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Vaginal Cytological Changes and Estrus Response Following Estrus Synchronization Using a Double Prostaglandin-$F_2\alpha$ and Gonadotropin Releasing Hormone in Pasundan Heifers

(*PERUBAHAN SITOLOGI VAGINA DAN RESPONS BERAHI PASCA-SINKRONISASI BERAHI MENGGUNAKAN PROSTAGLANDIN-$F_2\alpha$ GANDA DAN GONADOTROPIN RELEASING HORMON PADA SAPI DARA PASUNDAN*)

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ABSTRACT

Pasundan cattle, an indigenous breed native to West Java, Indonesia, demonstrate significant adaptability and reproductive potential. However, their declining populations pose a threat to genetic conservation. Effective estrus synchronization is essential for enhancing reproductive efficiency and supporting fixed-time artificial insemination (FTAI) programs. Vaginal cytology profiling offers a reliable, noninvasive method for assessing estrus by evaluating the dynamics of epithelial and leukocyte cells, which reflect hormonal fluctuations. This study was aimed to investigate the cytological dynamics of vaginal epithelial, leukocyte cells and estrus response in Pasundan heifers subjected to double injection of prostaglandin $F_2\alpha$ ($PGF_2\alpha$) and gonadotropin-releasing hormone (GnRH) synchronization. Nine Pasundan heifers, aged 1.5–2.0 years, were treated with $PGF_2\alpha$ with 2.75 μg/kg BW on days 0 and 11, followed by 2 μg/kg BW GnRH on day 12. Vaginal smears were collected daily from days 0 to 15, stained with 5% Giemsa, and examined microscopically to determine the proportions of parabasal, intermediate, superficial, keratinized, and leukocyte cells. The results indicated that the initial cytology showed a luteal phase dominated by parabasal (31.2%) and intermediate (30.6%) cells. Following hormonal treatment, superficial cells peaked at 70.9% on day 13, indicating estrus and

estrogen dominance. By days 14–15, keratinized and parabasal cells had increased, confirming post-ovulatory luteal activity. The protocol achieved complete (100%) estrus synchronization among all heifers. It can be concluded that the double PGF_2 α-GnRH protocol effectively synchronized estrus in Pasundan heifers. Vaginal cytology has proven to be a sensitive and practical tool for monitoring reproductive phases, thereby supporting breeding management and conservation of this native genetic resource.

Keyword: Pasundan cattle; estrus synchronization; $PGF_2\alpha$–GnRH protocol; vaginal cytology

ABSTRAK

Sapi pasundan, sapi lokal asal Jawa Barat, Indonesia, memiliki daya adaptasi dan potensi reproduksi yang tinggi. Namun, populasinya yang menurun mengancam kelestarian genetiknya. Sinkronisasi berahi (estrus) sangat penting untuk meningkatkan efisiensi reproduksi dan mendukung program *fixed-time artificial insemination* (FTAI). Penelitian ini bertujuan untuk mengevaluasi profil sitologi vagina, leukosit dan respons berahi sapi pasundan yang diberi perlakuan sinkronisasi injeksi ganda prostaglandin $F_2\alpha$ ($PGF_2\alpha$) dan *gonadotropin-releasing hormone* (GnRH). Sembilan ekor sapi psundan (umur 1,5–2,0 tahun) diberi dua kali injeksi $PGF_2\alpha$ (2.75 μg/kgBB pada hari ke-0 dan ke-11 diikuti 2 μg/kgBB GnRH pada hari ke-12. Sediaan apus vagina dikumpulkan setiap hari (hari ke-0 hingga ke-15), diwarnai dengan pewarna Giemsa 5% kemudian diamati di bawah mikroskop cahaya untuk menghitung proporsi sel parabasal, intermediet, superfisial, keratinisasi dan leukosit. Sitologi awal menunjukkan fase luteal yang didominasi sel parabasal (31,2%) dan intermediet (30,6%). Setelah perlakuan hormonal, sel superfisial mencapai puncak 70,9% pada hari ke-13 yang menandakan sapi sedang berahi dan dominasi estrogen. Pada hari ke-14 hingga 15 terdapat peningkatan sel keratinisasi dan parabasal akibat aktivitas luteal pascaovulasi. Protokol ini berhasil mencapai sinkronisasi estrus 100%. Protokol ganda $PGF_2\alpha$-GnRH efektif mensinkronkan berahi pada sapi pasundan dan sitologi vagina terbukti menjadi alat sensitif dan praktis untuk memantau fase reproduksi serta mendukung peningkatan manajemen reproduksi dan konservasi genetik sapi pasundan.

Kata-kata kunci: sapi pasundan; sinkronisasi estrus; protokol $PGF_2\alpha$–GnRH, sitologi vagina

INTRODUCTION

Pasundan cattle, native to West Java, Indonesia, represent a vital local genetic resource, esteemed for their adaptability to tropical climates and their notable reproductive capacity (Setiawati *et al.*, 2018). Traditionally managed within smallholder systems, cattle play a crucial role in supporting rural livelihoods and ensuring regional food security. Nevertheless, their population has experienced a decline with research by Arifin dan Sulasmi (2019) and Statistic Indonesia (BPS, 2024) indicating a 7.45% decrease in 2024. This decline underscores the urgent need to preserve genetic diversity, prevent inbreeding and enhance reproductive efficiency to ensure the long-term sustainability of the breed

Assisted reproductive technologies (ART) present a potent solution to these challenges by enhancing reproductive performance and bolstering genetic conservation initiatives (Widyastuti *et al*., 2021). Among these, estrus synchronization has emerged as a pivotal technique for regulating and optimizing breeding schedules through fixed-time artificial insemination/FTAI (Richardson *et al*., 2016). Research indicates that a double-injection prostaglandin F2α (PGF2α)

protocol effectively synchronizes estrus in Pasundan heifers, with day 14 identified as optimal for FTAI (Widyastuti *et al*., 2025). The combined application of PGF2α and gonadotropin-releasing hormone (GnRH) further refines synchronization accuracy: The PGF2α induces luteolysis of the corpus luteum, whereas GnRH stimulates the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), thereby facilitating follicular development and ovulation (Hassanein *et al*., 2024). However, successful estrus synchronization hinges on the precise identification of estrus. Therefore, it needs a technology to detect the estrus during this procedure.

Vaginal cytology is a reliable, non-invasive diagnostic method for assessing estrous cycle stages based on epithelial and leukocyte cell patterns (Cora *et al*., 2015). This technique offers valuable insights into hormonal fluctuations by evaluating the proportion of parabasal, intermediate, superficial and keratinized epithelial cells. Although its efficacy has been demonstrated in various species including cows, ewes and dogs (Ummaisyah *et al*., 2020), information on vaginal epithelial dynamics in Pasundan heifer remains limited. Given the unique physiological characteristics of Pasundan heifers (Setiawati *et al.*, 2018), This study was aimed to observe the estrous cycle response of Pasundan heifers through vaginal cytology assessment following synchronization using double $PGF_2\alpha$ injection combined with GnRH. These findings are expected to provide a cytological reference for evaluating estrus expression in Pasundan cattle and to support future reproductive management and conservation effort

RESEARCH METHODS

Animal and Ethical Approval

This study was conducted on nine clinically healthy Pasundan heifers, aged between 1.5 and 2.0 years, with body condition scores (BCS) ranging from 2.5-3.0. Animals were individually housed at the Beef Cattle Teaching Farm, Faculty of Animal Husbandry, Universitas Padjadjaran. Prior to the initiation of the estrus synchronization program, all heifers underwent a two-week acclimatization period to ensure adaptation to the management and feeding conditions. The experiment was conducted between January and February 2024 and laboratory analyses were performed at the Reproduction Laboratory in March 2024. All experimental procedures involving animals adhered to institutional ethical guidelines and were approved by the University Padjadjaran Health Research Ethics Committee (approval no. 1398/UN6.KEP/EC/2023).

Estrus Synchronization Protocol

The estrus synchronization protocol was designed to regulate luteolysis and ovulation using a combined hormonal approach. Each heifer received two intramuscular injections of prostaglandin F2α (Synchromate®, Bremer Pharma GmbH, Warburg, Germany) 0.25 mg/mL Cloprostenol sodium at a dose of with 2.75 μg/kg BW on days 0 and 11 to induce corpus luteum regression. This was followed by a single intramuscular injection of 2 μg/kgBW gonadotropin-releasing hormone/GnRH (Gonadorelin®, Veyx-Pharma GmbH, Schwarzenborn, Germany) per head on day 12 to stimulate ovulation.

Cervical Mucus Collection and Vaginal Cytology Evaluation

Vaginal smears were obtained daily during the 15-day observation period using sterile cotton swabs that were gently inserted into the vaginal canal. Each swab was rolled onto a methanol-treated glass slide to transfer cells before staining. The Romanowski method was employed using Giemsa staining for 10–15 min, followed by rinsing with distilled water and air-drying. Microscopic examination was conducted at 400× magnification and 100 epithelial cells were counted per sample to determine the proportion of each cell type. The cells were classified as parabasal, intermediate, superficial or cornified and their percentages were calculated to characterize the cytological profile of the vaginal mucosa (Widyastuti *et al.*, 2025).

Morphological changes in the epithelial and leukocyte populations were analyzed to describe the progression of estrous cycle phases, which were categorized as follows: proestrus (dominance of intermediate cells), estrus (predominance of superficial and keratinized cells), metestrus (decrease in superficial cells with an increase in leukocytes) and diestrus (presence of parabasal and keratinized cells) (Bayani *et al*., 2024)

Data Analysis

The data obtained from cytological observations were expressed as percentages. Descriptive analysis was performed to evaluate the temporal patterns of epithelial and leukocyte cell composition across individuals and to determine the cytological dynamics associated with each phase of the estrous cycle during the synchronization protocol.

RESULTS AND DISCUSSION

The cytological and reproductive changes observed during the synchronization period provide mechanistic insights into the endocrine pathways activated by the double injection $PGF_2\alpha$–GnRH protocol in Pasundan heifers. Continuous daily monitoring of vaginal epithelial smears revealed distinct temporal changes in cell composition that closely corresponded with the physiological progression of the estrus cycle (Prastowo *et al*., 2020). These cytological variations, reflected in the shifting proportions of parabasal, intermediate, superficial and keratinized cells, facilitated the precise identification of reproductive phases and offered a clear indication of the underlying endocrine environment. As expected, proestrus smears exhibited a mixed population of parabasal, intermediate and superficial cells, with parabasal cells remaining relatively abundant and intermediate cells increasing as epithelial maturation progressed. Small clusters of leukocytes were occasionally observed. During estrus, superficial and keratinized cells predominated, whereas intermediate and parabasal cells were scarce and leukocytes were largely absent. Advancement into metestrus was marked by the reappearance of parabasal and intermediate layers as superficial strata were shed, accompanied by a notable influx of leukocytes. Diestrus smears were dominated by parabasal and intermediate cells, with markedly reduced superficial cells populations, whereas leukocytes persisted at lower levels than those observed in metestrus (Figure 1).

These characteristic cytological profiles provided a reference against which temporal patterns during synchronization can be interpreted. On day 0, the smears consisted mainly of parabasal (31.2%) and intermediate (30.6%) cells, reflecting the initial distribution of diestrus and proestrus phases among heifers. After the first $PGF_2\alpha$ injecttion, a rapid shift occurred on days 1 and 2, characterized by steep reductions in parabasal (to 9%) and leukocyte (1.2%) populations and a pronounced increase in superficial (43.4%) and keratinized (6.8%) cells. By day 3, superficial cells predo-minated (44.8%), keratinized cells increased further (28.8%) and parabasal cells con-tinued to decline (13.9%). Between days 4 and 6, keratinized cells reached their highest value (48.8%), whereas superficial and intermediate cells fluctuated moderately. As synchronization progressed, the epithelial composition shifted again, with parabasal cells gradually re-emerging between days 7 and 11. By day 11, before the second $PGF_2\alpha$ injection, parabasal cells accounted for 51.8% of all cells, indicating a synchronized luteal phase. Following GnRH administration on day 12, a marked alteration in epithelial distribution occurred, reflected by a sharp increase in superficial cells to 70.9% on day 13. During the final observation period (days 14–15), keratinized cells rose to 56%, superficial cells declined to 5.8% and leukocytes increased to 2.2%, completing the cyclical pattern of remodeling expected

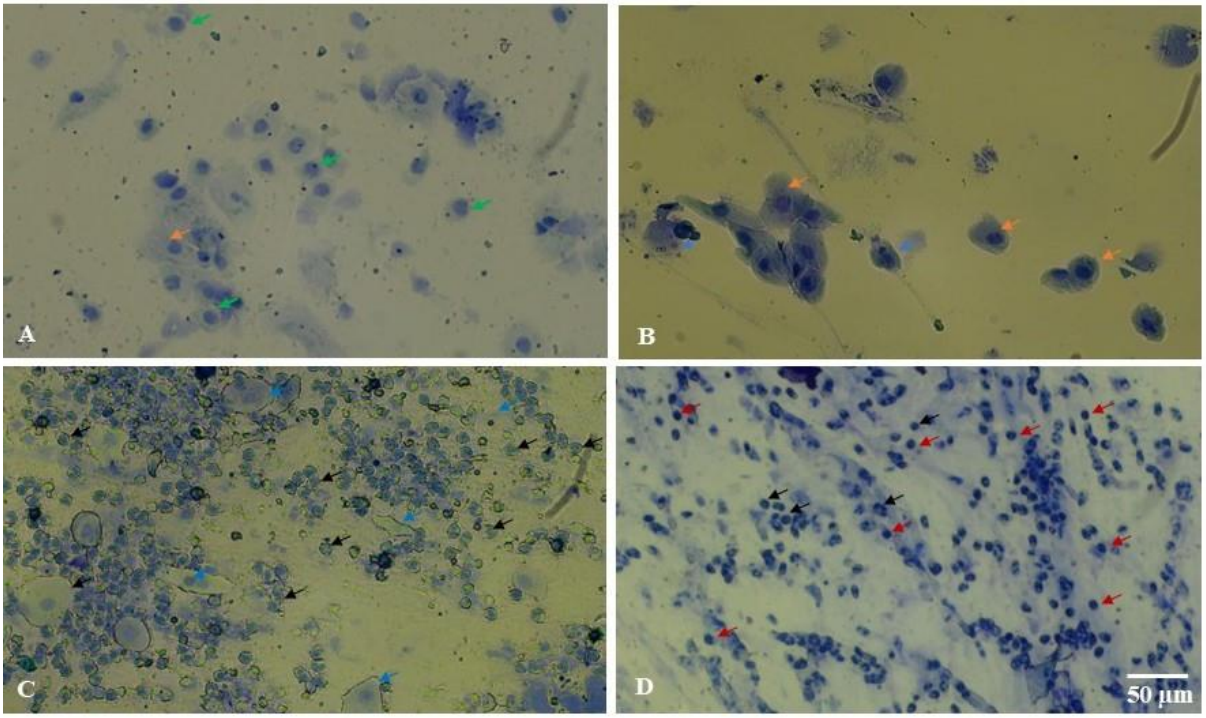

Figure 1. Mucosal Epithelial Vagina and leucocytes profile of Pasundan heifer during estrus cycle During Estrus Synchronization Using Double Injection PGF2α and GnRh. (A) pro estrus (B) estrus, (C) metestrus, (D) diestrus. Epithelial cells at parabasal, round, small cells with a large nucleus (red arrow), Intermediate cells, large round cells with a large nucleus (green arrow), Superficial cells, polygonal cells with a large nucleus (orange arrow) keratin cells, polygonal cells with a small or no nucleus (blue arrow), leucocyte (black arrow).

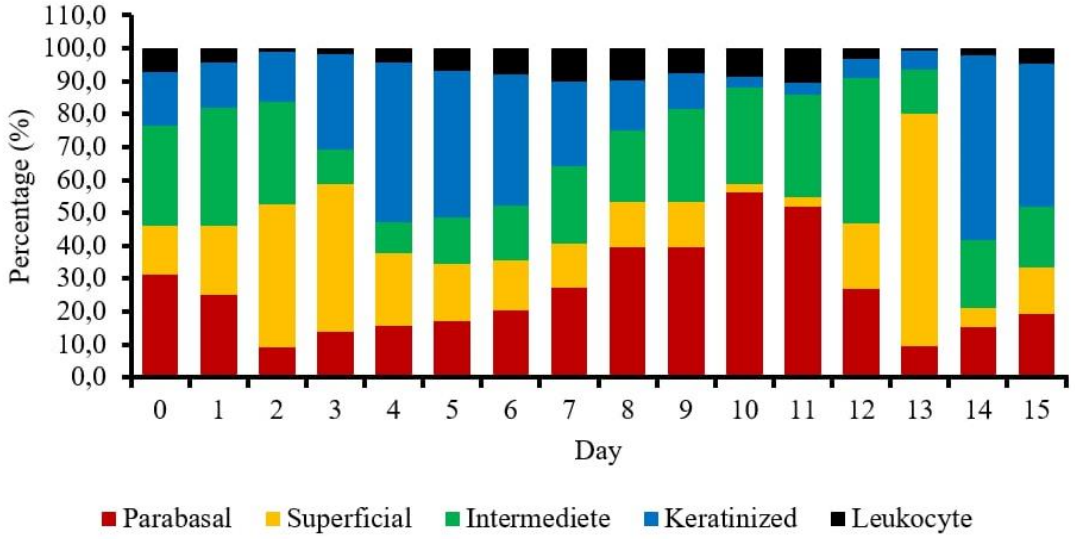

Figure 2. Mean Percentage of Vaginal Mucosal Epithelial and Leukocyte Cells During Estrus Synchronization Using Double Injection PGF2α and GnRh.

across the synchronization protocol (Figure 2).

The temporal progression of the estrous stages displayed a pattern consistent with the cytological findings (Figure 3). On day 0, four heifers were in the diestrus phase and five were in the proestrus phase, reflecting natural asynchrony. However, between day 1 and 3, most heifers transitioned into proestrus and estrus, indicating rapid luteolysis and follicular activation following $PGF_2\alpha$ injection. Between day 4 and 7, the

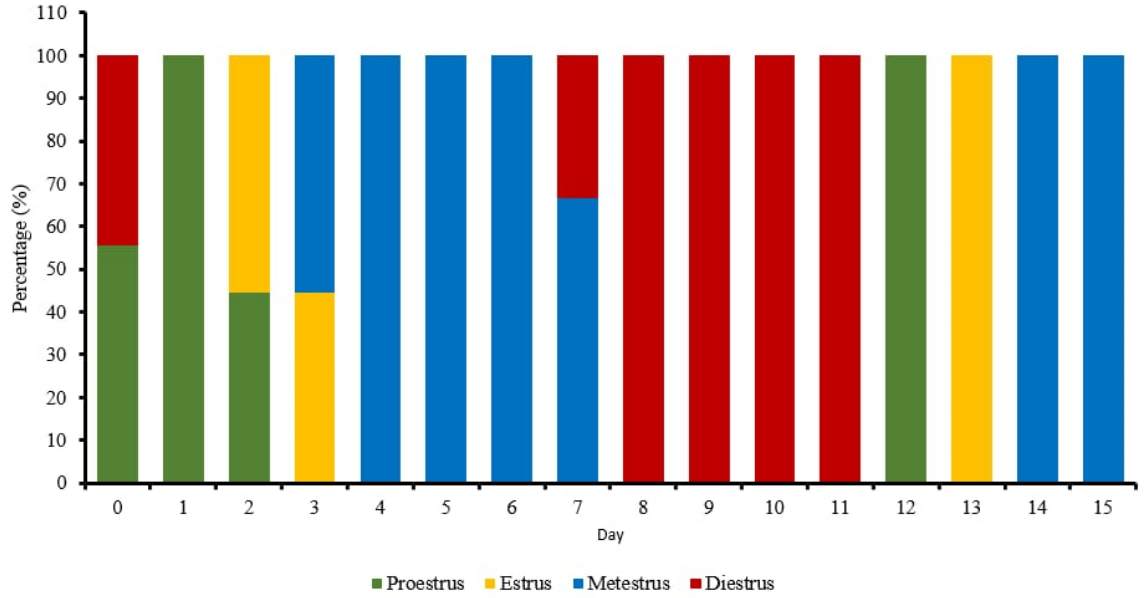

Figure 3. Estrus Cycle Phases During Estrus Synchronisation Using Double Injection PGF2α and GnRh.

majority progressed to metestrus, with a corresponding decline in the number of animals in estrus. All heifers uniformly entered diestrus between day 8 and 11, confirming strong synchronization of luteal activity before the second $PGF_2\alpha$ injection. After the second injection, the estrous phases shifted again; all heifers entered proestrus on day 12, expressed estrus on day 13, and transitioned into metestrus on Days 14–15, demonstrating the efficacy of the protocol in generating synchronized reproductive events.

These sequential cytological changes reflect the endocrine mechanisms underlying synchronization, consistent with the epithetlial remodeling described during luteolysis, follicular emergence and preovulatory maturation (Nagyová *et al.*, 2021; Abedel-Majed *et al*., 2019). The sharp decline in the parabasal and leukocyte populations between day 1 and 2 indicated effective luteolysis and progesterone withdrawal. The previous research reported that $PGF_2\alpha$ induces luteolysis within 48–72 h, supporting the estrus peak observed in this study (Setyawati, 2020). Moreover, $PGF_2\alpha$ facilitates corpus luteum regression by reducing luteal blood flow, inhibiting progesterone synthesis and inducing apoptosis of luteal cells (Monaco and Davis, 2023). The resulting decline in progesterone removes the inhibition on the hypothalamic–pituitary axis (Wen *et al.*, 2020), promoting GnRH secretion and the release of FSH and LH (Motta *et al*., 2020), which stimulate follicular growth (Monaco and Davis, 2023).

By day 3, the predominance of superficial cells signified an estrogen surge produced by the developing dominant follicles. The increase in superficial and keratinized cells from days 2 to 4 corresponds with estradiol secretion; estradiol promotes epithelial proliferation (Hewitt *et al.*, 2016), stratification and keratinization via the estrogen receptor alpha (ERα) pathway, as well as glycogen deposition and increased epithelial thickness (Wan *et al*., 2022), which create optimal conditions for sperm transport (Schulster *et al.*, 2016). The peak in keratinized cells observed on days 4–6 reflects maximal estrogen exposure during late proestrus, in line with the findings of Foeh *et al*. (2019) on estrogen-induced epithelial maturation in ruminants.

Between days 7 and 11, the gradual reappearance of parabasal cells reflected the establishment of a progesterone-dominated luteal phase following ovulation. The peak

proportion of parabasal cells on day 11 indicated strong synchronization of corpus luteum function across the group. GnRH administration on day 12 triggered a surge in LH levels, which is essential for final follicular maturation, meiosis resumption and ovulation. The sharp increase in superficial cells on day 13 corresponded with periovulatory estradiol secretion from the preovulatory follicle, reflecting precise hormonal coordination. By day 14–15, the rise in keratinized cells, decline in superficial cells, and increased leukocyte infiltration signify early post-ovulatory remodeling, consistent with the inflammatory-like response follo-wing follicular rupture (Monaco and Davis, 2023). Leukocyte recruitment, mediated by prostaglandins, facilitates tissue repair and epithelial restoration (Li *et al*., 2018).

Synchronization of estrus timing confirmed the physiological effectiveness of the protocol. The uniform expression of estrus on day 13 aligned with a synchronized preovulatory LH surge, allowing insemination to be timed 12–18 h after estrus onset or 16–20 h post-GnRH, matching the expected ovulation window of 24–32 h after the LH surge. These findings are consistent with those of Fauzi *et al*. (2017) and Handarini *et al*. (2017), who observed improved estrus uniformity and conception rates in cattle using combined $PGF_2\alpha$–GnRH strategies. Such alignment is particularly advantageous under smallholder field conditions, where predictable estrus expression facilitates efficient reproductive management.

Taken together, the integration of cytological dynamics, estrous phase transitions and endocrine responses highlights the effectiveness of the double $PGF_2\alpha$–GnRH protocol in eliciting a consistent reproductive response in Pasundan heifers. The strong correlation between epithelial cell remodeling and hormonal activity confirms that vaginal cytology is a reliable, noninvasive indicator of reproductive status during synchronization. Incorporating cytological evaluation alongside behavioral and hor-monal indicators may further enhance estrus detection accuracy and support more effi-cient reproductive scheduling in tropical, smallholder system

CONCLUSION

The implementation of an estrus synchronization protocol utilizing a dual $PGF_2\alpha$–GnRH regimen resulted in a consistent estrus response among Pasundan heifers, with all subjects uniformly exhibiting estrus on day 13. The presence of superficial cells during estrus and parabasal and keratinized cells during metestrus confirmed the hormonal patterns of luteolysis, estrogen elevation and corpus luteum regeneration.

SUGGESTION

To enhance the correlation between cytological and endocrine dynamics, future research should incorporate hormonal assays such as progesterone and estradiol profiling. Increasing the sample size across diverse age groups would improve the representativeness of our findings. The reproductive efficiency of this synchronization protocol should be assessed under field conditions using fixed-time artificial insemination. The integration of advanced reproductive technologies can yield deeper insights into the physiological responses. These efforts will contribute to refining reproductive management strategies and supporting the genetic conservation of Pasundan cattle, a crucial resource for livestock productivity in Indonesia.

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