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The Influence of Parity on Blood Metabolite Profiles and Reproductive Performance in Saanen-Etawah Crossbred Does

Dio Fico Felsidan Diatmono[1], Stefani Winda Paramita[1], Fransisca Gani Padmawati[1], Pradita Iustitia Sitaresmi[2], Seraphina Kumala[1], Megawati Andi[1], Budi Prasetyo Widyobroto[3], Yustina Yuni Suranindyah[3], Joana da Costa Freitas[4], Diah Tri Widayati[1]*

[1]Department of Animal Breeding and Reproduction, Faculty of Animal Science, Universitas Gadjah Mada, 55281 Sleman, Yogyakarta, Indonesia
[2]Research Center for Animal Husbandry, Cibinong Science Center, National Research and Innovation Agency, 16915 Bogor, Jawa Barat, Indonesia
[3]Department of Animal Production, Faculty of Animal Science, Universitas Gadjah Mada, 55281 Sleman, Yogyakarta, Indonesia
[4]Department of Animal Science, Faculty of Agriculture, Universidade Nacional Timor Lorosa'e, 10000 Dili, Dili, Timor-Leste

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* Corresponding author:
E-mail: widayati@ugm.ac.id

ABSTRACT

This study aimed to observe the effect of parity on the blood metabolite profiles and reproductive performance of Saanen-Etawah Crossbred does, maintained in smallholder farm. A total of 40 non-pregnant lactating does were divided into two groups: primiparous (n=16) and multiparous (n=24). The blood metabolite profiles, including total protein, glucose, cholesterol, and blood urea nitrogen (BUN), were assessed. Blood samples were collected from the jugular vein during the follicular phase, two hours prior morning feeding. Analysis of blood metabolites was carried out using photometric atomic absorption spectroscopy (AAS)-flame procedure. Furthermore, reproductive performance was observed through observing the characteristics of estrus and the length of the estrus cycle. The results indicated total protein in blood were significantly higher ($p<0.05$) in multiparous does (7.04±0.87 g/dL) compared to primiparous does (5.47±1.40 g/dL). In contrast, primiparous does exhibited significantly higher ($p<0.05$) blood cholesterol levels (122.33±30.55 mg/dL) than multiparous does (107.14±13.45 mg/dL). Multiparous does displayed typical estrus characteristics, including vulvar reddening, swelling, and vaginal mucus production, alongside a significantly shorter estrus cycle length. Parity was positively correlated with total protein levels (r=0.577), vulvar reddening (r=0.355), vulvar swelling (r=0.343), and vaginal mucus production (r=0.450), while negatively correlated with cholesterol levels (r=-0.330) and estrus cycle length (r=-0.532). These findings indicate that parity influences total protein and cholesterol levels, as well as several key estrus characteristics. Further investigation into optimized feed regimens and reproductive hormone fluctuations is warranted to enhance the reproductive efficiency of Saanen-Etawah Crossbred does.

Keywords: *Blood metabolites, Dairy goat, Parity, Reproductive performance, Saanen-Etawah Crossbred*

Introduction

Several goat breeds are valuable dual-purpose ruminants, known for producing both high-quality meat and milk. However, goat milk production in Indonesia remains relatively low and currently does not provide sufficient milk for domestic demand (Sumarmono, 2022). This problem arises from the predominantly traditional management of dairy goat farming in Indonesia, which faces several obstacles (Kumala *et al*., 2022). These obstacles include small dairy goat populations, limited information on production and business centers, complications in breeding programs, cultural preferences, and the financial accessibility of goat milk (Kumala *et al*., 2022; Suranindyah *et al*., 2018). Regardless of these challenges and limitations, goat milk production in Indonesia has significant potential to be optimized. All this is because goat milk provides potential health benefits in conjunction with a greater market price compared to cow's milk (Sumarmono, 2022). A particular one of the most commonly developed dairy goat breeds in Indonesia is the Saanen-Etawah Crossbred goat, which is known for its adaptability to tropical environments and relatively high milk quantity and quality (Widayati *et al*., 2024; Sumarmono, 2022; Suranindyah *et al*., 2018). The

potential of Saanen-Etawah Crossbred goats in Indonesia can be improved through increased reproductive efficiency.

Reproductive efficiency aims to enhance both milk production and the productivity of does. This efficiency is believed to be closely linked to the blood metabolite profiles of does and their reproductive performance during estrus (Diatmono *et al*., 2024a; Sitaresmi *et al*., 2023). Dairy goat's reproductive efficiency is heavily impacted by its blood metabolite profile, maintaining optimal levels of key metabolites is essential for normal reproductive hormonal mechanisms, supporting successful reproduction, and overall health (Sitaresmi *et al*., 2023; Kumala *et al*., 2022). This aims to avert a reduction in reproductive performance and doe productivity (Sitaresmi *et al*., 2020). Dairy goat's reproductive performance also directly impacts its overall breeding efficiency, as strong reproductive metrics including conception rate (CR), service per conception (S/C), and kidding interval (KI) are crucial for efficient breeding cycles and increased productivity (Magistrama *et al*., 2024; Suyadi *et al*., 2021). These reproductive performance indicators are closely related to the intensity of estrus symptoms (Diatmono *et al*., 2024b). Clear estrus signs in dairy goats are generally linked to strong reproductive outcomes, reflecting a healthy, fertile estrus cycle essential for successful breeding and conception (Diatmono *et al*., 2024b; Kumala *et al*., 2021).

The blood metabolite profiles and reproductive performance of does are strongly suspected to be closely related to their parity. Parity, which reflects the number of parturitions or births a doe has had, is an important indicator in her reproductive history (Ralević *et al*., 2021). Classifying does as primiparous (having given birth once) or multiparous (having given birth more than once) can provide insight into their physical maturity and metabolic profile, which can influence their reproductive performance and productivity (Diatmono *et al*., 2024b; Ralević *et al*., 2021). Increased parity, a consequence of aging in does, correlates with changes in their reproductive performance and milk yields (Briggs *et al*., 2023; Kumala *et al*., 2022). Beyond this, previous study indicates that multiparous cows are known to produce a higher quantity of milk compared to primiparous cows (Walter *et al*., 2022). Several previous studies have investigated the influence of parity on the blood metabolite profile of dairy goats (Kumala *et al*., 2022), milk production in Saanen goats (Ralević *et al*., 2021), and the productivity of the local Nigerian goats (Briggs *et al*., 2023). However, study exploring the correlative relationship between parity with the blood metabolite profile and reproductive performance of does has not yet been conducted. Given this, the current study is essential, and its findings can serve as a basis for evaluating husbandry practices, reproductive management, and doe selection to enhance the reproductive efficiency of Saanen-Etawah Crossbred does in smallholder farms.

Materials and Methods

Study period and location

This study was conducted from July to December 2023 at Sahabat Ternak Farm, a dairy goat farm located in Girikerto Village, Turi District, Sleman Regency, Special Region of Yogyakarta, Indonesia (7°36'24.7"S 110°24'12.4"E). Blood sample preparation, vaginal epithelial cell cytology observation, and salivary ferning observation were performed at the Laboratory of Animal Physiology and Reproduction, Faculty of Animal Science. Meanwhile, blood metabolite analysis was carried out at the Integrated Laboratory for Research and Testing (LPPT), Universitas Gadjah Mada.

Experimental animals and feeding regimen

This study used 40 non-pregnant lactating Saanen-Etawah Crossbred does, which were grouped by parity into primiparous (n=16) and multiparous (n=24) individuals. All does were housed in individual pens, received no estrus synchronization treatment, and were not mixed with bucks. Feed was provided twice daily, in the morning (08:00 WIB) and afternoon (14:00 WIB). The entire sample of does was provided with the same dietary composition and amount. The diet consisted of 1000 grams commercial concentrate, 1000 grams dried water spinach (*Ipomoea aquatica*), and 500 grams soybean hulls (*Glycine max*), with *ad libitum* drinking water. Feed ingredients were proximately analyzed to determine the nutrient composition of the diet provided by the farmer (AOAC, 2005). Daily feed intake was monitored by subtracting the refusal from the amount offered. The nutrient composition of the diet provided by the farmer is presented in Table 1.

Table 1. The proportion of feed ingredients and total nutrient composition of farmer-provided feed

Feed Proportions and Nutrient Compositions	Daily Feed Offered (per head per day)
Feed proportions (%)	
Commercial concentrate	40.00
Dried water spinach (*Ipomoea aquatica*)	40.00
Soybean hulls (*Glycine max*)	20.00
Total nutrient compositions (%)	
Dry matter (DM)	75.74
Ash	10.74
Crude protein (CP)	19.40
Ether extract (EE)	4.08
Crude fiber (CF)	22.81
Nitrogen-free extract (NFE)	42.96

Total digestible nutrient (TDN)	64.32

Blood sampling and preparation

Blood samples were collected during the follicular phase, two hours prior to morning feeding, via the jugular vein. Samples were drawn using a needle, holder tube, and approximately 6 mL of ethylenediaminetetraacetic acid (EDTA) tubes. Blood samples were centrifuged at 3,000 rpm for 15 minutes to separate plasma from other blood components. The blood plasma was aliquoted into four microcentrifuge tubes and stored at -20°C until the analysis of blood metabolite profiles was conducted (Diatmono *et al*., 2024a; Kumala *et al*., 2022).

Blood metabolite analysis

Doe plasma samples were analyzed using photometric procedures. Specifically, total protein level was analyzed by the biuret method. Blood urea nitrogen (BUN) level by the urease-glutamate dehydrogenase (urease-GLDH) method, glucose level by the glucose oxidase-peroxidase aminoantipyrine (GOD-PAP) method. Blood cholesterol level by the cholesterol oxidase-peroxidase aminoantipyrine (CHOD-PAP) method.

Observation of reproductive performance in does

Reproductive performance of the does was observed, including the scoring and assessment of estrus characteristics, and the observation of estrus cycle length. Scoring of estrus characteristics was performed when the does showed signs of estrus for two consecutive estrus cycles. The estrus characteristic variables observed included changes in vulva color, vulva swelling, and vaginal mucus secretion. Estrus characteristics was classified on a 1–3 scale, with increments of 0.5, where 1 represented none, 2 represented a medium score, and 3 signified a high score. In addition to scoring these variables, observations were made on vaginal mucus pH, vaginal temperature, vaginal epithelial cell population, and salivary ferning scoring. Salivary ferning was evaluated using a 1–6 scale, with 1 denoting the absence of ferning and 6 representing maximal ferning score (Diatmono *et al*., 2024b; Magistrama *et al*., 2024). Estrus cycle length was determined by calculating the interval between one estrus and the next, which was corroborated by observing the dominance of vaginal epithelial cells every two days for two estrus cycles.

Statistical analysis

Blood metabolite profiles and reproductive performance data obtained from the does were analyzed using an Independent-Samples T-Test. The relationships between all variables were analyzed using bivariate Pearson correlations with a two-tailed test of significance, with the significance threshold was set at $p<0.05$. All statistical analyses were performed using IBM SPSS version 23 software. Results are expressed as mean±standard deviation (SD).

Results and Discussion

Feed and nutrient intakes

The results shown in Table 2 indicates that, the nutrient intake of dry matter (DM), ash, crude protein (CP), ether extract (EE), crude fiber (CF), nitrogen-free extract (NFE), and total digestible nutrient (TDN) did not differ significantly among the various parity groups of does. This homogeneity in nutritional intake can be attributed to the does being housed on the same farm and fed a uniform daily feed ratio (Kumala *et al*., 2022). This result is attributed to a complex interplay of several factors, including body weight, milk yield, environmental conditions, and the physiological status of the does (Hudaya *et al*., 2020; de Oliveira *et al*., 2014). Multiparous does generally exhibit higher consumption rates due to their larger body size and higher milk production (Widayati *et al*., 2024; Kumala *et al*., 2022). However, no significant difference in intake between the parity groups is suspected to be due to nutrient allocation for body growth, mammary gland development, and milk production in the primiparous group (Salomone-Caballero *et al*., 2024; de Oliveira *et al*., 2014). This relatively high nutrient demand leads to an increase in feed consumption by primiparous does, thereby closing the gap with multiparous goats (de Oliveira *et al*., 2014). However, it is crucial to maintain optimal nutrient intake in does, as excessive consumption, such as high protein intake, can lead to elevated BUN levels, which can negatively impact reproductive performance of the does, including embryonic implantation failure and repeat breeding (Diatmono *et al*., 2024a; Sitaresmi *et al*., 2023).

Table 2. Daily nutrient intake of does across different parities (mean±SD)

Nutrient Intake (g per head per day)	Parity Groups		*p* Value
	Primiparous (n=16)	Multiparous (n=24)	
Dry matter (DM)	1975.10±250.73	2006.87±232.81	0.684
Ash	224.36±37.08	236.89±30.96	0.254
Crude protein (CP)	256.25±8.25	262.96±14.03	0.093
Ether extract (EE)	94.59±7.07	92.90±7.31	0.473
Crude fiber (CF)	481.31±125.79	494.89±119.91	0.733
Nitrogen-free extract (NFE)	932.15±122.49	949.75±110.79	0.640
Total digestible nutrient (TDN)	1037.80±39.59	1035.80±42.94	0.883

n: Number of sample (head) and *p* Value: Represent the probability of observing the sample data

Blood biochemical profile of does and its correlation with parity

Observations of blood metabolite levels are presented in Table 3. Based on these results, it was found that differences in parity (primiparous and multiparous) significantly affected total protein and cholesterol levels in the blood of Saanen-Etawah Crossbred does ($p<0.05$). Multiparous does exhibited higher blood protein levels compared to primiparous does, whereas primiparous does showed higher blood cholesterol levels than their multiparous counterparts. However, these results also indicated that no significant impact of parity was observed on plasma glucose and BUN levels ($p>0.05$). The correlation results (Table 4) showed that, parity exhibited a strong positive correlation with blood total protein levels in the does ($p<0.01$). Furthermore, parity was also found to be negatively correlated with blood cholesterol levels ($p<0.05$). Total protein levels were found to correlate strongly with BUN levels ($p<0.01$), with increasing protein levels associated with elevated BUN levels. The results also revealed no significant correlation between parity and blood glucose or BUN levels.

Table 3. Blood metabolite profile of Saanen-Etawah Crossbred does at each parity (mean±SD)

Blood Metabolite Profiles	Parity Groups		*p* Value
	Primiparous (n=16)	Multiparous (n=24)	
Total protein (g/dL)	5.47±1.40*	7.04±0.87*	0.001
Glucose (mg/dL)	60.36±6.92	63.42±7.29	0.194
Cholesterol (mg/dL)	122.33±30.55*	107.14±13.45*	0.038
BUN (mg/dL)	20.99±3.66	23.24±4.53	0.107

*: Superscript within the same row indicate a significant difference ($p<0.05$), BUN: Blood urea nitrogen, n: Total number of sample (head), and *p* Value: Represent the probability of observing the sample data

Blood metabolite profiles are known to be related to feed intake and rumen metabolism, as blood reflects how animals process nutrients from feed to meet their energy and physiological requirements (Arias-Islas *et al*., 2020; Widayati *et al*., 2017). Blood plays a crucial role in the transport of nutrients, metabolites, and hormones throughout the body (Widayati *et al*., 2024; Arias-Islas *et al*., 2020). Previous studies have indicated that plasma total protein levels in doe of different parities do not show significant differences (Widayati *et al*., 2024; Kumala *et al*., 2022). The results (Table 3), showing plasma protein levels still within the normal range (Sitaresmi *et al*., 2024; Widayati *et al*., 2024). Higher total protein levels (Table 3), correlating with increased BUN levels (Table 3) in multiparous does (Sitaresmi *et al*., 2023). Furthermore, previous study in dairy cows has demonstrated that multiparous cows exhibit higher total protein levels compared to primiparous cows, along with elevated albumin and globulin levels (Rocha *et al*., 2019). This can be attributed to multiparous does having developed more efficient metabolic responses, differing nutritional requirements, and a higher capacity for milk production (Rocha *et al*., 2019; Safayi *et al*., 2010). Multiparous does possess a greater ability to produce milk during lactation than primiparous does, due to several factors such as larger udder cisterns, enhanced mammary gland development, and increased feed intake (Boshoff *et al*., 2024; Safayi *et al*., 2010). This is further supported by the pivotal role of adequate plasma total protein levels in overall maternal lactation performance, directly contributing to amino acid availability and the synthesis of milk components such as milk fat, milk protein, and lactose (Letelier *et al*., 2022; El-Tarabany *et al*., 2018; Meng *et al*., 2016). The observed positive correlation between total protein and BUN levels (Table 4) suggests that maintaining both parameters within optimal ranges is crucial to prevent adverse effects on reproductive performance (Sitaresmi *et al*., 2024; Sitaresmi *et al*., 2023).

Table 4. Relationship between parity and blood metabolite profiles of Saanen-Etawah Crossbred does

r	Parity	Total protein	Glucose	Cholesterol	BUN
Parity	1	0.577**	0.210	-0.330*	0.259
Total protein	0.577*	1	-0.160	-0.048	0.619**
Glucose	0.210	-0.160	1	-0.157	0.080
Cholesterol	-0.330*	-0.048	-0.157	1	0.249
BUN	0.259	0.619**	0.080	0.249	1

*: Superscript indicated correlation is significant at the 0.05 level (2-tailed), **: Superscripts indicated correlation is significant at the 0.01 level (2-tailed), and r: Correlation coefficient of the sample

Cholesterol is a crucial component in the synthesis of steroid hormones in dairy goats, including estrogen (estradiol) and progesterone (progestin), which are categorized as reproductive steroid hormones (Mondragón *et al*., 2012). The observed blood cholesterol levels (Table 3) were within the normal range (Sitaresmi *et al*., 2024; Widayati *et al*., 2024). Higher plasma cholesterol levels in primiparous does compared to multiparous does align with previous studies (Widayati *et al*., 2024; Kumala *et al*., 2022). The lower cholesterol levels in multiparous does indicate a high utilization of stored body nutrients to support milk production performance (Sitaresmi *et al*., 2023; Sitaresmi *et al*., 2020). Furthermore, the utilization of blood cholesterol as energy to support lactation performance also contributes to lower blood cholesterol levels in multiparous does compared to primiparous does (Widayati *et al*., 2024). The results are corroborated by a negative correlation between parity and blood cholesterol levels (Table 4). Multiparous does are presumed to

have reached an optimal stage of body and mammary gland development, enabling them to produce higher milk yields and utilize more cholesterol than primiparous does (Widayati *et al*., 2024; Kumala *et al*., 2022; Lang *et al*., 2012). Optimal blood cholesterol levels are essential as they influence milk composition (Tosto *et al*., 2021; El-Tarabany *et al*., 2018). Lower blood cholesterol levels have been correlated with better goat milk production and healthier milk components (El-Tarabany *et al*., 2018). The importance of adequate plasma cholesterol levels for reproductive performance in does is also highlighted, specifically in their role as a steroid hormone precursor and their contribution to oocyte development, ovulation, embryo development, and the overall health of the reproductive system (Diatmono *et al*., 2024a; Sitaresmi *et al*., 2023).

The results (Table 3) showed that blood glucose and BUN levels in Saanen-Etawah Crossbred does, sampled during the follicular phase, were within the normal range (Diatmono *et al*., 2024a; Widayati *et al*., 2024). The non-significant blood glucose levels among the does were likely due to all does being in the same physiological state (lactating and non-pregnant), receiving the same feeding ratio (Table 1), and similar feed consumption (Table 2). This is consistent with previous studies indicating that lactation and pregnancy periods demand higher energy and glucose levels (Lunesu *et al*., 2021; Sitaresmi *et al*., 2020). Furthermore, blood glucose levels are more significantly influenced by factors such as diet and the post-parturition transition period (Arias-Islas *et al*., 2020; Zamuner *et al*., 2020). Additionally, complex metabolic regulation occurs within the body to maintain glucose homeostasis (Sitaresmi *et al*., 2024; Abbas *et al*., 2020). Glucose homeostasis plays a crucial role as glucose serves as an energy source and the primary substrate for lactose synthesis, which is the main osmotic regulator of milk volume and yield (Abbas *et al*., 2020; Cai *et al*., 2018; Liu *et al*., 2013). Moreover, improper glucose regulation has been shown to reduce overall milk production and compromise doe health (Hamzaoui *et al*., 2020; Liu *et al*., 2013). Similar to blood glucose, BUN levels in the does (Table 3) did not show significant differences. This is because BUN levels tend to be influenced by a combination of diet, body condition score (BCS), and physiological status (Sitaresmi *et al*., 2024; Widayati *et al*., 2024). Optimal BUN levels are essential, as an imbalance in dietary protein and energy will decrease does productivity (Sitaresmi *et al*., 2023; Oliveira *et al*., 2021). Beyond reduced productivity, elevated blood BUN levels, resulting from increased hepatic urea production, can negatively impact does' reproductive performance, leading to decreased fertility and conception rates, altered reproductive hormones, and early embryonic death (Sitaresmi *et al*., 2023; Widayati *et al*., 2019; Widayati *et al*., 2017).

Reproductive performance of does and its correlation with parity

The results indicate that the difference in parity (primiparous and multiparous) significantly influenced ($p<0.05$) several reproductive performance parameters during the estrus cycle phase. These included reddening of vulva, vulvar swelling, vaginal mucus production, and estrus cycle length. Multiparous does are known to exhibit more pronounced estrus symptoms and shorter estrus cycles. However, several variables showed no significant difference ($p>0.05$), such as vaginal mucus pH, vaginal temperature, superficial cell population, and salivary ferning scores. The estrus characteristics, representing the reproductive performance of Saanen-Etawah Crossbred does, are presented in Table 5. The correlation between parity and reproductive performance variables is presented in Table 6. Our findings indicate that parity was positively correlated with reddening of vulva, vulvar swelling, and vaginal mucus production, but negatively correlated with estrus cycle length. Several estrus characteristics also exhibited positive inter-correlations: reddening of vulva correlated positively with vulvar swelling, vaginal mucus production, and mucus pH value. Vulvar swelling was positively correlated with vaginal mucus pH, and vaginal mucus production showed a positive correlation with vaginal temperature. Beyond its negative correlation with parity, estrus cycle length also correlated negatively with vaginal mucus production, vaginal temperature, and salivary fern patterns.

The results (Table 5) indicate that the estrus characteristics showed by multiparous does were within a similar range compared to previous studies (Diatmono *et al*., 2024b; Kumala *et al*., 2021). However, these estrus characteristics were lower when compared to does that received estrus induction treatment (Olurode *et al*., 2020). The more pronounced estrus characteristics observed in multiparous does are attributed to a combination of several factors related to hormonal changes, prior parturition experience, more developed uterine and ovarian organs (Cano-Suarez *et al*., 2024; Madureira *et al*., 2015). It has been established that multiparous does, possessing a longer reproductive history, exhibit a more mature and finely tuned hormonal feedback system and a potentially higher sensitivity to estrogen, resulting in a more pronounced expression of estrus (Li *et al*., 2025; Madureira *et al*., 2015). The intensity of estrus characteristics, such as the reddening, swelling of the vulva, and increased vaginal mucus production, which are correlated with other estrus characteristic variables (Table 6) are manifestations of elevated blood estrogen and follicle stimulating hormone (FSH) levels (Kumala *et al*., 2021; Sitaresmi *et al*., 2020; Sitaresmi *et al*., 2019). Furthermore, estrogen is recognized as a vasodilator and a blood pressure lowering agent (Foeh *et al*., 2022; Sitaresmi *et al*., 2019). Based on its role, estrogen induces vascular relaxation through the activation of endothelial nitric oxide

synthase and the release of nitric oxide (NO) or by directly acting on vascular smooth muscle (Foeh *et al*., 2022; Nicholson *et al*., 2017). Increased blood circulation in the reproductive organs during the estrus phase leads to swelling and reddening of the vulvar area (Diatmono *et al*., 2024b; Magistrama *et al*., 2024), as well as the development of the cervical tissues to produce mucin (Setiatin *et al*., 2023; Sitaresmi *et al*., 2019). This is further supported by the correlations among estrus characteristics obtained (Table 6), where adequate estrogen promotes more discernible estrus signs (Magistrama *et al*., 2024; Mondragón *et al*., 2012).

Table 5. Characteristics and estrus cycle length of Saanen-Etawah Crossbred does at each parity (mean±SD)

Estrus Parameters	Parity Groups		*p* Value
	Primiparous (n=16)	Multiparous (n=24)	
Estrus characteristics:			
Reddening of vulva	2.00±0.63*	2.45±0.58*	0.024
Vulvar swelling	1.87±0.61*	2.33±0.63*	0.030
Mucus production	1.68±0.47*	2.25±0.60*	0.004
Mucus pH value	8.65±0.47	8.81±0.32	0.222
Vaginal temperature (°C)	38.35±0.36	38.41±0.37	0.659
Superficial cell population (%)	82.57±5.40	83.67±5.23	0.525
Salivary ferning	4.62±1.14	4.75±1.03	0.722
Estrus cycle length (days):	27.68±1.99*	24.87±2.40*	0.001

*: Superscript within the same row indicate a significant difference (p<0.05), n: Total number of sample (head), and *p* Value: Represent the probability of observing the sample data

Table 6. Relationship between parity and reproductive performance of Saanen-Etawah Crossbred does

r	Parity	RV	VS	MP	MpH	VT	SCP	SF	ECL
Parity	1	0.355*	0.343*	0.450**	0.198	0.072	0.104	0.058	-0.532**
RV	0.355*	1	0.384*	0.370*	0.587**	0.123	-0.193	-0.139	-0.245
VS	0.343*	0.384*	1	0.116	0.691**	-0.006	-0.111	0.029	-0.192
MP	0.450**	0.370*	0.166	1	0.185	0.440**	0.131	-0.066	-0.316*
MpH	0.198	0.587**	0.691**	0.185	1	-0.054	-0.136	-0.153	-0.112
VT	0.072	0.123	-0.006	0.440**	-0.054	1	0.201	0.177	-0.339*
SCP	0.104	-0.193	-0.111	0.131	-0.136	0.201	1	0.245	-0.205
SF	0.058	-0.139	0.029	-0.066	-0.153	0.177	0.245	1	-0.587**
ECL	-0.532**	-0.245	-0.192	-0.316*	-0.112	-0.339*	-0.205	-0.587**	1

*: Superscript indicated correlation is significant at the 0.05 level (2-tailed), **: Superscripts indicated correlation is significant at the 0.01 level (2-tailed), r: Correlation coefficient of the sample, RV: Reddening of vulva, VS: Vulvar swelling, MP: Mucus production, MpH: Mucus pH value, VT: Vaginal temperature, SCP: Superficial cell population, SF: Salivary ferning, and ECL: Estrus cycle length

The estrus cycle length of multiparous does was within the normal to slightly longer range, typically 17–25 days. In contrast, primiparous does exhibited a prolonged estrus cycle, exceeding 25 days (Alves *et al*., 2018). This difference is likely due to multiparous does having a more established reproductive cycle (Cano-Suarez *et al*., 2024). Having undergone the hormonal changes associated with pregnancy and parturition multiple times, multiparous does may exhibit a more regular and predictable estrus cycle, potentially leading to a shorter duration (Li *et al*., 2025; Cano-Suarez *et al*., 2024; Madureira *et al*., 2015). This is also supported by a negative correlation between estrus cycle length and several estrus characteristics (Table 6), indicates that more pronounced estrus signs are associated with a shorter estrus cycle length (normal). Moreover, normal reproductive cycles are contingent upon typical fluctuations in reproductive hormones, which subsequently impact estrus manifestations (Li *et al*., 2025; Sitaresmi *et al*., 2020). Furthermore, several estrus characteristics, including vaginal mucus pH value, vaginal temperature, superficial cell population, and salivary ferning score, did not differ significantly between groups (p>0.05). All observed estrus manifestations are linked to the concentrations of estrogen in the blood circulatory system (Setiatin *et al*., 2023; Kumala *et al*., 2021; Sitaresmi *et al*., 2019). Fluctuations in estrogen levels throughout the estrus cycle (follicular and luteal phases) lead to morphological changes in vaginal epithelial cells (Figure 1) and alterations in salivary ferning patterns (Figure 2), consistent with prior studies (Diatmono *et al*., 2024b; Magistrama *et al*., 2024; Sitaresmi *et al*., 2019). The systemic circulation of estrogen in follicular phase influences the regulation of vaginal mucus pH into more alkaline, increased vaginal temperature, stimulation of vaginal wall thickening and keratinized squamous epithelium (Figure 1), as well as salivary fern patterns (Figure 2), resulting in no significant differences observed for these parameters (Setiatin *et al*., 2023; Magistrama *et al*., 2024; Sitaresmi *et al*., 2019).

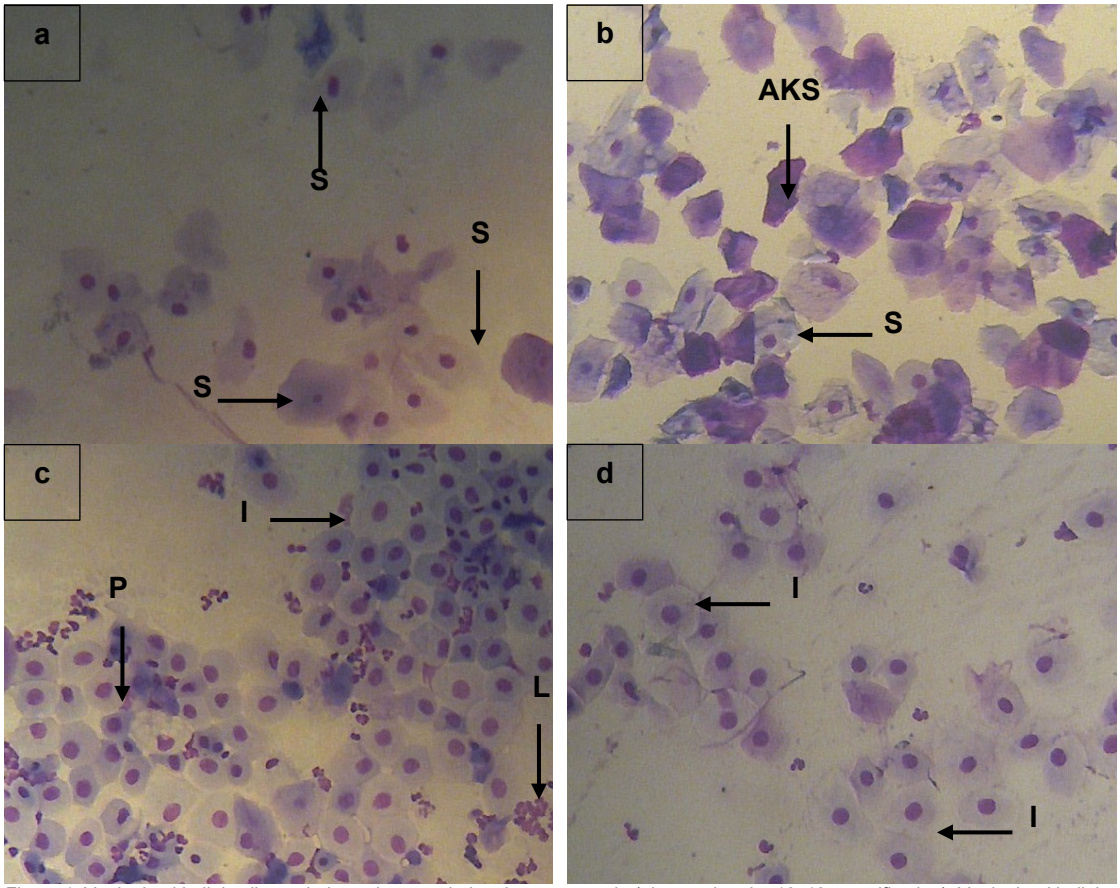

Figure 1. Vaginal epithelial cell morphology changes during the estrus cycle (observed under 10x10 magnification): Vaginal epithelial cell cytology in the follicular phase (a, b) and vaginal epithelial cell cytology in the luteal phase (c, d). P: Parabasal cells, I: Intermediate cells, S: Superficial cells, AKS: Anucleated and keratinized superficial cells, and L: Leukocytes

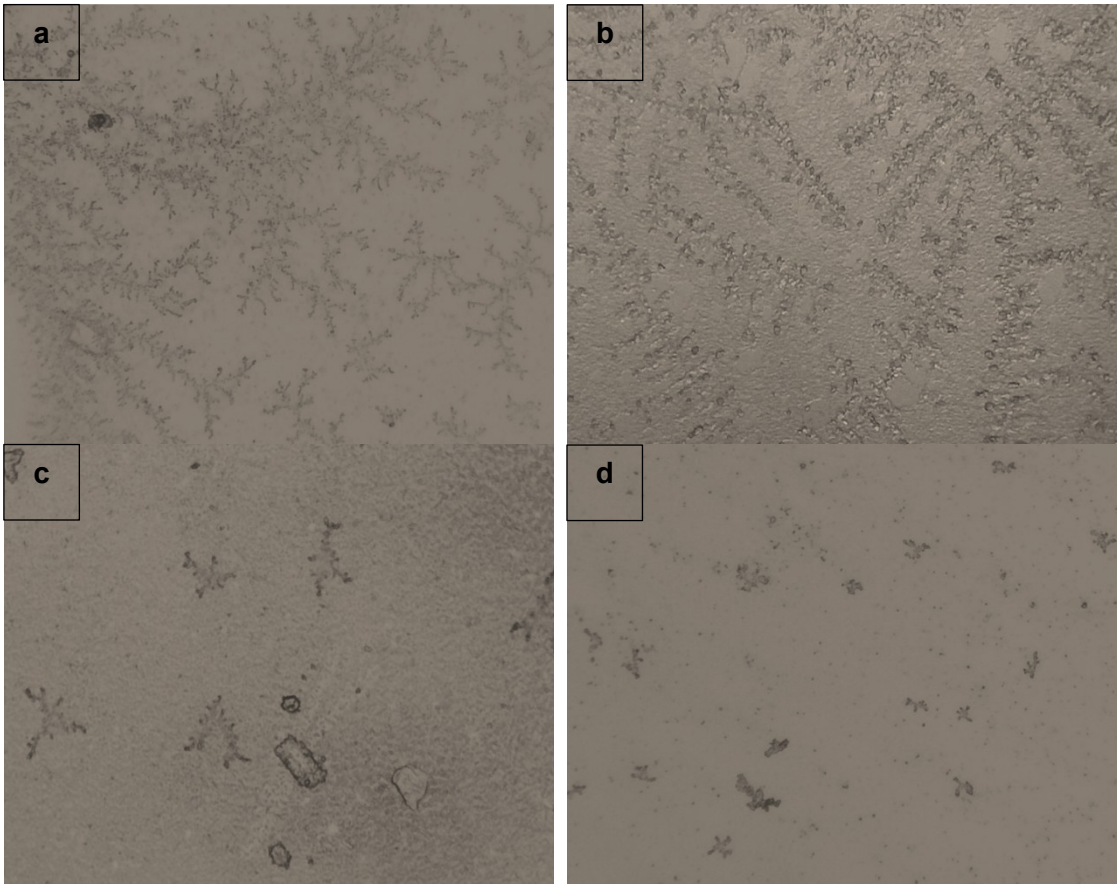

Figure 2. Changes in salivary ferning patterns during the estrus cycle (observed under 10x10 magnification): Salivary ferning patterns in the follicular phase (a, b) and salivary ferning patterns in the luteal phase (c, d)

Conclusion

The study demonstrated that multiparous does exhibited higher blood total protein levels, which were positively correlated with parity, reflecting metabolic adaptations to increased lactation demands. Conversely, primiparous does showed higher blood cholesterol levels, negatively correlated with parity, likely due to lower utilization of cholesterol for milk production. Blood glucose and BUN levels did not differ significantly between parity groups. Reproductive performance parameters, including reddening of the vulva, vulvar swelling, vaginal mucus production, and estrus cycle length, were significantly affected by parity, with multiparous does showing more pronounced estrus characteristics and shorter estrus cycles. These findings highlight the importance of parity-related metabolic and physiological changes in optimizing reproductive efficiency and lactation performance in Saanen-Etawah Crossbred does. Maintaining balanced protein and cholesterol levels is essential to support both reproductive health and milk production. Further study on feeding strategies and hormonal regulation is recommended to enhance reproductive outcomes in this breed.

Conflict of Interest

The authors declared there is no potential conflict of interest related to this article.

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Author's Contribution

The authors contributed to the manuscript as follows: DTW, BPW, and DFFD designed the study. DFFD, SK, SWP, and MA performed the fieldwork administered the experiments. DFFD, PIS, and FGP conducted the statistical analysis. DTW, PIS, DFFD, JdaCF, and FGD performed data interpretation. DFFD, SWP, SK, and PIS drafted and edited the original manuscript. DTW, BPW, YYS, and PIS supervised the study. DTW, YYS, BPW, JdaCF, and PIS reviewed and validated the manuscript. All authors read and approved the final manuscript at its concluding stage.

Ethics Approval

The animal maintenance, data collection, and blood sampling procedures received ethical approval from the Research Ethics Committee, Faculty of Veterinary Medicine, Universitas Gadjah Mada (No. 3/EC-FKH/int./2024). During data collection, does were handled with utmost care to minimize stress. This approval was effective for the duration of the study.

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