Original Article

Comparison of Different Oestrus Synchronisation/Induction Protocols on Fertility and Prolificacy Rates in Ewes

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Abstract

Background: Oestrus synchronisation is an important reproductive management strategy for improving fertility and productivity in sheep. **Aims and Objectives:** This study evaluated the effectiveness of two alternative oestrus synchronisation protocols, double prostaglandin F2α (PGF2α) injections and a combined gonadotropin releasing hormone (GnRH)–PGF2α protocol, compared with the conventional controlled internal drug release (CIDR) based protocol. **Materials and Methods:** Twenty four ewes were allocated into three groups (n = 8). Group 1 received CIDR for 14 days, followed by 400 IU pregnant mare serum gonadotropin (PMSG) at removal. Group 2 received two PGF2α injections 11 days apart, followed by 400 IU PMSG, while Group 3 received GnRH followed by PGF2α and PMSG. Oestrus was detected, and ewes were naturally mated. Blood samples were collected before, after, 2 days after treatment and 17 days post mating to determine serum oestradiol and progesterone concentrations. **Results:** The CIDR group achieved the highest lambing rate (100%), followed by the PGF2α group (85.71%), whereas the GnRH group showed a significantly lower lambing rate (33.33%) ($P \leq 0.05$). Litter size was numerically larger in the CIDR group (1.37) than in the PGF2α (1.16) and GnRH (1.00) groups, although differences were not significant. Oestradiol concentrations increased following hormonal treatment in all the groups, with the greatest increase observed in the PGF2α group (33.07–209.91 pg/mL). At day 17 post mating, the CIDR group exhibited significantly higher oestradiol concentrations than the other groups ($P \leq 0.05$). Progesterone concentrations increased in all the groups without significant differences among protocols. **Conclusion:** the CIDR protocol showed the highest reproductive performance, while the PGF2α protocol provided comparable and cost effective reproductive outcomes.

Keywords: Controlled internal drug release, gonadotropin-releasing hormone, prostaglandin $F_2\alpha$, synchronisation

Introduction

Oestrus synchronisation is one of the most important reproductive management strategies used to improve fertility and productivity in sheep.(1) It plays a crucial role in facilitating controlled breeding, enhancing reproductive efficiency, reducing labour and production costs and shortening the breeding season.(2) Among the available methods, the use of progestogen-containing intravaginal devices is the most widely applied technique for oestrus synchronisation in ewes.(3) These devices, including controlled internal drug release (CIDR) systems containing progesterone (P4) and intravaginal sponges impregnated with synthetic progestogens such as medroxyprogesterone acetate or fluorogestone acetate, are extensively used worldwide due to their effectiveness in regulating the oestrous cycle.(4)

In addition to progesterone-based methods, hormonal protocols involving prostaglandin F2α ($PGF_2\alpha$), pregnant mare serum gonadotropin (PMSG) and gonadotropin-releasing hormone (GnRH) are commonly used either alone or in combination to control follicular dynamics and luteal function.(1,5,6) For instance, the administration of two doses of $PGF_2\alpha$ at 9–11-day intervals is a common and effective approach for oestrus synchronisation in ewes.(7)

Furthermore, previous studies have shown that combining progesterone with $PGF_2\alpha$ can shorten the interval to oestrus onset compared to progesterone treatment alone.(8) Despite the effectiveness of progesterone-based protocols, their

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relatively high cost and longer treatment duration represent important limitations under practical field conditions. Therefore, exploring alternative synchronisation protocols that are both effective and economically feasible is of significant importance. Accordingly, the present study aimed to evaluate the effectiveness of two alternative oestrus synchronisation protocols, double $PGF_2\alpha$ injections and a combined GnRH–$PGF_2\alpha$ protocol, and to compare them with the conventional CIDR-based protocol in ewes. The comparison was based on fertility and prolificacy rates indicators, including lambing rate and litter size, as well as serum concentrations of oestradiol (E2) and progesterone.

Materials and Methods

Ethical approval

Ethical approval for this study was obtained from the College of Veterinary Medicine, University of Tikrit, Salah Al-Deen, Iraq (Approval No. Tu. Vet. 186; Approval Date: 23 August 2025), in accordance with the relevant Iraqi legislation.

Experimental animal

The present study was conducted at the animal house of the College of Veterinary Medicine, Tikrit University, from August 2025 to March 2026. A total of 24 ewes and 8 rams, aged 2–3 years and weighing 50-60 kg, were used in this study. The animals were housed in semi-open barns under standard management conditions. Before the start of the experiment, all animals underwent clinical examination to ensure they were healthy. Before the experimental procedures, ewes were separated from rams for 30 days. Pregnancy status was confirmed using ultrasonography with a 3.5 MHz probe (Chison, China) to ensure that all animals were non-pregnant at the beginning of the study.

Experimental design

The experimental animals in the present study were randomly divided into three equal groups (n = 8 each) as follows [Figure 1]:

- Group 1 (G1) was treated with a CIDR device (CIDR; NWECIDAR, Iran) for 14 days, followed by an intramuscular injection of 400 IU PMSG at the time of CIDR removal(9)
- Group 2 (G2) received an intramuscular injection of 1 mL $PGF_2\alpha$ (Galapan, Spain) on day 3, followed by a second dose after 11 days. Subsequently, 400 IU PMSG was administered 2 days after the second $PGF_2\alpha$ injection(10)
- Group 3 (G3) was administered 2 mL GnRH (Receptal, Spain) on day 7, followed by an injection of $PGF_2\alpha$ 5 days later and then 400 IU PMSG 2 days after $PGF_2\alpha$ administration.(11)

Blood sample collection

Blood samples were collected from all ewes included in the experiment to determine progesterone and oestrogen concentrations. Samples were collected at four time points: before treatment, at the end of treatment, 2 days after the end of treatment and on day 17 post-mating. Approximately 5 mL of blood was collected, and the separated serum was stored at −20°C until hormonal analysis.(12)

Hormonal assay

Serum concentrations of progesterone and oestradiol were evaluated in serum from whole blood samples using a fully automated enzyme immunoassay analyser (Cobas System, Roche Diagnostics, Switzerland) per the manufacturer's recommendation. Whole blood samples were separated into serum through the use of a centrifuge and then stored at −20°C until analysed. Analytes were measured via commercial human

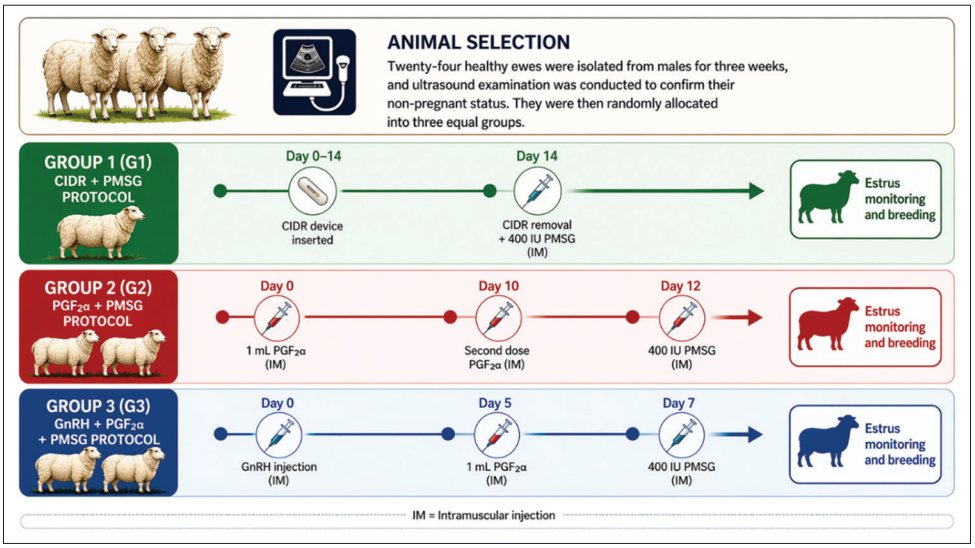

Figure 1: Comparative efficacy of conventional controlled internal drug release-, prostaglandin $F_2\alpha$- and gonadotropin-releasing hormone-based protocols for oestrus synchronisation in sheep. CIDR: Controlled internal drug release, $PGF_2\alpha$: Prostaglandin F2α, GnRH: Gonadotropin-releasing hormone, PMSG: Pregnant mare serum gonadotropin

progesterone and oestradiol reagent kits designed for use on a Cobas analyser. The analyser evaluated analyte concentration using manufacturer-specific calibration curves and standard quality control methods. Progesterone concentrations were reported as ng/mL and oestradiol concentrations as pg/mL.(12) Briefly, serum samples and standards were added to microplate wells pre-coated with specific antibodies against the target hormones. After incubation, unbound components were removed by washing. An enzyme-conjugated secondary antibody was then added, forming hormone–antibody complexes. Following a second incubation and washing step, a chromogenic substrate solution was added, resulting in a colour change proportional to the hormone concentration. The reaction was stopped using a stop solution, and absorbance was measured using a microplate reader at an appropriate wavelength (typically 450 nm). Hormone concentrations were calculated using a standard calibration curve generated from known standards provided in the kit. All samples were analysed in duplicate to ensure accuracy and reproducibility, and mean values were used for statistical analysis

Reproductive efficiency parameter

To evaluate the reproductive efficiency of ewes subjected to different synchronisation protocols and to enable comparison among groups, the following parameters were assessed:

Lambing rate

Lambing rate was calculated as the percentage of ewes that successfully gave birth relative to the total number of ewes mated in each group, according to Kumar,(13) using the following equation:

$$\text{Lambing rate} = \frac{\text{number of ewes given birth}}{\text{number of ewes serviced}} \times 100$$

Litter size

Litter size was determined as the average number of lambs born per ewe that lambed, according to Kumar,(13) using the following equation:

$$\text{Litter Size} = \frac{\text{total number of lambs born}}{\text{number of ewes that lambed}}$$

Statistical analysis

Statistical analysis was performed using Statistical analysis was performed using SAS software.(14) Lambing rate (%) was analysed using the Chi-square test to compare proportions among the groups. Data on litter size and hormonal concentrations (oestrogen and progesterone) were analysed using one-way analysis of variance. Differences among means were determined using appropriate *post hoc* tests. A probability level of $P \leq 0.05$ was considered statistically significant.

Results and Discussion

Lambing rate

The results showed that oestrus synchronisation protocols had a significant effect on lambing rate [Table 1]. The CIDR group achieved the highest lambing rate (100%), followed by the $PGF_2\alpha$ group (85.71%), while the GnRH group exhibited a markedly lower lambing rate (33.33%). Statistical analysis revealed no significant difference between the CIDR and $PGF_2\alpha$ groups ($P > 0.05$), as both shared the same significance letter (a). However, the GnRH group differed significantly from the other two groups ($P < 0.05$), indicating reduced reproductive efficiency.

Table 1: Effect of different oestrus synchronisation protocols on lambing rate

Group	Protocol	Lambing ewes (*n*)	Lambing rate (%)	Significance
Group 1	CIDR	8	100	a
Group 2	$PGF_2\alpha$	6	85.71	a
Group 3	GnRH	2	33.33	b

Different letters between rows mean significant differences at $P \leq 0.05$.
CIDR: Controlled internal drug release, $PGF_2\alpha$: Prostaglandin $F_2\alpha$, GnRH: Gonadotropin-releasing hormone

These findings suggest that CIDR and $PGF_2\alpha$ protocols are more effective than GnRH in improving lambing rate under the conditions of this study. The present results are in agreement with Farrag,(9) who reported a higher lambing rate in ewes treated with progesterone (100%), followed by prostaglandin (87.5%), and a lower rate in the GnRH protocol. The findings are also consistent with those of Waheeb *et al*.,(10) who reported comparable pregnancy and lambing rates. Similarly, Akoz *et al*.(11) observed an 85.7% lambing rate in Akkaraman crossbred ewes treated with progesterone and 700 IU PMSG. Furthermore, Kulaksiz *et al*.(15) reported lower oestrus, conception and lambing rates with GnRH-based protocols compared to long- and short-term progesterone sponge plus PMSG treatments.

In contrast, the present results disagree with Almadaly *et al*.,(16) who reported higher lambing rates in ewes treated with $PGF_2\alpha$ compared to progesterone and GnRH protocols. These variations may be attributed to differences in breed, environmental conditions, management practices and the type, dose and duration of hormonal treatments. The superior performance of the CIDR protocol may be explained by the role of exogenous progesterone in mimicking the luteal phase, thereby regulating follicular dynamics and ensuring proper oestrus expression, ovulation and subsequent conception. This hormonal environment enhances reproductive efficiency and ultimately increases lambing rate.(17)

Litter size

Litter size varied among the experimental groups, with the CIDR group showing the highest mean value (1.37), followed by the $PGF_2\alpha$ group (1.16), while the GnRH group recorded the lowest value (1.00) [Table 2]. Despite these numerical differences, statistical analysis revealed no significant differences among the groups ($P > 0.05$), as all the groups shared the same significance letter (a). This indicates that although CIDR and $PGF_2\alpha$ protocols tended to produce slightly higher litter sizes, these differences were not statistically significant,

suggesting that the oestrus synchronisation protocols did not markedly influence litter size under the conditions of this study. These findings are consistent with Abu *et al*.,(18) who reported a litter size of 1.4 in Rahmani ewes synchronised using a CIDR protocol. Similarly, Ashour *et al*.(19) reported a comparable litter size (1.4) in ewes treated with progesterone sponges. Furthermore, Koyuncu and Ozis Alticekic(20) indicated that PMSG administration enhances follicular development and ovulation rate in ewes, which may contribute to improved litter size. In addition, Alkass *et al*.(21) reported that the use of PMSG, alone or in combination with Vitamin A, significantly increased lambing and twinning rates compared to untreated ewes. In contrast, the present results disagree with Abu *et al*.,(18) who reported a higher litter size (1.58) in ewes treated with a GnRH protocol. These variations may be attributed to differences in breed, environmental conditions, nutritional status, management practices and hormonal treatment regimens. It is well established that litter size is influenced by multiple factors, including genetic background, environmental conditions and management systems.(22)

Progesterone concentration

Progesterone concentrations did not differ significantly among the three experimental groups (G1, G2 and G3) at any of the studied time points ($P > 0.05$) [Table 3]. Before treatment, progesterone concentrations were 6.88 ± 0.52, 6.48 ± 0.41 and 11.18 ± 2.35 ng/mL in G1, G2 and G3, respectively, with no significant differences among the groups ($P > 0.05$). Following hormone administration, progesterone concentrations increased in all the groups, reaching 11.93 ± 1.38 (G1), 15.89 ± 1.81 (G2) and 19.81 ± 3.60 (G3). Despite these numerical differences, the variation remained statistically non-significant ($P > 0.05$), suggesting a comparable luteal response among the applied synchronisation protocols. At 2 days post-treatment, progesterone levels remained relatively stable across the groups (12.57 ± 1.17, 13.35 ± 2.55 and 15.55 ± 2.24 for G1, G2 and G3, respectively), further confirming the absence of significant differences and indicating a consistent endocrine response during the early post-treatment phase. Similarly, at 17 days after treatment, progesterone concentrations showed a numerical increase in G1 (18.37 ± 3.98) compared to G2 (16.16 ± 0.95) and G3 (15.64 ± 4.56); however, these differences were not statistically significant ($P > 0.05$). This overall increase across all the groups likely reflects the formation and maintenance of functional corpora lutea following mating and early pregnancy. The absence of significant differences among the groups suggests that the different oestrus synchronisation protocols exerted comparable effects on luteal function and progesterone secretion. These findings are consistent with those reported by Abu *et al*.(18) and Naderipour *et al*.,(23) who observed no significant differences in progesterone concentrations among various synchronisation protocols at different stages of the oestrous cycle. Similarly, Kanduri *et al*.(24) reported comparable progesterone profiles when using similar hormonal treatments. Although the CIDR-treated group exhibited relatively higher progesterone concentrations at later stages, this may be attributed to the sustained release of exogenous progesterone from the device, which supports luteal function and mimics the natural oestrous cycle.(25) However, as these differences were not statistically significant, their biological impact appears to be limited under the conditions of the present study. In contrast, the slightly lower progesterone concentrations observed in the $PGF_2\alpha$ and GnRH groups may be related to their mechanisms of action, as these protocols primarily influence luteal regression and follicular dynamics rather than directly supplying exogenous progesterone. The increase in progesterone levels at 17 days post-treatment is consistent with previous reports,(9,26,27) which demonstrated elevated progesterone concentrations during early pregnancy due to active corpus luteum function.

Oestrogen concentration

Oestradiol concentrations showed significant variations among experimental groups at specific time points, reflecting changes in follicular activity and ovarian physiological

Table 2: Effect of different oestrus synchronisation protocols on litter size

Group	Protocol	Number of lambing ewes	Number of lambs	Litter size (%)	Significance
Group 1	CIDR	8	11	1.37	a
Group 2	$PGF_2\alpha$	6	7	1.16	a
Group 3	GnRH	2	2	1.00	a

Different letters between rows mean significant differences at ($P \leq 0.05$). CIDR: Controlled internal drug release, $PGF_2\alpha$: Prostaglandin $F_2\alpha$, GnRH: Gonadotropin-releasing hormone

Table 3: Progesterone concentration (mean±standard error of mean) in the different groups at different time points

Time of measurement	G1 ($n=8$)	G2 ($n=8$)	G3 ($n=8/2$*)	*P*
Before treatment	6.88±0.52 (a)	6.48±0.41 (a)	11.18±2.35 (a)	0.052 (NS)
After hormone injection	11.93±1.38 (a)	15.89±1.81 (a)	19.81±3.60 (a)	0.101 (NS)
2 days after treatment	12.57±1.17 (a)	13.35±2.55 (a)	15.55±2.24 (a)	0.581 (NS)
17 days after treatment	18.37±3.98 (a)	16.16±0.95 (a)	15.64±4.56 (a)	0.834 (NS)

*The sample size in G3 was reduced at day 17 due to lambing outcomes (only lambed ewes were included in the analysis), Means within the same row sharing the same letter are not significantly different ($P>0.05$). Values are expressed as mean±SEM. NS: Not significant, SEM: Standard error of mean

Table 4: Oestradiol concentration (mean±standard error of mean) in the different groups at different time points

Time of measurement	G1 ($n=8$)	G2 ($n=8$)	G3 ($n=8/2$*)	*P*
Before treatment	23.66±5.90 (b)	33.07±3.09 (b)	88.14±19.49 (a)	0.0019
After hormone injection	101.31±4.68 (a)	209.91±112.78 (a)	106.08±4.64 (a)	0.427 (NS)
2 days after treatment	96.03±5.45 (a)	96.13±11.74 (a)	108.63±6.81 (a)	0.491 (NS)
17 days after treatment	101.79±7.54 (a)	69.80±6.54 (b)	58.37±3.00 (b)	0.0062

*The sample size in G3 was reduced at day 17 due to lambing outcomes (only lambed ewes were included), Means within the same row with different letters indicate significant differences ($P \leq 0.05$), while similar letters indicate no significant difference. Values are expressed as mean±SEM. NS: Not significant, SEM: Standard error of mean

responses [Table 4]. Before treatment, a significant difference was observed among the groups ($P \leq 0.05$), with G3 exhibiting the highest oestradiol concentration (88.14 ± 19.49) compared to G1 (23.66 ± 5.90) and G2 (33.07 ± 3.09), which did not differ significantly from each other. This elevated level in G3 likely reflects greater follicular activity or the presence of dominant follicles before synchronisation.

Following hormone administration, oestradiol concentrations increased in all the groups; however, no significant differences were observed among treatments ($P > 0.05$). A similar pattern was observed 2 days after treatment, where oestradiol levels remained comparable across all the groups. This convergence in hormone levels indicates that the synchronisation protocols were effective in standardising follicular dynamics and minimising variability among animals, which represents a key objective of oestrus synchronisation. These findings are consistent with those reported by Naderipour *et al*.(23) who observed similar oestradiol profiles following CIDR and $PGF_2\alpha$ protocols. At 17 days after treatment, significant differences reappeared ($P \leq 0.05$), with G1 showing the highest oestradiol concentration (101.79 ± 7.54), while G2 (69.80 ± 6.54) and G3 (58.37 ± 3.00) exhibited significantly lower values. This pattern may be attributed to differences in reproductive status among the groups, particularly the establishment of pregnancy and luteal activity. Increased progesterone levels during early pregnancy can suppress GnRH secretion, thereby reducing follicular development and oestradiol production.(28) Similar observations were reported by Al-Marzani and Barwary,(27) who documented reduced oestradiol concentrations during later reproductive stages. However, the present findings differ from those of Naderipour *et al*.(23) who reported higher oestradiol concentrations in $PGF_2\alpha$-treated ewes immediately after treatment. Such discrepancies may be explained by differences in breed, environmental conditions and management practices.

Conclusion

The CIDR protocol revealed the highest reproductive performance in terms of lambing rate and overall efficiency. However, the $PGF_2\alpha$-based protocol showed comparable results with no significant difference, indicating that it can be considered a reliable and cost-effective alternative for oestrus synchronisation in ewes. In contrast, the GnRH-based protocol exhibited lower reproductive efficiency under the conditions of this study.

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Conflicts of interest

There are no conflicts of interest.

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