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Research

Estrus Quality of Garut Sheep after Estrus Synchronization using Prostaglandin, Progesterone, and Their Combination

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ABSTRACT

Estrus synchronization is a technique used to stimulate estrus and ovulation in a group of animals using hormones. This study aimed to compare three estrus synchronization methods. Fifteen female garut sheep were used and treated with: progesterone-CIDR, double injection prostaglandins, and their combination. At standing heat, ewes were mated with a buck. The parameters measured include the onset estrus, duration estrus, pattern of electrical impedance in the vaginal mucus, number and size of follicles, and pregnancy status. All treated ewes responded to estrus (100%). There were different onset of estrus, namely CIDR (54h), CIDR+PGF2α (41h), and double injection of PGF2α (49.4h), respectively. Meanwhile, the Duration of estrus of CIDR (30,6h), CIDR+PGF2α (29.8h), and double injection of PGF2α (22.4h), respectively. Furthermore, electrical impedance of vaginal mucus at standing heat were CIDR (208Ω), CIDR+PGF2α (189Ω) and double injection of PGF2α (305Ω). The follicle size on H_0 to *standing heat* in each group was significant ($p<0.05$), while the follicle count was significant only ($p<0.05$) in the CIDR group. Pregnancy rate was achieved by synchronization with CIDR and double injection prostaglandin (100%) compared to CIDR+PGF2α (40%). It's concluded that the third method of synchronization could result in the best estrus response with slight different characteristics.

Keywords: Synchronization, CIRD, Prostaglandins, Garut sheep

ABSTRAK

Sinkronisasi estrus merupakan teknik penyerentakan sekelompok hewan dengan menggunakan preparat hormon. Tujuan penelitian ini untuk membandingkan tiga metode sinkronisasi estrus. Sebanyak lima belas domba garut betina yang dikelompokkan dalam tiga kelompok yaitu Progesteron_CIDR, double injeksi prostaglandin dan Kombinasi dari kedua hormon. Pada saat estrus domba dikawinkan dengan pejantan yang sudah disiapkan. Parameter yang diukur meliputi onset estrus, durasi estrus, pola hambatan arus listrik lendir vagina, jumlah dan ukuran folikel serta kebuntingan. Semua hewan yang yang diberi perlakuan merespon (100%). Ada perbedaan dalam onset estrus pada masing-masing kelompok yaitu CIDR (54 jam), CIDR+$PGF_{2\alpha}$ (41 jam) dan double injeksi $PGF_{2\alpha}$ (49,4 jam). Sementara itu, durasi estrus CIDR (30,6 jam), CIDR+$PGF_{2\alpha}$ (29,8 jam) dan double injeksi $PGF_{2\alpha}$ (22,4 jam). Selain itu hambatan arus Listrik lendir vagina pada kelompok CIDR (208Ω), CIDR+$PGF_{2\alpha}$ (189Ω) dan double injeksi $PGF_{2\alpha}$ (305Ω). Ukuran folikel dari H_0 ke *standing heat* pada masing-masing kelompok signifikan ($p<0.05$) sedangkan jumlah folikel hanya signifikan ($p<0.05$) pada kelompok CIDR. Keberhasilan kebuntingan pada sinkronisasasi dengan CIDR dan double injeksi prostaglandin (100%), dibandingkan dengan kombinasi CIDR+$PGF_{2\alpha}$ (40%). Dapat disimpulkan bahwa ketiga metode sinkronisasi dapat menghasilkan respon estrus terbaik dengan karakteristik yang sedikit berbeda.

Kata kunci : Sinkronisasi, CIDR, Prostaglandin, Domba Garut

INTRODUCTION

Garut sheep is a local Indonesian sheep that has the advantage of being an agile sheep in addition to having good meat quality. Garut sheep also have prolific traits, namely, having more than one fetus (Somanjaya *et al.* 2025)Therefore, this breed is highly desirable for increasing livestock populations. Reproductive efficiency is a crucial factor in enhancing sheep livestock productivity. Livestock productivity can be improved by synchronization techniques.

Estrus synchronization is a technique to synchronizes estrus in a group of animals using a hormone to induce estrus behavior and ovulation (Julanov *et al.* 2024). The advantage of uniformity of estrus and proper mating can result in relatively equal offspring, allowing for efficient livestock management. The effectiveness of estrus synchronization depends on the body condition score, estrus cycle, and hormones used (Hameed 2025). An understanding of the estrus cycle and the precision of hormone administration can help with timing and phases in the estrus cycle because hormones can only work in specific phases of the estrus cycle. There are two phases in an estrus cycle: the follicular and luteal phases. The time required for the luteal phase is longer than the follicular phase. The synchronization of estrus is related to hormonal mechanisms such as *gonadotropin-releasing hormone* (GnRH), *follicle-stimulating hormone* (FSH), *luteinizing hormone* (LH), ovarian hormones such as estrogen and progesterone, and uterine hormone prostaglandins (Abu El-Ella *et al.* 2016).

Prostaglandins and progesterone are the two hormones that are often used for estrus synchronization. There are two principal methods for estrus synchronization by either shortening the luteal phase by regressing of corpora lutea with PGF2α or prolonging it by providing exogenous progesterone (Hameed 2025). The *Corpus luteum* (CL) is a temporary endocrine gland formed from a previous follicle that plays a role in the synthesis of progesterone, which functions to maintain early pregnancy in all mammalian species. Regression *of the Corpus luteum* (*Luteolytic*) can initiate a new cycle (Seto and Bogan 2015). Progesterone suppresses follicle development by inhibiting GnRH activity (Waqas and Tibary 2025). The use of progesterone hormone (CIDR) aims to manipulate the luteal phase, thereby inhibiting estrus behavior and ovulation and increasing the number of females that exhibit estrus sign after progesterone withdrawal (Knickmeyer *et al.* 2019) After removal, there is no more inhibition to gonadotropins, which triggers to increase FSH and LH, leading to estrus. Knickmeyer *et al.* (2019) reported that more precise control of estrus behavior can be achieved through the use of a combination of progesterone and prostaglandins.

The onset of estrus and the signs of estrus vary between animals. The estrus sign is often used as an indicator to perform other actions, such as artificial insemination, to determine a fertile animal status, and other indications. The quality of estrus greatly determines the success of animal mating. Synchronization with progesterone hormones, prostaglandins, and the combination of these two hormones are expected to obtain the best quality of estrus, so it will increase the success of mating. The quality of estrus can be characterized by mucus production and an increased number and size of follicles. The high level of estrogen correlated with the presence of large follicles, accompanied by large and clear mucus amounts indicates the best estrus quality (Balumbi *et al.* 2019).

Estrus quality can be measured in various ways. Abundant estrus mucus during estrus correlates with the quality of estrus and has a specific pattern. In addition, the quality of estrus can also be observed by counting the number and quality of developing follicles using ultrasonography. Therefore, the present study was conducted to observe the response and characteristics of estrus that occurred after synchronization, onset of estrus, estrus duration, number and size of follicles.

MATERIALS AND METHODS

Research Location and Ethical Clearance

The study was conducted from November 2024 to April 2025 at the Reproductive Rehabilitation Unit (URR), School of Veterinary Medicine and Biomedical Sciences, IPB University. The research was approved by the Animal Ethics Commission, School of Veterinary Medicine and Biomedical Sciences, IPB University, No. 279/KEH/SKE/XII/2024.

Experimental Animals Selection

The experimental animals were selected by physical examination and based on health condition, aged 1.5-2 years, had given birth once, ± weight 30–40 kg, and were not pregnant. Meanwhile, the buck was checked for its health condition and semen quality before being used as a male teaser.

Animals were kept in the same environment with standard feed and grass and drinking water *ad libitum*. Animals were adapted previously several weeks before treated.

Estrus Synchronization Treatment

Ewes were divided into three groups, with each group containing 5 heads. The first group was implanted with progesterone (Eazi-Breed™ CIDR® sheep and goat devices) for 13 days (Yasa et al. 2018). The second group was treated with a prostaglandin cloprostenol (Synchromate® 0.250 mg/ml, Bremer Pharma GMBH 34414 Warburg, Germany) double injection intramuscularly with an interval of 11 days (Besufkad et al. 2020), and the third group was treated a combination of CIDR® and Prostaglandin (cloprostenol 0.250 mg/ml). The CIDR implant was applied for 13 days and injected of prostaglandins at the CIDR removal (Figure 1, 2, and 3).

Observation of Estrus

Estrus was observed three times a day (Cox *et al.* 2024) starts from 06.00-07.00, 12.00-13.00, and 18.00-19.00 for five days (Roman *et al.* 2016) consecutively. The estrus detection was done by inserting a male teaser wearing an apron to observe the behavior of estrus, such as *standing heat* (Balan-may *et al.* 2021). An ewe showing standing heat indicated that the female was in estrus (Abu El-Ella *et al.* 2016). *Onset of estrus* was measured as an interval time after the CIDR removal (group 1), injection of the second prostaglandin (group 2), and injection of prostaglandins (group 3) until showing the estrus sign for the first time (Balan-may *et al.* 2021). *The duration of estrus* was calculated from the first estrus sign appearance to the end of estrus, characterized by the absence of signs of estrus (Mohan dan Kumar 2023).

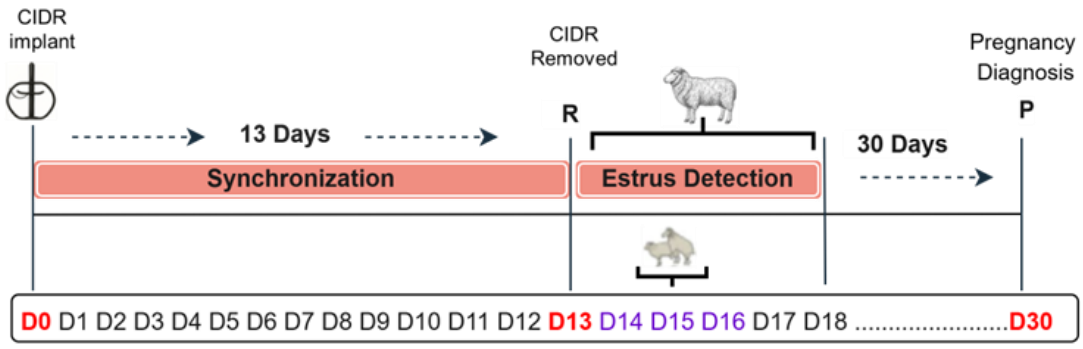

Figure 1. Progesterone (CIDR) Implant (Do (CIDR implant), D13 (CIDR Removed (R)), D13-D18 (Estrus detection), D14-D16 (mating), D30 (Pregnancy test (P))

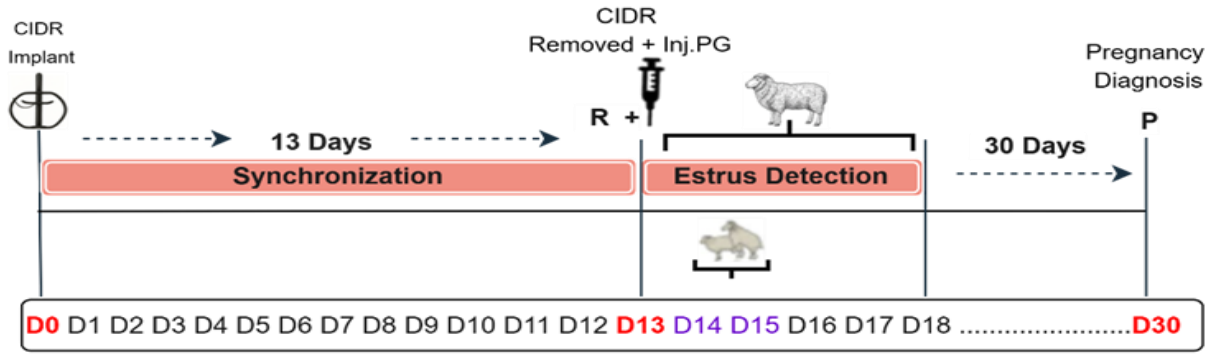

Figure 2. Combination of the hormone progesterone (CIDR) and prostaglandin (PGF2α) Do (CIDR implant), D13 (CIDR Removed (R+ PGF2α injection), D14-D18 (Estrus detection), D14-D15 (mating) and D30 (Pregnancy test (P))

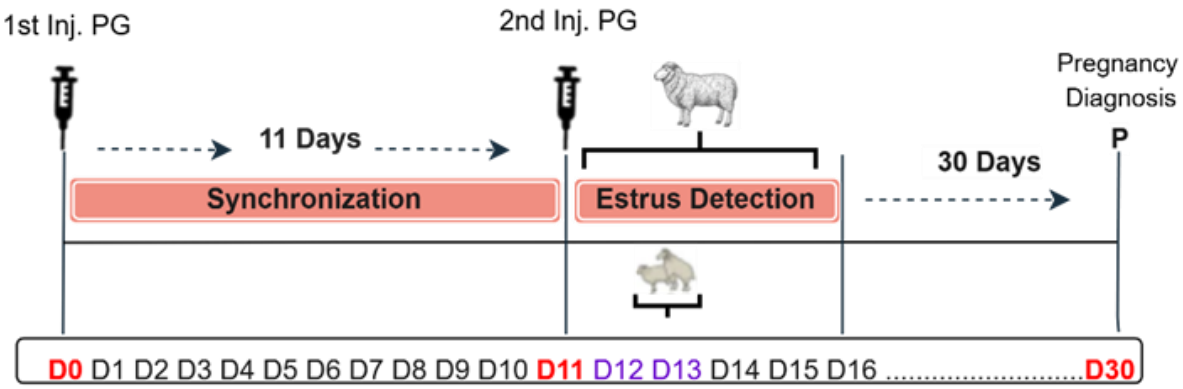

Figure 3. Prostaglandin (PGF2α) double injection (Do (1st injection), D11 (2nd injection), D12-D16 (Estrus Detection), D12-D13 (mating), D30 (Pregnancy test (P))

Measurement of Electrical Impedance of Vaginal Mucus

Electrical impedance was measured by inserting a probe of an estrus detector (*Draminski® Estrous Detector for sheep*) into an area of the vagina, and by measuring the electrical potential on the surface (Řezáč 2008). The tool was equipped with a probe, which has two electrodes at the end that are parallel and serve to measure the resistance value of the electric impedance of the estrus mucus, a screen, and a handle equipped with a standard 9-volt battery. The measurement was carried out by pressing the button behind the handle, after showing the line 2 signs that the tool can be used, then inserting a probe about ±15 cm from the vulva. Measurements were carried out twice at each examination.

Follicle Development Observation

Follicle development was observed using an ultrasound (Edan DUS 60 VET 100V-240V~50 Hz/60 Hz) equipped with a 5 MHz linear probe to measure the diameter of the dominant and subordinate follicles. Observations were conducted after the last injection of PGF_{2a} or the removal of the CIDR device. Observations were performed on both ovaries daily for five consecutive days. The diameter of the follicle was measured using an internal caliper on ultrasound. Follicle sizes were grouped based on their size, namely small follicles (2–3.4 mm in diameter), medium follicles (3.5–4.9 mm in diameter), and large follicles (≥ 5 mm in diameter), according to Brogni and Mezzalira (2024) with slight modifications.

Estrus Quality Parameters

1. Response of estrus (%): Percentage of sheep that showed symptoms of estrus among all experimental animals.
2. Onset of estrus: the interval from the last injection (cloprostenol) or withdrawal (CIDR) until the first sign of estrus
3. Duration of estrus: The time interval from the first signs of estrus to the absence of signs of estrus
4. Vaginal mucus electrical impedance (R) was obtained by measuring the voltage developed across the monitored tissue in response to sinusoidal electric current excitation
5. Follicle diameter: is the size of the follicle measured using an internal *caliper* with ultrasound

Data Analysis

The data on response of estrus, onset, duration of estrus, electrical impedance value of the vaginal mucus, number and size of follicles were tested for normality, and the results were shown as Mean±SD. *One-way ANOVA (Welch's)* and *Tukey Post Hoc Tests* were used to test the significance between treatments and individuals in a group. *T-tests* (*paired samples t-test*) were used for the analysis of follicular parameters. The significance confidence level was set at $p<0.05$.

RESULTS

Response, onset, and duration of estrus

Estrus synchronization treatment with CIDR, double injection with prostaglandins, and a combination CIDR+PGF2α resulted in maximum estrus response (100%) in all treated sheep. The estrus response, onset of estrus, and estrus duration for each treatment are presented in Table 1. The maximum estrus response in the group was in line with the results of a study in sheep (Yasa et al. 2018), which reported a 100% estrus response. This shows that CIDR is a suitable hormone for estrus synchronization in sheep.

Table 1. Response, onset, and duration of estrus

Treatment	n	Response estrus (%)	Onset of estrus (hours)	Duration of estrus (hours)
CIDR	5	100	54.0 ± 13.21	30.6 ± 4.93
CIDR + $PGF_2\alpha$	5	100	41.0± 12.81	29.8 ± 7.36
$PGF_2\alpha$ + $PGF_2\alpha$	5	100	49.4 ± 8.88	22.4 ± 5.81

Mean±SD: average onset and duration of estrus, Estrus response.

The fastest onset of estrus in this study was observed in the group of combination CIDR+$PGF_2\alpha$ (41 h), followed by the double injection $PGF_2\alpha$ group (49.4 h) and CIDR group (54 h). According to (Waqas dan Tibary 2025), onset of estrus on average lasts 36 hours (24-72 hours). These finding is supported by Omontese *et al.* (2010) ; Lima Meneses *et al.* (2024) That occurring estrus within 72 hours is classified as synchronized estrus. In addition, the duration of estrus was 30.6 h in the CIDR group (30.6 hours), CIDR+$PGF_2\alpha$ group (29.8 hours), while the double injection of $PGF_2\alpha$ group (22.4 hours), respectively.

Electrical Impedance of Vaginal Mucus

The electrical impedance of vaginal mucus is the resistance value of the electric current from the detector of estrus that passes through the vaginal mucus. Measurements were observed in three phases: before *standing heat*, during *standing heat*, and after *standing heat*. The data on electrical impedance are expressed as mean ± standard deviation (SD). The resistance patterns of the three treatments are presented in figure 4, 5, and 6.

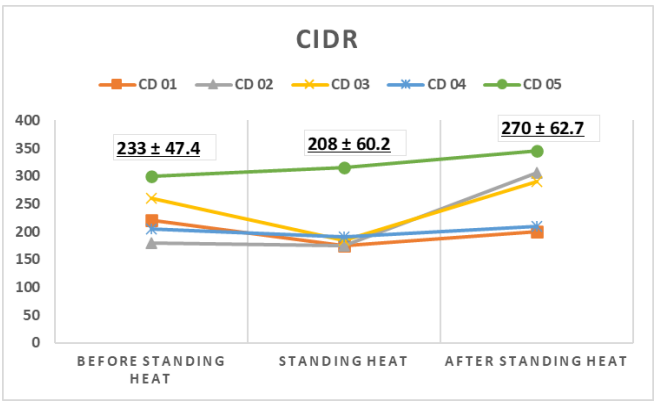

Figure 4. Vaginal mucus electric impedance in CIDR Group

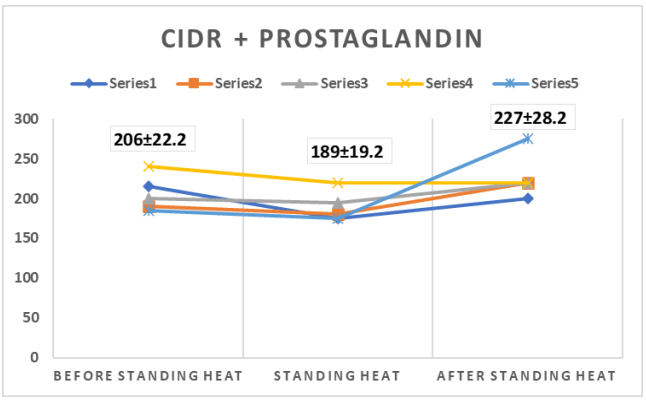

Figure 5. Vaginal Mucus Electrical Impedance CIDR + PGF2α Group

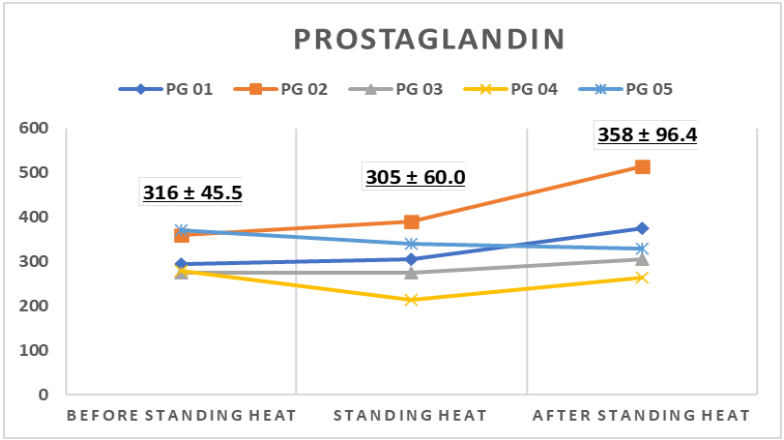

Figure 6. Vaginal Mucus Electrical Impedance in the Double Injection of PGF2α Group

The electrical resistance of vaginal mucus during *standing heat* was 208 Ω in the CIDR group, 189 Ω in the CIDR+PGF2α group, and 305 Ω in the double injection PGF2α group, respectively. CIDR, either alone or in combination with prostaglandins, showed lower values of electrical impedance of vaginal mucus than the double injection of prostaglandins.

Development of Follicles and Pregnancy

The size and number of follicles play an important role in producing estrogen hormone, which can cause estrus signs appearance. In this study, the number and size of follicles were calculated at the time of CIDR removal or the last injection with prostaglandins (H0), and at standing heat. Pregnancy diagnoses were done 30 days after mating. All the data are presented in Table 2.

Table 2. Number and size of follicles (H0) ~ *Standing heat,* Pregnancy

Treatment	n	Number of follicles		Follicle size		Pregnancy (%)
		H0 (Σ)	*Standing Heat* (Σ)	H0 (mm)	*Standing Heat* (mm)	
CIDR	5	2.60±1.34b	5.6±3.13a	2.5±0.73b	4.48±1.11a	5 (100)
CIDR+PGF2α	5	5.20±1.30	6.0±1.87	1.3±0.13b	3.76±0.38a	2 (40)
PGF2α+PGF2α	5	3.40±1.14	4.4±1.82	2.8±0.49b	4.24±0.64a	5 (100)

Remarks: Different superscript letters (a,b) on the same line show a significant difference between H0 and SH on the same treatment (independent *t-test*, $p<0.05$). *H0* (time of CIDR removed/last injection of prostaglandins). Superscript [a] (for large numbers), superscript [b] (for small numbers).

In general, the number of follicles increased after the last treatment until *standing heat* occurred. A significant ($p<0.05$) increase in follicle number was observed in the group treated with CIDR alone. Changes in follicle size during *standing heat* showed a significant ($p<0.05$) in all treatments. Successful pregnancy in synchronization with CIDR alone and double prostaglandin injection resulted in a 100% pregnancy rate (Table 2). Although the response to estrus showed 100% in synchronization with the combination of CIDR+$PGF_2\alpha$, the pregnancy rate was only 40% (Table 2).

DISCUSSION

Response, Onset, and Duration of Estrus

The progesterone method for synchronization mimics the conditions in the luteal phase, where implanted progesterone has a negative feedback effect on *the anterior pituitary*, which suppresses cyclical activity by inhibiting the release of gonadotropins. When the exogenous supply of progesterone is terminated at removal, the inhibition process ceases to occur, allowing cyclical activity to resume (Noakes *et al.* 2018), resulting in estrus. Meanwhile, estrus synchronization with double injection of PGF2α is based on prostaglandin F2α as the main of luteolytic factor in ruminants, (Abecia *et al.* 2012). PGF2α or its analogs only work on the functional *Corpus luteum* (López-Gatius 2022; Lima Meneses *et al.* 2024). Animals in the anestrus phase, or at the beginning or end of the luteal and follicular phases, do not respond to the exogenous administration of prostaglandins or their analogs (Abecia *et al.* 2012). It is therefore, not all females respond to a single injection of PGF2α, double injections are administered with an interval of 11 days (Somanjaya *et al.* 2025). Thus, in the second injection, all animals were in the mid-luteal phase, which were responsive to exogenous prostaglandin, thus causing estrus signs simultaneously. The estrus occurs is caused by the lysis of CL due to the action of PGF2α, which causes vasoconstriction in CL through the release of endothelin-1. Furthermore endothelin-1 to bind the receptors on the large luteal cell, triggering functional luteolytic, resulting in a decrease in progesterone levels (Waqas dan Tibary 2025). As a result, there is an increase in gonadotropin hormones (FSH and LH) along with the maturation of the de Graff follicle, which secretes large amounts of estradiol, triggering the onset of estrus and followed by LH surge that causes ovulation (Layek *et al.* 2013). This was shown by a 100% of estrus response. The higher estrus response (100%) also reported in Barbados Black Belly sheep using the same method (Mohan dan Kumar 2023). Combination of CIDR+PGF2α in this study also resulted a maximum estrus response (100%). It is known, CIDR (progesterone) can provide negative feedback to the anterior pituitary (Noakes *et al.* 2018) and, by PGF2a injection, can lyse the *Corpus luteum*, thus shortening the diestrus phase and then followed by new estrus cycle

(Lima Meneses *et al.* 2024), which is characterized by several estrus signs. The earliest onset of estrus was observed in the CIDR+$PGF_2\alpha$ group where the CIDR implanted mechanism was based on the luteal phase (13-14 days). When the CIDR implant was removed on day 13 and followed by prostaglandin injection then the concentration of progesterone began to decrease drastically. This decrease was related to increased estradiol secretion from the follicular waves, thus causing LH surge, preparing for the next follicular phase (Talebi *et al.* 2018). Exogenous prostaglandins accelerate the transition from the luteal phase to the follicular phase, so that the occurrence of estrus faster compared to CIDR without prostaglandins.

CIDR (progesterone) implanted for 13 days releases progesterone slowly, mimicking the luteal phase and suppressing estrus activity. Meanwhile, $PGF_2\alpha$ causes hypoxia in the *Corpus luteum,* which causes CL regression. Prostaglandins also interfere with the binding of the LH at the cell surface receptors, triggering estrus (Roy *et al.* 2014; Walker *et al.* 2025). The onset of estrus in combination on CIDR and prostaglandin group took faster than the onset of estrus in the CIDR group and double injection of prostaglandins. This is because the injection of the second prostaglandin soon stops the luteal phase so that the animal immediately enters estrus.

However, double injection of prostaglandin showed a late onset of estrus compared to the combination CIDR+PGF2α. It is presumably because the double injection of prostaglandins resulted in fewer follicles compared to the CIDR group (Table 2), so the onset of estrus was slightly slower.

The duration of estrus in the CIDR only and with prostaglandins lasted longer than in the double injections of prostaglandin. This is understandable because in the CIDR group, progesterone can stimulate follicle development (Table 2) better, so that the duration last longer. Meanwhile, in the double injection of the prostaglandin group, there is no special stimulation of follicle development, so that the duration of estrus was the shortest compared to the CIDR group. However, the duration of estrus in all treatments was still within the normal range, as stated by Mohan and Kumar (2023) that the duration of estrus ranges from 24-72 hours.

Electrical impedance of vaginal mucus

The electrical current resistance of vaginal mucus is highest in the luteal phase and lowest in the follicular phase (Řezáč 2008). The CIDR group resulted electrical impedance value was lower compared to the double injection of prostaglandin. It seems that CIDR can stimulate more vaginal mucus than double injection of prostaglandin. The abundance of mucus production in synchronization using CIDR is suspected that progesterone stimulate the numerous and development of follicles. Furthermore Verma *et al.* (2014) states that cervical secretion becomes more copious and watery during the estrogen dominating phase of the ovarian cycle, while the viscosity of mucus increases under high progesterone concentration. This data is evidenced by the lowest value of vaginal mucus electrical impedance during estrus, as reported by Olawole *et al.* (2024).

Furthermore, cervical secretion is mostly composed of water, lipids, cholesterol, carbohydrates, proteins and ions, (Huang *et al.* 2024). The higher water and ion content in the mucus causes lower electric impedance (Verma *et al.* 2014). This situation can be one of the indicators in determining the optimal time for insemination and natural mating.

Contrary to the above-mentioned matter, the prostaglandin group showed the electrical impedance of the vaginal mucus was higher value compared to CIDR group. This is suspected because prostaglandin does not stimulate abundant mucus. This data is supported by Yu *et al.* (2022) that double injection of prostaglandins does not stimulate the formation of more follicles but only lyse the CL.

Development Follicles and Pregnancy

The increase in the number of follicles in the CIDR group alone was higher than prostaglandin group as shown in the Table 2. This is suspected to be related to the role of progesterone which stimulate follicles according to Walker *et al.* (2025).

Meanwhile, in the CIDR group combined with prostaglandins, there was no significant increase in the number of follicles at standing heat. This is likely due to the fact that at the time of removal, the follicle growth is immediately stopped by the injection of prostaglandins as a luteolytic agent (Fierro *et al.* 2013). A further insignificant increase in the number of follicles also occurred in the prostaglandin double injection group. This is understandable because prostaglandin injections do not cause an increase in the number of follicles, but follow a normal cycle (Talebi *et al.* 2018). However, the presence of follicles is influenced by the status of the individual (Spanner *et al.* 2024).

The follicle diameter at *standing heat* showed a significant greater than at the last treatment time (H_0) in all groups. According to Cox *et al.* (2024), large follicles in sheep that are capable of ovulating are follicles with ≥4.0 mm in diameter. This was demonstrated by the CIDR group and double injection of prostaglandins with follicle diameter >4.0 mm, resulted 100% pregnancy. Contrary to the opinion, Fernandez *et al.* (2018) stated that follicles above 2 mm in sheep have the potential to ovulate. It is proven by the results of combination CIDR and prostaglandins that resulted in pregnancy, even though it is only 40%.

The estrus response to the three treatment methods resulted in a maximum response (100%), but the success of pregnancy in the CIDR+$PGF_2\alpha$ combination group was only 40%. There are various allegations of pregnancy failure in this group. First, this group has a follicle diameter when *standing heat* smaller than the other group at 3.76 mm. The second suspicion of low fertility rates in this group may be related to the status of animals that previously had pseudopregnancy cases. According to (Almushawwah *et al.* 2025) pseudo-pregnant Animals are capable to produce the large follicles showed estrus sign, but the follicles are unable to ovulate.

In summary, it can be concluded that the estrus response rate from the method of CIDR alone, CIDR+PGF2α, and double injection with prostaglandins could induce optimum response estrus, with differences on the onset and duration. The lowest electrical impedance vaginal mucus was obtained in the group of CIDR combined with prostaglandins. Furthermore, the third synchronization treatments could improve the number and diameter of follicles at *standing heat* and support a twin pregnancy rate.

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