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Research Article

# Cyto-morphological Changes in Exfoliated Vaginal Cells and Thermal Rhythms of Red Sokoto does during the Oestrous cycle

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**Abstract** | In order to provide a more reliable, non-invasive method for defining the reproductive phase and determining the optimum time of ovulation, fifteen (15) apparently normal, non-pregnant cycling Red Sokoto does were used to evaluate exfoliative vaginal cytology sequential pattern; as well as temperature (rectal and vaginal) changes relative to various stages of the oestrus cycle. The does were synchronized by treatment with two luteolytic dosages of $PGF_{2\alpha}$ analogue (Lutalyse®, *Dinoprost tromethamine*), 12.5mg administered intramuscularly in the gluteal region, 11 days apart. Vaginal smears were stained using a modified Papanicolaou method while the vaginal or rectal temperatures (°C) was recorded with a standard (dry-bulb) clinical thermometer taken from 07:30 to 08:30 hrs daily and monitored throughout the period of experiment. In vaginal cytological smear examination, five exfoliated cell types were identifiable viz; anucleated superficial cells, superficial nucleated cells, intermediate cells, parabasal cells and leucocytes. Typically, the proestrus witnessed an increase in the proportion of anucleated superficial and superficial nucleated cells, with marked reduction in both intermediate and parabasal cells. Oestrous vaginal smear cells were highly cellular and consisted predominantly of superficial nucleated cells. Metestrus and Diestrus phases were markedly similar with respect to cell population and distribution. Metestrus phase showed drastic reduction in number of superficial cells and the proportion of leucocytes in smears began to rise while the desquamation of parabasal and intermediate cells with leucocytosis characterized the diestrus phase. In thermal study, there was significant ($P< 0.05$) difference in both mean rectal temperature (MRT) and mean vaginal temperature (MVT) values on day of estrus, the same not the case during the three other stages of the oestrous cycle, however, there was no significant ($P> 0.05$) difference between the mean rectal temperature (MRT) and mean vaginal temperature (MVT). Taken together, these results indicate that vaginal cytologic evaluation and temperature changes can be used as an adjunct tool during oestrus detection and for predicting time of ovulation in this breed; and furthermore, either of diurnal temperature rhythms, MRT or MVT can be used, in combination with other diagnostic tools, as a potential on-farm indicator of oestrous stage in Nigerian indigenous breeds of Red Sokoto does.

**Keywords** | Exfoliative vaginal cytology, Oestrus cycle, Red Sokoto does, Rectal temperature, Vaginal temperature

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## INTRODUCTION

Reproduction is one of the most important considerations in determining the profitability of small ruminant production (Dávila and Muñoz, 2020) in the tropics, and there exist possibilities that valuable improvements in the biological and economic efficiency can be achieved through the appropriate application of modern reproduction techniques such as oestrus synchronization, pregnancy diagnosis and artificial insemination (Alexander et al., 2010; Andrabi et al., 2015; Sen and Onder, 2016; Llanes et al., 2019). Therefore, the successful application of above techniques, requires good knowledge of reproductive cycle, especially the stages of oestrus cycle and the optimal time for mating.

The estrous cycle represents a complex neurohumoral process characterized by morphological and physiological changes in some of the reproductive organs, such as the vagina. These cyto-morphological changes in exfoliated cells in the vaginal smears, fairly reflect the underlying endocrine events of the reproductive cycle, due to sensitivity of vaginal epithelium to stimulation by sex hormones (Kinoshita et al., 2012; Peña-Corona et al., 2019; Holumbiyovska et al., 2021) and are, thus, considered as a better predictor of the breeding time.

It is generally accepted that exfoliated cell types in the vaginal smear are related to the stage of the estrous cycle (Cora et al., 2015); which makes the vaginal exfoliative cytology, a simple, non-invasive technique routinely employed by clinicians for reproductive diagnostics (Moxon et al., 2010; Zohara et al., 2014; Sharma and Sharma, 2016; Siregar et al., 2016; Antonov, 2017; Sitaresmi et al., 2018; Reckers et al., 2022).

Research relating to cyclical changes in exfoliated vaginal cytology used to characterize the estrous cycle have been reported in women (Papanicolau, 1963; Berga et al., 2019), rats (Paccola et al., 2013; Cora et al., 2015) and also, in a variety of domestic animal species, including the bitch (Pérez et al., 2005; Maenhoudt et al., 2018), queen (Kustritz, 2012; Kanca et al., 2014), bovine (Siregar et al., 2016; Holumbiyovska et al., 2021), ewes (Solis et al., 2008; Zohara et al., 2014), does (Ola et al, 2006; Leigh et al, 2010; Ribeiro et al., 2019). The results of some of these studies were used clinically in the detection of estrus and early pregnancy (Reddy et al., 2011; Sharma and Sharma, 2016; Antonov, 2017), and as well as, in management of reproductive disorders and genital tract infections (Sharma and Sharma, 2016; Llazani et al., 2021).

Also, thermal changes have been monitored throughout the oestrus cycle. For instance, a rise in vaginal temperature during estrus has been reported by various workers (Ali et al, 2013; Indira et al., 2014; Sakatani et al., 2016). Others have reported results showing that a preovulatory vaginal temperature as well as rectal temperature increase, may be taken as a reliable indicator of the luteinizing hormone (LH) surge (Clapper et al., 1990; Rajamahendran and Taylor, 1991; Mosher et al., 1999; Fisher et al., 2008; de Freitas et al., 2018). It is well established that the knowledge concerning thermal changes--rectal and/or within the female genitalia (vagina) during the estrous cycle, may be of practical significance for optimizing estrus detection efficiency and optimal timing regimes for artificial insemination (Ali et al., 2013; Anggriawan et al., 2017), hence, thermal variations may be related to animal physiological status and hormonal activity (Sakatani et al., 2016).

Despite the vast application and optimization of these reproductive techniques in the breeding protocols and for gynecological examination in canine and bovine species, there are dearth of literature on the exfoliative vaginal cytology, as well as temperature changes, particularly in the does, relative to the stages of the estrous cycle (Ola et al., 2006; Indhumathi et al., 2020). Therefore, the aim of the present study was to characterize cyto-morphological alterations in exfoliated vaginal smears associated with each stage of the does' oestrus cycle; and to determine whether, rectal and/or vaginal temperature is predictive of any particular stage(s) of the oestrus cycle in the Red Sokoto does.

## MATERIALS AND METHODS

### Location and Climatology

The experiment was carried out at the National Animal Production Research Institute (NAPRI), Shika-Zaria, Nigeria. Shika is located on longitude 7° 3' E, and the latitude of 11° 12'N, and at altitude of 610 m above mean sea level. The climate is sub-humid and vegetation zone in Northern Guinea Savannah. The mean annual rainfall and ambient temperature recorded at the meteorological unit of the Institute of Agricultural Research (IAR) Samaru, (i.e 10 km from Shika), were 1107mm and 24.4°C respectively. The seasonal distribution of rainfall is approximately 0.1% in the late dry season (January-March), 25.8% in the early wet season (April-June), 69.6% in the late wet season (July-September) and 4.5% in the early dry harmattan (October-December) season (Odekunle, 2004)

### Animals and Management

A total of fifteen (15) apparently normal multiparous Red Sokoto does (mostly di- and tri-parous) and weighing between 20-35kg; with an average body score condition score of 3.0 (Mendizabal et al., 2011) and ranging in age between 3½ to 4years, were used for the study. The animals were individually identified by means of numbered plastic

ear tags. Prior to the commencement of the experiment, the animals were grazed daily from 0900 hrs to 1600 hrs on improved *Digitaria spp* and *Brachiara decumbens* pastures, with a daily supplementary concentrate ration of groundnut and wheat offal of 250g/head/day. Water and mineral salt licks were provided to the animals *ad libitum*. Also, strategic anthelminthic and prophylactic antibiotic treatment regimes using Wormex® [Piperazine adipate, Pfizer Nigeria Limited] administered at the dose rate of 250mg/kgbwt and Tetroxy LA® [Oxytetracycline, Bimeda Pharmaceuticals, Holland] administered at the dose rate of 10mg/kg bwt were given respectively, to ensure the animals were in healthy condition throughout the study. During the period of heat observation, all the does remained in the fenced paddock continuously and were not taken out for grazing but fed with sown improved pastures from the field.

The non-pregnant cycling (i.e open) does selected for the experiment were determined on the basis of kidding records and external abdominal digital palpation. The stages of the oestrus cycle were determined on the basis of standing oestrus and other stages of were determined relative to standing oestrus.

## Oestrus synchronization

All the does selected for the experiment were synchronized using two injections of $PGF_{2\alpha}$ [Lutalyse® *Dinoprost tromethamine*, UpJohn Limited, Crawley, UK] 12.5mg/head. The does (n=15) received 12.5mg of $PGF_{2\alpha}$, was administered (11 days apart) to the does, that does not exhibit behavioral symptoms of oestrus. In this study, estrous periods occurring within 1 to 7 days post-injection were classified as synchronized, although other investigators (Omontese et al., 2014, 2016; Famakinde et al., 2017; Bello et al., 2019; Parmar et al., 2020) have reported oestrus to begin, after an average interval of ≤50 hrs.

## Oestrus detection and mating

Following each treatment with $PGF_{2\alpha}$, the does were visually observed for oestrus continuously for about a week (Fonseca et al., 2012). Throughout the period of experiment, an active apronized buck about 4yrs of age and weighing 32kg, fitted with breeding harness crayon, was allowed to run freely into the doe's pen at least twice daily for the purpose of heat detection. All the does were visually observed for oestrus (i.e as evident by the crayon markings around the tail region) in the mornings (from 7.00 hrs - 9.30 hrs) and in the evenings (from 16.30 -17.30 hrs). Standing to be mounted by the apronized buck or by other does (homosexual mounting) was the only criterion used to consider a doe as having been in oestrus. All the oestrus observations through secondary signs of oestrus (i.e. vaginal discharge, behavioral changes) and the reaction of the does to the buck were noted and recorded.

## Sampling schedule

**Vaginal smear collection:** Reproductive cycles were monitored using the exfoliative vaginal cytology method (Leigh et al., 2010). For each day of the oestrus cycle, starting from first day of oestrus (Day 0), the vaginal smear was taken. The doe was restrained in standing position by lifting the hindquarter, and the external genitalia was thoroughly cleaned with a sterile gauze swab soaked in a diluted Savlon®[Chlorhexidine] solution. A sterile self-retaining vaginal speculum, Cusco® (bi-valve type) was then inserted obliquely into the vagina, to reach the anterior vagina about 5cm caudad to the opening of the cervix. Using a clean, sterile moistened Cytobrush™ inserted about 3 inches into the vagina, a smear (Figure 1) was taken and gently smeared on a clean fat-free, labelled microglass slides and air-dried immediately, before fixing in a Coplin jar containing equal volumes of diethyl ether and 95% ethanol. The smears were stained in the laboratory within 1hr of collection using a modified cytological stain such Papanicolau method (Pérez et al., 2005). Stained smears were cleared in xylene, mounted with Depex® mounting media and the examination of the smears were achieved using a bright-field Leitz™ Microscope (Leitz GmbH, Germany) at higher magnification of X400. The proportion of exfoliative vaginal cells during the various stages of oestrus cycle were classified according to morphological criteria described by Siregar et al (2016). A calibrated eyepiece graticule™ [Wild Heergrugg Ltd, Switzerland] with a fixed square of $1.89 \times 10^{-6}$ (fitted to a microscope eyepiece), was used to analyze the mean (arithmetic) counts of the vaginal smear cells. Ten blocks of the squares were identified and the cells that fell within these blocks were identified and counted. The average reading from four different fields on each stained microslides were recorded and the proportion-- expressed in percentage (%) of each of the cell types on the vaginal smear, was used to characterize the phases of the oestrus cycle.

## Temperature measurement

Temperatures (Rectal and vaginal) measured in °C using a digital clinical thermometer were also taken and recorded for each doe daily, starting from the day 0 [day of oestrus] and throughout the oestrus cycle. The Rectal and vaginal temperatures were monitored in the mornings between 7.30-8.30 hrs daily throughout the period of the experiment. The daily mean rectal (MRT) and mean vaginal (MVT) temperatures for each doe, relative to the stage of the oestrus cycle were calculated and analyzed.

## Data Analysis

The proportion and arithmetic mean (expressed in %) of each type of vaginal cells in the smear obtained, relative to

**Table 1:** Mean values (±SEM) of exfoliated vaginal cell types in Vaginal smears of Red Sokoto does at various stages of the Oestrus cycle.

| Cell Types | Proestrus | Oestrus | Metestrus | Diestrus (Early) | Diestrus (Late) |
|---|---|---|---|---|---|
| Superficial Nucleated (*Sp*) | 25.17 ±7.90 | 52.69± 7.36* | 23.01± 8.65 | 20.98 ± 7.43 | 22.69 ± 8.52 |
| Anucleated Superficial (*An*) | 51.57 ± 3.20** | 24.20 ±8.21 | 11.01 ± 7.65 | 8.58 ± 6.10 | 12.48 ± 6.25 |
| Intermediate (*It*) | 9.72 ± 6.75 | 16.00 ± 8.21** | 14.39 ± 9.28 | 7.50 ± 4.61 | 5.69 ± 2.56 |
| Parabasal (*Pb*) | 7.98 ± 4.82 | 5.18 ± 3.04 | 9.75 ± 6.46 | 12.36 ± 7.43 | 23.77 ± 7.20* |
| Leucocytes (*Leu*) | 5.62 ± 2.76 | 2.23 ± 0.03 | 41.84± 3.12 | 50.64 ± 10.63*** | 35.37 ± 9.76 |
| No of smears evaluated | 8 | 21 | 16 | 30 | 17 |

*Differences are significant between the values indicated with a superscript ($P< 0.001$);
**Differences are significant between the values indicated with a superscript ($P< 0.01$) ;
***Differences are significant between the values indicated with a superscript ($P< 0.05$)

**Table 2:** Comparison of Mean (±SEM) of Rectal and Vaginal temperature values of Red Sokoto does (n=15) at various stages of the Oestrus cycle.

| Stages of Oestrus cycle | Temperatures (°C ) | |
|---|---|---|
| | Mean Rectal Temperatures (MRT) | Mean Vaginal Temperatures (MVT) |
| Proestrus | $38.7^{a}$ ±0.04 | 38.6 ± 0.04 |
| Oestrus | $39.2^{b}$ ±0.08 | 39.2 ± 0.06 |
| Metestrus | $38.5^{c}$ ± 0.04 | 38.5 ± 0.03 |
| Diestrus (Early) | 38.2 ±0.05 | $38.2^{d}$ ± 0.05 |
| Diestrus (Late) | $38.3^{e}$ ± 0.04 | 38.2 ± 0.02 |

$^{a,b,c,d,e}$ means with different letter superscripts in each row are significantly different ($P < 0.05$).

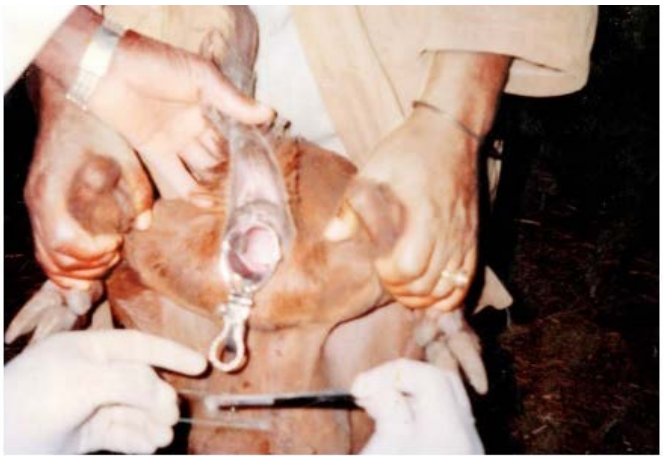

**Figure 1**: Exfoliated vaginal smear taken from the anterior vagina (5cm caudad to cervical opening) of Red Sokoto doe, using a self-retaining bivalved (BicuscoTM) speculum and with the aid of cytobrushTM, which then smeared onto a clean fat-free labelled micro glass slide.

the stages of the oestrus cycle were subjected to two-way ANOVA test using the General Linear Model Procedure of SAS Version 16. Least square means were compared through use of the probability of difference option of SAS, subsequently, the data were subjected to Duncan multiple range F-test to determine the differences between the mean values of various stages of the oestrus cycle. Graphical analysis was done using Sigma Plot™ (Jandel Scientific Software®, GmbH, version 12) and MS Excel 2019 Software. The value level of $P< 0.05$ was considered statistically significant.

## RESULTS

### Exfoliative Vaginal Cytology

A total of 92 vaginal smears obtained from the Red Sokoto does (n=15) were evaluated to determine the proportion of different exfoliated vaginal cell types in the smear, relative to the various stages of the oestrus cycle. The analysis of variance (ANOVA) for each of the five cell types was conducted and the mean (±SEM) values for the five stages of oestrus cycle (ie. proestrus, oestrus, metestrus, diestrus (early and late) were as shown in Table 1. The variance ratio for each of the five vaginal cell types (i.e superficial nucleated, *Sp*; anucleated superficial, *An*; intermediate, It; parabasal, *Pb*; and leucocytes, *Leu*) seen at various stages of the oestrous cycle was highly significant $P< 0.01$ (Table 1). During the proestrus period, the smears obtained were very scanty, superficial cells formed about two-thirds of the total cells present. Of this, anucleated superficial cells accounted for 51.6%, while the superficial nucleated cells accounted for 25.2%. On the other hand, intermediate cells, parabasal and leucocytes were comparative low, representing a mean proportion of 9.7%, 7.9%, and 5.6% respectively (Table 1 and Figure 2a). The superficial anucleated cells (*An*) were large and polygonal (Figure 2a). The cytoplasm

of these cells contains dark granules, which according to Papanicolaou (1963) are predominantly glycogen granules. The superficial nucleated (*Sp*) cells looked generally the same but contained centrally located pyknotic nuclei.

The vaginal cytologic smears obtained at oestrous stage (Figure 2b), were characteristically, highly cellular and free from debris. Superficial squamous cells were abundant with few parabasal and leucocytes. As depicted in Figure 3, there was a significant level of *Sp* i.e. 52.7% in the oestrus vaginal smears. Consequently, there was decrease in the number of the *An* to about 24.2%, *It* cell counts was highest (15.8%) at oestrus, while the *Pb* and *Leu* cell counts remained low (5.2% and 2.2% respectively), compared to their proestrual value.

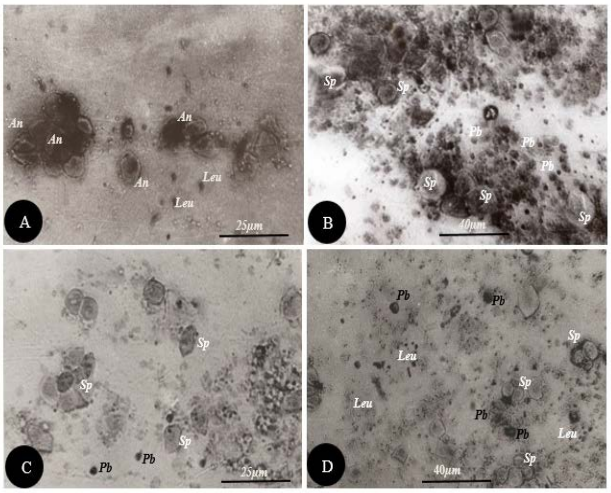


**Figure 2:** Photomicrographs of exfoliated vaginal cells smear taken during various stages of the estrous cycle A) Proestrus phase- showing several polyhedral superficial nucleated cells (An), single or in clusters and containing dark (glycogen) granules. Leucocytes (Leu) are seen but very scanty. B) Oestrus phase- Note the characteristic highly cellular nature of smear which is deeply basophilic stained. Superficial nucleated cells (Sp) are abundant, few parabasal (Pb) and Leucocytes (Leu) are seen. C) Metestrus phase- Note few superficial cells (polygonal in outline) and parabasal (Pb) cells, Leucocytes (Leu) begin to appear in this phase. D) Diestrus phase- Note the abundant leucocytes (Leu) i.e marked leukocytosis which is predominantly seen in the smear, also few superficial nucleated cells (Sp) are seen. Modified Papanicolaou stain.

Metestrus period witnessed a gradual reduction in the number of S*p* to a value of about 23%. A slight increase in the number of *Pb* cells, 9.8% was noted. The proportion of *Leu* (ie. neutrophils) present in vaginal smears at metestrus was more than that, seen during the proestrus and estrus periods (Figure 2c and 3). Interestingly, there was a sharp increase in the numbers of leucocytes during the early part of diestrus (50.6%), than at any particular stage of the oes

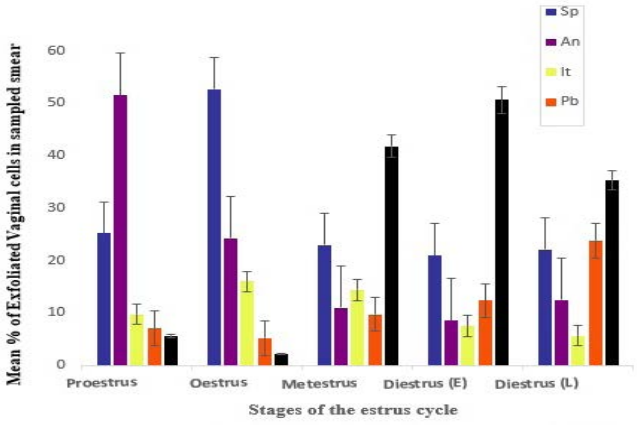


**Figure 3:** Percentage of epithelial vaginal cells (mean ± SEM) of the vaginal smears from Red Sokoto does (n=15) PGF2induced estrus at different stages of the Oestrus cycle. Legend: Sp- Superficial nucleated cells; An- Anucleated superficial; It- Intermediate cells; Pb - Parabasal; Leu- Leucocytes.

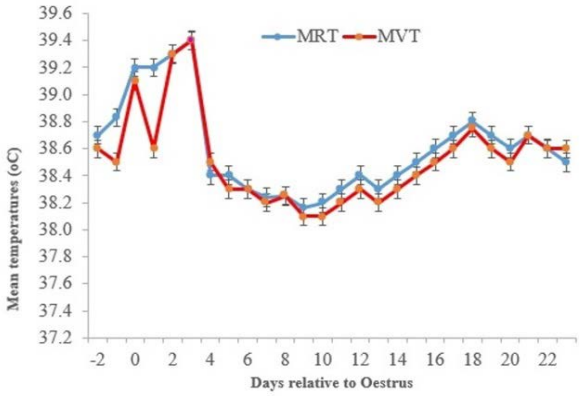


**Figure 4:** The mean (± SEM) of rectal and vaginal temperature values of Red Sokoto does (n=15) PGF2α-induced estrus at various stages of the Oestrus cycle. Legend: Mean Rectal temperature (MRT), Mean Vaginal temperature (MVT).

trous cycle (Table 1). *An* cells however, remained significantly low (8.6%) while *It* cells (7.5%) appeared to drop in their numbers. In contrast, *Pb* cells during this period showed an increase in number from metestrual value of 9.8% to 12.4% (Figure 2d and 3). The general smear picture during this stage was typically basophilic. Though, the *It* cells also stained basophilic, they were very much smaller than the *Sp* cells, although also polygonal in outline, but with slight variations. During the latter part of diestrus, the vaginal smear witnessed a significant increase in the number of parabasal (23%). Also, there was a concomitant increase in the number of *Sp* and *An* cells compared to their early diestrual value. Leucocytes counts show a gradual decline from early diestrual value of 50.6% to 35.4% in the late diestrus phase (Figure 3).

### Temperature changes

During the period of study, the temperatures (i.e rectal and vaginal) were taken early in the mornings from the Red Sokoto does throughout the oestrus cycle. The mean rectal temperature (MRT) and mean vaginal temperature (MVT) for the does (n=15) at different stages of the oestrus cycle were as shown in Table 2. In addition, the analysis of variance (ANOVA) showed that no significant ($P$> 0.05) difference in both MRT and MVT values during each stage of the oestrus cycle. However, there was significant ($P$< 0.05) difference between the MRT and MVT values at various stages of the oestrus cycle (Table 2).

At proestrus, the mean values of rectal (MRT) and vaginal temperatures (MVT) were 38.7 °C and 38.6 °C respectively, but rose steadily to 39.2 °C (i.e difference of 0.5% for MRT and 0.6% for MVT values (Figure 4). During the oestrus period, there was a significant estrual rise in both MRT and MVT values, i.e. from the preovulatory values of 38.7 °C and 38.6 °C respectively to 39.2 °C, prior to ovulation. At metestrus, there was a sharp decline in MRT and MVT values from the estrual value of 39.3 °C (for both) to about 38.5 °C (Table 2), however, there was no significant ($P$> 0.05) difference between the mean values of rectal (MRT) and vaginal (MVT) temperatures during the early and late part of diestrus (Figure 4 and Table 2).

## DISCUSSION

### Vaginal exfoliative cytology

In the earlier exfoliative vaginal study, Schutte (1967) reported six (6) vaginal exfoliated cell types for the dog, while Pérez et al. (2005), using an ultrafast Papanicolau staining protocol reported five (5) cell types for the dog. Also, Kanca et al (2014) reported five (5) cell types for the queens. Similarly, Leigh et al. (2010) in does, Zohara et al. (2014) and Ribeiro et al. (2019) in ewes, reported about 5 or 6 cell types. In the present study, however, it is possible to classify the cells in vaginal smears of Red Sokoto does into 5 main types viz: the superficial anucleated, superficial nucleated, intermediate, parabasal and leucocytes based on the classification (Siregar et al., 2016) criteria. Although, the intermediate cells did not vary much in size to warrant their classification into small and large, as reported by (Schutte, 1967).

In the present study, vaginal smears at the proestrus stage, showed a rise in the proportion of anuclated superficial cells and nucleated superficial cells, with a reduction in intermediate and parabasal cells. This observation is in agreement with the reports (Reddy et al., 2011; Holumbiyovska et al., 2021) for the dog and Sitaresmi et al. (2019) in does. Also, the few numbers of parabasal and intermediate cells that were observed, is consistent with the findings of Siregar et al. (2016); but these observations were at variance with that of Kustritz (2012), who reported very high proportions of intermediate cells and few nucleated superficial cells at proestrus stage. But, what explains the remarkable increase in the proportion of anucleated superficial cells observed at the proestrus phase, may be related to concomitant rise in concentrations of circulating estrogen (E2) hormone levels released from the pre-ovulatory follicles, increasing activeness in uterus and vagina; and thereby causing vaginal epithelium (mucosa) to become hyperplastic, with subsequent hypersecretion of epithelial cells and cornification (Zohara et al., 2014; Sitasremi, 2019).

Also, at oestrus stage, vaginal smears were characteristically, highly cellular and consisting predominantly of superficial nucleated cells followed by anucleated superficial cells. These findings corroborate with the observations, in rats (Cora et al., 2015; Mohammed and Sundaram, 2018), in bitches (Pérez et al., 2005; Peña-Corona et al., 2019), in humans (Berga et al., 2019), in cows (Indira et al., 2014; Widyaningrum and Marhendra, 2020), in ewes (Solis et al., 2008; Zohara et al., 2014) and, in does (Fatet et al., 2010; Sitaresmi et al., 2018). Apparently, less leucocytes are encountered in this stage, which is at variance with the findings and observations of (Akusu, 1987; Ola et al., 2006) in a related breed: West African dwarf [WAD] does, depicting that mainly leucocytes and small epithelial cells were present at the oestrus stage. Similarly, Siregar et al (2016) in cow, Kustritz (2012) in bitch and Kanca et al (2014) in queens, have reported the absence of leucocytes in the vaginal smears during the oestrus stage. Noticeably, the gradual increases in E2 level makes the vaginal epithelium to become very thick and leading to increased cornification (tissue cellularity). Again, the oestrus stage predominantly is a period when the Graafian follicle is the most mature and ovulation is imminent (Santos et al., 2016). It is a period of maximum cellular proliferation due to increase in estrogen level (Guimarães et al., 2011; Indira et al., 2014) with influence on oestrus cyclicity.

The Metestrus period represents the transition from the estrogen (E2) hormone dominance to progesterone (P4) dominance. There is precipitous decline in the number of superficial cells and re-appearance of intermediate and parabasal cells. This reduction in number of superficial epithelial layer as witnessed in metestrus phase, from this study, may be due to a diminished estrogen level (Gaafar et al., 2005). Kustritz (2012) in bitch and Solis et al. (2008) in ewes, also reported the intermediate cells and parabasal cells to predominate in Metestrus. Also, Metestrus is characterized by marked vaginal leucocytosis, as evident in this present study, perhaps, the leucocytic invasion was due to interaction between estrogen and progesterone hormone (Kinoshita et al., 2012), and this probably serves as normal physiological response to clear the desquamated cells

which are recognized as foreign body. Also, the vaginal epithelium is continuously being sloughed off (desquamated), as a result of drop in the level of estrogen hormone in the blood. More leucocytes migrate from the sub-epithelial microvasculature through the vaginal epithelium and are released into the lumen (Reddy et al., 2011).

Diestrus smears were markedly similar, in smears seen at metestrus period in the doe, with respect to cell population and distribution. The proportion of leucocytes (i.e. neutrophils) observed in smears peaked its maximum at diestrus i.e indicative of vaginal leucocytosis. Also, there was desquamation of parabasal and intermediate cells to slightly higher value in diestrus. This perhaps may be linked with the influence of functional corpus luteum which is predominant (Kinoshita et al., 2012) at this stage, as it serves as a mechanism to clear the desquamated cells in preparing for the next oestrus cycle.

A plausible explanation for the cyto-morphological changes i.e. exfoliation process observed in the doe-goat during the various stages of the reproductive (oestrous) cycle, may be attributable to cyclic changes occurring in the reproductive organs ie. ovaries and the tubular genitalia (Santos et al., 2016; Sitaresmi et al., 2018; Indhumathi et al., 2020). It is well established that the variations of vaginal mucosa at each phase of estrus cycle occurred, primarily, under the effect of estrogen (E2) and progesterone (P4) hormones that is secreted from the ovary (Gaafar et al., 2005; Fatet et al., 2010; Zohara et al., 2014). Exfoliated cells observed in the vaginal lumen, are direct result of rising peripheral estrogen (E2) hormone, which causes the vaginal wall (epithelium) to become thickens. As the outermost layers moves further from the vascular supply, the cells keratinize and detach (exfoliate) from the vaginal wall (Guimarães et al., 2011; Cora et al., 2015; Santos et al., 2016; Sitaresmi et al, 2018, 2019; Indhumathi et al., 2020).

Also, it is noteworthy to mention that, there are discrepancies and fluctuations in the relative number of exfoliated vaginal smears as reported by different authors. In a related study, Ola et al. (2006) reported that epithelial cells and leucocytes were present in the vaginal smears throughout the oestrus cycle of the WAD does and the daily variations in vaginal smears, were not striking enough to distinguish between the different stages of the cycle. Nevertheless, this observation is at variance with the sequential pattern of vaginal cytological smears as observed in our present study. Again, such discrepancies may be attributed to the differences in the period of observation of the vaginal smears; or variability in reporting of different cell types and/or the technical experience in interpretation of the vaginal smears (Moxon et al., 2010; Arlt, 2018), since the exfoliated cells once removed from their natural environment may assume forms differing from similar cells as they appear in sections; and therefore, change shape and structure and may become dehydrated. Some authors (Ducatman and Wang, 2002; Reckers et al., 2022), have also noted that the cellular picture as revealed by vaginal smear was never exactly the same in any two oestrus cycles.

## Temperature changes

In the thermal study, the mean rectal temperature (MRT) and mean vaginal temperature (MVT) changes in the Red Sokoto does observed in this study, varied slightly throughout the phases of the oestrus cycle. There was no significant difference ($P>0.05$) between the MRT and MVT, used as a variable to indicate any particular stage of the oestrus cycle. In other words, neither of the two (i.e. MRT nor MVT) can be applied as a variable for the predictor of a particular stage of the oestrus cycle, other than oestrus.

Remarkably, there was significant rise in both temperatures (MRT and MVT) values, from Day -2 to Day 3 (proestrus and estrus)—the proliferative phases, when estrogen hormone concentration is predominant, and thereafter, the temperatures drop sharply at the beginning of metestrus—the secretive phase, when progesterone (P4) hormone concentration, synthesized by the corpus luteum steadily begins to rise. On the basis our results and the timing of the rise in temperature, we postulated that this pattern is more likely to be as a result of localized effect of P4 secreted during the luteal phase, on the vaginal tissues that contributed to the rise in body temperature (Kyle et al., 1998; Acosta et al., 2003; Suthar et al., 2012), than the consequence of generalized thermogenic actions (Ali et al., 2013; Abdulhay et al., 2014). This observation is in agreement with the findings of (Clapper et al., 1990; Rajamahendran and Taylor, 1991; Mingoas and Ngayam, 2009) in bovines, (Anggriawan et al., 2017; de Freitas et al., 2018) in ewes and that of (Samuel et al., 2018) in does, who reported an average estrual rise in rectal temperature; and that of (Fisher et al., 2008; Vickers et al., 2010; Suthar et al., 2011; Sakatani et al., 2016) in bovines, who also noted a rise in vaginal temperature, on the day of oestrus. Elevated levels of estrogen (E2) hormones are responsible for vascular changes, such as increase in blood flow, that occur in the vaginal and vulvar tissues, causing an increase in vaginal temperature during estrus. Also, such elevation in MRT (39.2 ± 0.8°C) values, as observed in present study, perhaps could be attributed partly to increase in physical and/or muscular activities, that are usually seen during estrus (Clapper et al., 1990; Mosher et al., 1990; Vickers et al., 2010).

However, MRT and MVT values continue to vary inconsistently throughout the stages of the Oestrous cycle. The rise and dip in both temperatures as observed, in this study, therefore make thermal changes as a predictor of various

stages of oestrus cycle--in this specie, very inconclusive. Similar observations were also reported by Rajamahendran and Taylor (1991); Mingoas and Ngayam (2009) and Vickers et al. (2010) in the bovines, who noted that the body temperature was not a practical tool for determining breeding time in cattle; and that of Piccione et al. (2003) who also noted that heat and temperature phenomenon in animals, were inconclusive. Therefore, as revealed from the present study, it can be said that MRT and MVT are predictive of the oestrus stage, only.

## CONCLUSION

Taken together, the results of the present study indicate the sequential pattern of exfoliation of vaginal cells, rectal and/or vaginal temperatures during the does oestrus cycle, can be used as a cost effective, non-invasive methods of determining the oestrus stage, and invariably-- the optimal mating time in this breed. This will elucidate our understanding of the reproductive morpho-physiology and oestrus cycle phenomenon in the Red Sokoto does, which, consequently, will be beneficial to the growing Artificial Insemination (AI) sub-sector of the livestock industry in Nigeria.

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### Ethical Approval

All experimental procedures and animal protocols were carried out in accordance with the guidelines and approval of the Institutional Animal Ethics Committee (AEC) of the Ahmadu Bello University, Zaria [vide Protocol No.V2001-83-19].

## CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

## AUTHORS' CONTRIBUTION

UMB, SAO conceived the study, participated in its design and conception. AG, AA(Jr) involved in experimental design/field study and coordination. UMB performed the laboratory work, statistical analysis of data, and wrote the entire manuscript. MNB involved in vaginal smears collection, animal welfare/handling. COI had a consultative role and involved in critical review/editing of the revised manuscript. All authors read and approved the final version of the manuscript.

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