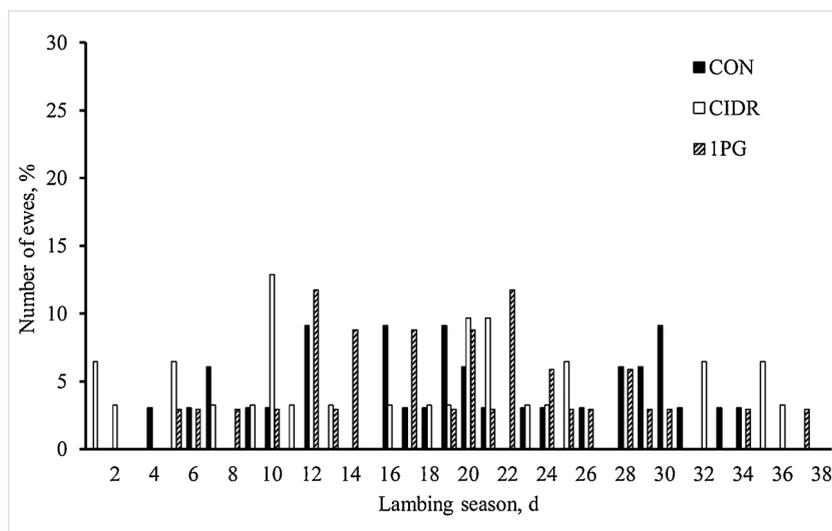


Fig. 3. Distribution of lambing in ewes estrous-synchronized using a CIDR protocol, a single injection of PG at d 2.5 after the initiation of the breeding season, or in untreated control ewes; Note lambing season represents days after the first lamb was born (d 1).

Table 3
Effect of estrous synchronization protocol on lamb production in Rambouillet ewes.

Item	CON ¹	CIDR ²	1 PG ³	SEM	P-value
Cost of estrous synchronization, \$	0.00	7.37	1.64	–	–
Gross profit per ewe ⁴ , \$/ewe	99.33	100.70	98.10	6.17	0.95
Net profit per ewe ⁵ , \$/ewe	99.33	93.33	96.46	6.17	0.78

¹ CON = untreated ewes.

² CIDR = ewes treated with a 12 d CIDR insert.

³ 1 PG = ewes treated with a single injection of prostaglandin 2.5 d following initiation of the breeding season.

⁴ Gross profit per ewe was based on kilograms weaned per ewe multiplied by the average lamb price.

⁵ Net profit per ewe was calculated based on the total cost of estrous synchronization labor and materials subtracted from the gross profit per ewe.

mated, indicating effectiveness of the 1 PG protocol. Control ewes, however, had a similar lambing distribution, as well as a lesser cost due to the lack of labor and materials costs needed for estrous synchronization compared to both CIDR- and 1 PG-treated ewes. Overall, results indicate there is no economic advantage of utilizing either a progestin- or PG-based protocol in a farm flock.

Declaration of Competing Interest

The authors have no conflicts of interest to report.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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