Research Article

Optimizing Estrus Detection Techniques in Tropical Saanen Does (*Capra aegagrus hircus*): A Comparative Study

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Abstract | Estrus detection has been a major challenge in Saanen goat farm due to the inconsistent expression of estrus signs (silent heat) and the farmer's inability to detect these signs. This issue led to mating failure and adversely affected the reproductive efficiency of tropical Saanen does. This study focused on saliva fern patterns and vaginal cytology as an alternative estrus detection method. Twelve non-pregnant Saanen does with a body condition score (BCS) of 2.5 were used in this study and without estrus synchronization. Vaginal mucosa and saliva samples were collected every two days in the morning before feeding. Vaginal smear were obtained using the swab method on vaginal epithelium, while saliva was collected by swabbing the lower mouth of does. Additionally, vaginal pH was measured as an additional estrus detection method using a pH indicator paper. The parameters observed included the composition of vaginal epithelial cells (parabasal, intermediate, and superficial), the score of salivary fern patterns, and vaginal pH value. All the data were analyzed using One-Way ANOVA. The results showed significant differences in vaginal epithelial cells, salivary fern patterns scoring, and vaginal pH values across the different estrus phase (p<0.05). In conclusion, vaginal cytology, salivary ferning, and vaginal mucus pH value were effective in identifying the estrus cycle in tropical Saanen does. Among these method, salivary ferning was the easiest to perform and demonstrated high validity for detecting estrus.

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Keywords | Does, Estrus detection, Saanen, Salivary ferning, Vaginal pH, Vaginal cytology

Introduction

Saanen goats have been one of the leading livestock commodities developed in Indonesia for nearly two decades. Originating from Western Switzerland, these goats are known for their high milk production, excellent genetics and impressive productivity (Miller and Lu, 2019). Saanen goats exhibit high fertility,

short calving intervals, rapid breeding capabilities, and efficient utilization of various forages types (Varlyakov *et al*., 2018). However, in the tropical environments of Indonesia, the hot weather often leads to mating failures in Saanen does due to improper detection of estrus, even though estrus is the main factor in the success of a matings (Ridlo *et al*., 2019). Estrus determination on the right time is the key component of successful mating program then leads in improvement of conception rates in Saanen goat. Currently, estrus determination in Saanen goat was majorly based on behavioral and physiological signs. However, visual failure to detect estrus can occur, particularly because goats may experience silent heat, making it difficult for farmers to determine the optimal mating time (Danso *et al*., 2024; Singh *et al*., 2020). This results in prolonged empty period and suboptimal milk production. Therefore, it is necessary to develop an accurate, non-invasive, and simple method estrus detection that is not affected by silent heat.

An effective method involves utilizing of vaginal smear analysis (Sitaresmi *et al*., 2019, 2020) and saliva fern patterns (Ravinder *et al*., 2016; Tanjung *et al*., 2015; Ondho *et al*., 2019), as the estrus phase holds significant importance in the management of ruminant reproduction. The vaginal smear method has been applied to various livestock, including goats (Sitaresmi *et al*., 2019). Significant hormonal changes, particularly the surge estrogen during estrus, impact the biophysical and biochemical conditions of the body and reproductive tract (Rutllant *et al*., 2002), leading to specific estrus signs (Sitaresmi *et al*., 2020). Moreover, the rise in estradiol levels triggers increased salt concentration in body fluids, including saliva during estrus (Terzano *et al*., 2012). The elevated sodium content in the saliva results in the formation of a fern/crystallization pattern, leading to the development of salivary ferning method for estrus detection techniques. This method has been found applicable in human (Surla *et al*., 2021) and buffaloes (Ravinder *et al*., 2016).

Observation of saliva crystallization patterns has predominantly focused on large ruminants (Ravinder *et al*., 2016), with limited exploration in Saanen goats from tropical regions. Vaginal cytology changes (Sitaresmi *et al*., 2019; Indira *et al*., 2014), salivary ferning (Ravinder *et al*., 2016; Wijayanti *et al*., 2014), and pH mucus (Widayati *et al*., 2018; Diatmono *et al*., 2024) remain unaffected by silent heat, as their mechanism are regulated by estradiol and other steroid hormones, even when hormonal surges do not reach peak levels. Therefore, it is essential to conduct thorough research on combined estrus detection methods, including salivary fern pattern, vaginal smear, and vaginal mucus pH to identify the most effective and practical options for identifying estrus sign in tropical Saanen goats within smallholder farming, especially in goats with unclear signs of visual estrus or experiencing silent heat.

Materials and Methods

Research location

The experiment was conducted at a dairy goat teaching farm of "Pusat Pengembangan Ternak (PPT)/Center for Livestock Development" is being made by adding the phrase "Center for Livestock Development" as the English translation for "Pusat Pengembangan Ternak." Faculty of Animal Science, Universitas Gadjah Mada (7°46'8"S and 110°23'10"E). Sample preparation and observation were performed at the Laboratory of Animal Physiology and Reproduction, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia (Figure 1).

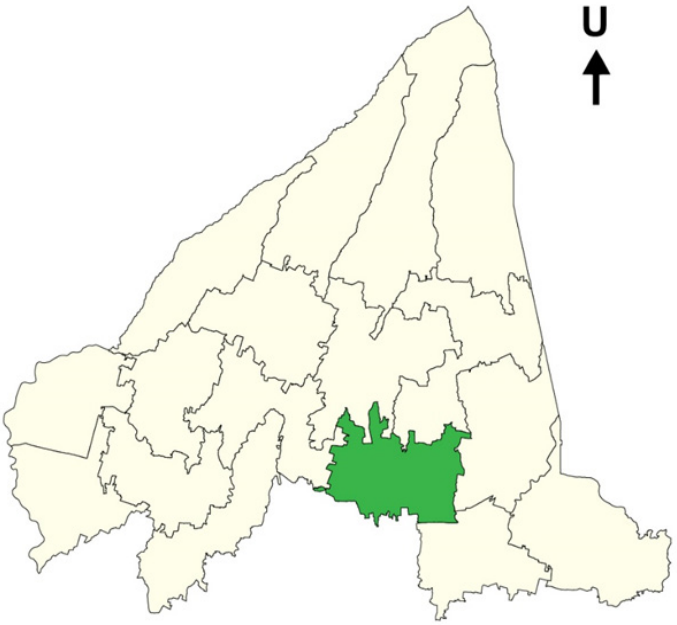

Figure 1: *Research location, in Center for Livestock Development (PPT), Faculty of Animal Science, Universitas Gadjah Mada, Sleman Regency, Daerah Istimewa Yogyakarta Province (in tropical climate).*

Animal used

This study utilized 12 non-pregnant Saanen does with body condition score (BCS) of 2.5, assessed on a scale from 1 to 5 (Ghosh *et al*., 2019). The Saanen does

receive standard maintenance management without any special treatment and without estrus synchronization. The does were kept in individual pens without tethering and prioritize livestock comfort to reduce stress in animals. Feeding occurred twice daily, in the morning (07:30am) and afternoon (02:00pm), consisting of commercial concentrates and fresh forage (*Pennisetum purpureum*), and an *ad libitum* access to drinking water. Feedstuffs was analyzed using the proximate method to determine the nutrients composition (AOAC, 2005). Nutrient composition were showed in Table 1. All research methods and animal care procedure were approved by the Ethics Committee, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta (No: 3/EC-FKH/int./2024).

Salivary fern patterns determination

Saliva collection was conducted using a cotton bud moistened with aquadest. The cotton bud was gently swabbed on the lower part of the mouth, in front of the teeth or under the tongue, to collect the saliva. Subsequently, the saliva-coated cotton bud was lightly pressed onto an object glass to deposit the saliva sample. The saliva smear was then air-dried at room temperature (27 to 28°C) for approximately five hours to facilitate the crystallization process (Badr *et al*., 2017). Observation of salivary fern pattern were performed using a microscope at a magnification of 10x10. The scoring of salivary fern pattern was conducted using a scale ranging from 1 to 6 (Wijayanti *et al*., 2014; Tanjung *et al*., 2015).

Vaginal smear determination

Vaginal smear samples were obtained by swabbing vaginal epithelial cells approximately 2 cm from vulva using a cotton bud moistened with aquadest solution. The swab result was transferred on to object glass and air-dried in room temperature (Amrullah *et al*., 2018). To prepare the vaginal epithelial cells for cytological examination, the dried smears were fixed by dipping the glass in alcohol 70% for 7 minutes. Alcohol fixation enhances the absorption of color by the cell nucleus and cytoplasm, while also rendering bacteria colorless (Ardina and Rosalinda, 2018). The fixed smears were then stained by immersion in 3% Giemsa (Merck, Germany) solution for 45 minutes, followed by washing with aquadest. Subsequently, the stained vaginal smear preparations were air-dried at room temperature. Observation of vaginal cytology were conducted using a microscope with a magnification of 10x10 (Sitaresmi *et al*., 2019).

Mucus pH determination

Vaginal pH values were measured by swabbing vaginal mucus using pH indicator paper (MQuant®, Germany), which has a scale ranging from 1 to 14. This measurement was conducted during each estrus response observation (Kumala *et al*., 2021; Diatmono *et al*., 2024). The pH value was determined by comparing the color changes on the pH paper with standard indicator or using a pH meter for precise calibration (Kumala *et al*., 2022; Sitaresmi *et al*., 2019).

Statistical analysis

The composition data of salivary ferning scores, vaginal epithelial cells, and mucus pH value on each estrus phases were analyzed using One-way analysis of variance (ANOVA) from the statistical package for the social sciences (SPSS) 26.0 software. The results are presented as mean±standard deviation (SD).

Results and Discussion

Score of salivary crystallization on each estrus phases

The results of salivary fern patterns, vaginal epithelial cells, and mucus pH on each estrus phases are showed in Table 2. Significant differences (p<0.05) were observed in salivary ferning score, vaginal cytology, and mucus pH in each estrus phases, indicating the influence estrogen level during the estrus cycle. The highest ferning score were observed during heat estrus, while the lowest score were seen during diestrus phase. This finding aligns with previous studies that reported an increase in estrogen levels corresponding to elevated NaCl level, which affect salivary crystallization (Ravinder *et al*., 2016; Priya *et al*., 2020). Saliva composition is closely related to estrogen levels in livestock. Elevated estrogen and adrenocorticotropic hormone (ACTH) can stimulate aldosterone production, which regulates electrolytes and fluid balance, thereby influencing sodium chloride (NaCl) levels in saliva (Priya *et al*., 2020). Photomicrographs illustrating salivary fern pattern scoring in Saanen does were showed in Figure 2.

Table 1: *Feed nutrient composition.*

Ingredients	Nutritional contents (%)					
	DM	CP	CF	Crude fat	NFE	TDN
Commercial concentrate	89.49	14.58	16.66	6.42	54.36	66.96
Pennisetum purpureum	25.57	7.98	29.44	2.28	47.96	46.25

DM: dry matter, CP: crude protein, CF: crude fiber, NFE: nitrogen-free extract, TDN: total digestible nutrient.

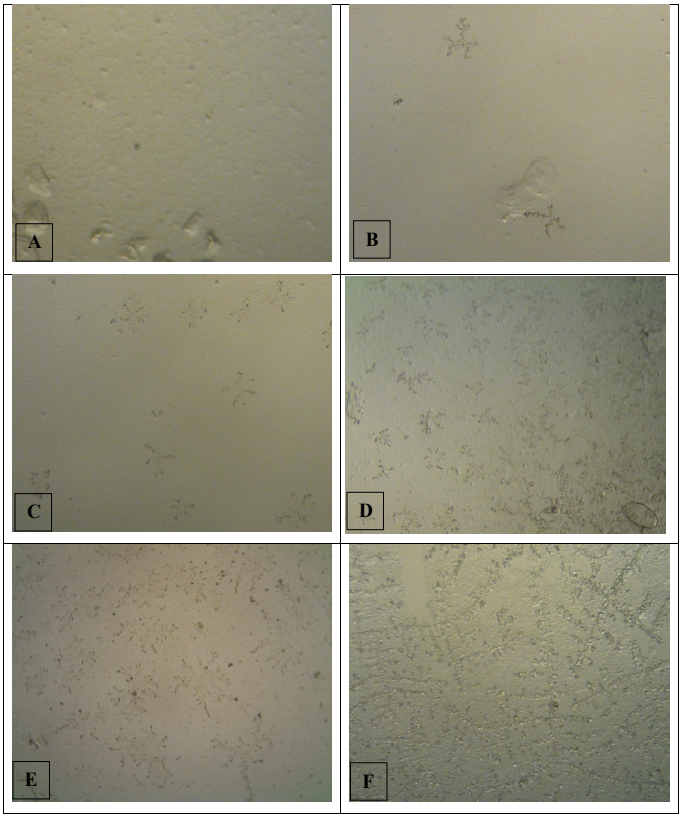

Figure 2: *Photomicrograph of salivary fern pattern's scoring with magnification of 10x10; score of 1 (A), score of 2 (B), score of 3 (C), score of 4 (D), score of 5 (E), and score of 6 (F).*

The substantial differences in saliva crystallization reflected changes in steroid hormone levels in saliva and blood circulation which correlated with ovarian functional activity (Badr *et al.*, 2017). An increase in estradiol, which triggers the LH surge, affected the body's performance through the action of steroid hormones such as cortisol and aldosterone. These hormones raise blood pressure by stimulating the kidney to absorb sodium chloride into the bloodstream as a response to regulate blood pressure and body electrolytes, ultimately leading to an increase in salt levels in various body fluids (Terzano *et al.*, 2012). The higher the salt content, the higher the fractal dimension value in saliva crystallization, resulting in more complex crystallization patterns (Ravinder *et al.*, 2016; Gonçalves *et al.*, 2020). This complexity of saliva crystallization is what characterizes the ferning score. The absence of crystals in dry saliva smears indicated a low level of sodium salt, thus reducing the ferning score, while increasingly complex crystallization enhances the ferning score (Wijayanti *et al.*, 2014). Salivary fern patterns on each estrus phases showed in Figure 3. Based on the results, there were no significant differences in salivary crystallization between large and small ruminants, suggesting that this method can be used validly across different species.

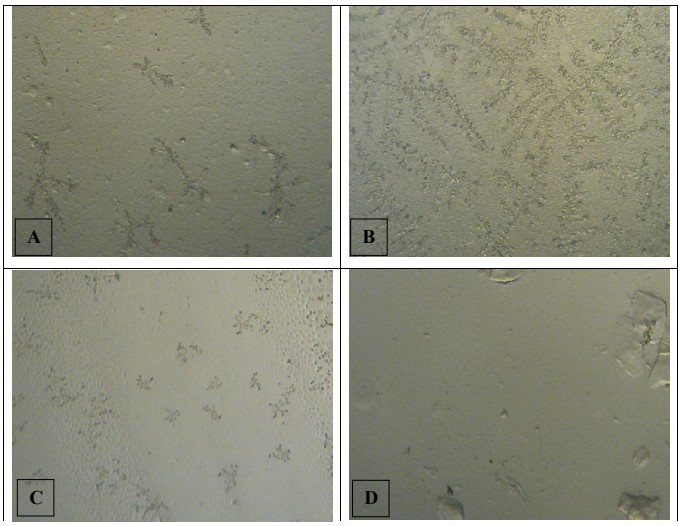

Figure 3: *Photomicrograph of salivary fern pattern on each estrus phases with magnification of 10x10; proestrus (A), estrus (B), metestrus (C), and diestrus (D).*

Exfoliative vaginal cytology on each estrus phases

The results in Table 2, also indicates that vaginal epithelial cells exhibit different characteristics during each phase of the estrus cycle ($p<0.05$). Vaginal epithelial cells are classified based on the morphology, including shape, size, and the presence of the cell nucleus (Figure 4).

Table 2: *Results of estrus characteristics of Saanen does on each estrus phases.*

Estrus characteristics (n=12)	Estrus phases			
	Proestrus	**Estrus**	**Metestrus**	**Diestrus**
Vaginal cytology				
Parabasal	23.83±8.36^{b}	0.79±1.05^{a}	28.08±13.62^{b}	69.16±7.39^{c}
Intermediate	68.83±9.65^{c}	4.66±2.10^{b}	9.04±5.08^{a}	30.04±7.30^{b}
Superficial	7.33±3.89^{a}	94.54±2.33^{c}	66.29±9.34^{c}	0.79±1.67^{a}
Salivary fern patterns	3.75±0.62^{y}	5.41±0.66^{z}	2.83±0.57^{x}	1.08±0.28^{w}
Vaginal pH value	8.16±0.32^{y}	8.91±0.28^{z}	7.87±0.37^{x}	7.08±0.35^{w}

^{a,b,c} Different superscript in the same column indicated significant differences ($p<0.05$). ^{w,x,y,z} Different superscript in the same row indicated significant differences ($p<0.05$).

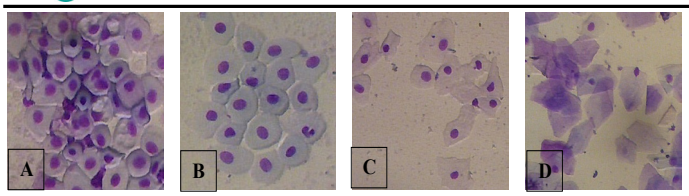

Figure 4: *Photomicrograph of vaginal epithelial cells with magnification of 10x10; parabasal (a), intermediate (b), superficial (c), and superficial with keratinization (d).*

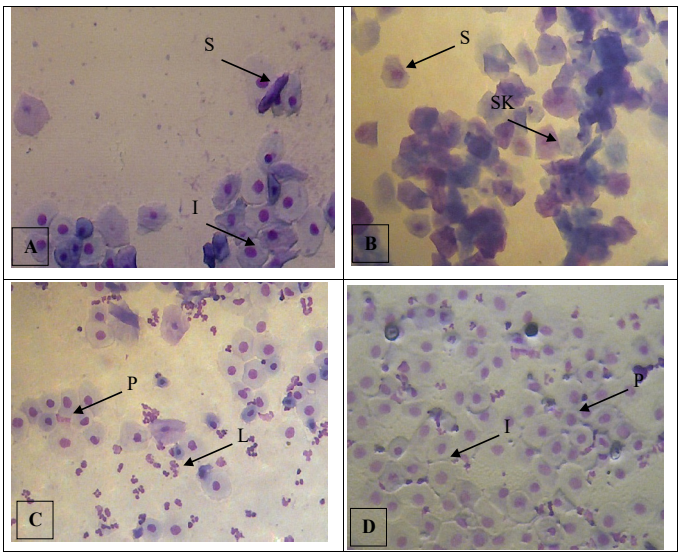

Figure 5: *Photomicrographs of vaginal smear on each estrus phases with magnification of 10x10; proestrus (A), estrus (B), metestrus (C), diestrus (D), also various of epithelial cells in vaginal cytology; parabasal cell (P), intermediete cell (I), superficial cell (S), superficial with keratinization (SK), and leucocyte (L).*

The differences in the characteristics of vaginal epithelial cells reflect the hormonal changes that occur in each phase of the estrus cycle (Figure 5). In the proestrus phase (Figure 5A), the cell population is dominated by intermediate and parabasal cells, with a small proportion of superficial cells. Parabasal cells (Figure 4A) have a round or oval shape with a large cell nucleus, and the ratio of the cell nucleus size to the cytoplasm is relative balanced. The presence of superficial cells during proestrus phase is due to the increasing concentration of estrogen (Sitaresmi *et al.*, 2019). During estrus phase (Figure 5B), the epithelial cells are predominantly superficial cells (Famakinde *et al.*, 2017; Sitaresmi *et al.*, 2019; Kumala *et al.*, 2021). The transition from parabasal to superficial cells occurs due to elevated estrogen levels in the blood. Increased estrogen levels during estrus phase enhance the vascularization of the vagina, thicken the vaginal wall, and cause the outermost layer of vaginal epithelial cells to lose nourishment. This results in the exfoliation of parabasal cells, transforming them into polygonal, nucleus-lacking superficial cells (Indira *et al.*, 2014; Sitaresmi *et al.*, 2019).

Superficial cells (Figure 4C) are characterized by their flat, polygonal shape with irregular edges and abundant cytoplasm. These cells undergo cornification and keratinization due to the high concentration of estrogen during the estrus phase (Figure 4D). The cornification and keratinization of superficial cells protect the vaginal mucosa from irritation during the copulation (Sitaresmi *et al.*, 2019). In the metestrus phase (Figure 5C), a mixture of superficial, parabasal cells, intermediates, along with the presence of leukocytes is observed. In the diestrus phase (Figure 5D) the vaginal epithelial cells are dominated by intermediate and parabasal cells (Sharma and Sharma, 2016; Sitaresmi *et al.*, 2019). Intermediate cells (Figure 4B) are distinguished by their large, polygonal nucleus and abundant cytoplasm. In goat vaginal smears, intermediate cells typically have an oval shape with a prominent nucleus or a polygonal shape with abundant cytoplasm and a small nucleus (Ola *et al.*, 2006; Sitaresmi *et al.*, 2019). During the metestrus and diestrus phases, the formation of the corpus luteum in the metestrus phase increased the concentration of progesterone and decreases the concentration of estrogen. This hormonal dynamic results in higher proportion of intermediate and parabasal cells during the luteal phase, under the influence of progesterone (Widayati *et al.*, 2010; Sitaresmi *et al.*, 2019).

Vaginal mucus pH values on each estrus phases

Based on the results presented in Table 2, there was a significant difference in pH level in each estrus phases. These differences were attributed to hormonal changes throughout the estrus cycle. The highest pH value occurred during the estrus phase, while the lowest pH value was observed in the diestrus phase. This change in pH is due to the vagina becoming more alkaline in response to increased estrogen levels (Ondho *et al.*, 2019; Widayati *et al.*, 2018; Sitaresmi *et al.*, 2019). Under normal conditions, the vaginal environment tends to be acidic due to the activity of normal flora in the vulva. Lactic acid bacteria actively secrete lactic acid, which acts as a bio protective agent to prevent the activity of pathogenic microorganisms. The fluctuations in vaginal pH are influenced by steroid reproductive hormones. Increased estrogen levels stimulate the secretory cells of the endometrium to produce more mucus. This mucus helps sperm to survive longer and maintain their motility (Sitaresmi *et al.*, 2019).

Vaginal mucus pH values are related to biochemical changes and are controlled by hormonal dynamic during the estrus cycle, leading to different pH conditions in each phase (Rutllant *et al*., 2002; Diatmono *et al*., 2024). During the estrus phase, the pH value of the vagina increases along with rising estrogen levels. Estrogen contains OH-ions, which cause an increase in vaginal pH. This increase in vaginal pH also occurs as a preparation for the spermatozoa medium when ejaculated into the female reproductive tract (Sitaresmi *et al*., 2019; Widayati *et al*., 2018). The elevated pH of vaginal mucus creates an optimal environment for spermatozoa to live and move within the female reproductive tract. Additionally, the higher pH of vaginal mucus inhibits the growth of endogenous bacteria by preventing their metabolism. Outside the estrus phase, the vaginal pH decreases due to the accumulation of ions such as hydrogen and sodium chloride (Gaafar *et al*., 2005), as well as glycogen and protein (Nakano *et al*., 2015). These biochemical changes contribute to the acidic environment observed during other phases of the estrus cycle.

Conclusion and Recommendations

In conclusion, salivary fern pattern scoring, vaginal smears, and vaginal mucus pH values can be effectively used as methods for estrus identification in tropical Saanen goats. Among these, the observation of salivary fern patterns stands out as the easiest and most cost-effective method with high validity. However, further studies are needed to explore the effectiveness of these methods when combined with estrus synchronization, to validate and enhance the information available on estrus detection techniques.

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Novelty Statement

This study examines advanced approaches for detecting estrus in Saanen goats, focusing on overcoming reproductive efficiency challenges and refining detection methods for application in tropical climates.

Author's Contributions

Muhammad Evan Magistrama: Conducted the fieldwork study, literature search, paper write-up, and performed statistical analysis.
Dio Fico Felsidan Diatmono: Performed the statistical analysis, data interpretation, literature search, and paper write-up.
Mira Tsurayya Masruroh: Conducted the fieldwork study, literature search, and paper write-up.
Fransisca Gani Padmawati: Performed literature search and paper write-up.
Pradita Iustitia Sitaresmi: Performed literature search, reviewed the manuscript, and supervised the fieldwork study.
Yustina Yuni Suranindyah: Performed data analysis, reviewed the manuscript, and supervised the study.
Diah Tri Widayati: Designed the study, data analysis and interpretation, literature search, reviewed the manuscript, and supervised the study.

Conflict of interest

The authors have declared no conflict of interest.

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