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Exfoliative Vaginal Cytology and Serum Progesterone during the Estrous Cycle of Indigenous Ewes in Bangladesh

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ABSTRACT

A study was carried out on 16 indigenous ewes in Bangladesh in order to assess the reproductive physiology, the pattern of vaginal cell exfoliation and progesterone profiles during the estrous cycle period. The mean estrous cycle length and duration of estrus were 15.8±0.12 days and 31.1±0.57 h respectively. The exfoliated epithelial cells were categorized into parabasal, intermediate, superficial and keratinized and their relative occurrences. The percentages of parabasal, intermediate and superficial cell type during proestrus were similar. The percentage of superficial cell type during estrus was 61.7%, which was significantly (p<0.01) differ from other types of cells and stages of estrus cycle. Metoestrus was predominant with neutrophils in addition with other cell types. Dioestrus was dominated by neutrophils. On days 0 to 5 of the cycle the progesterone concentration was 0.09 to 1.6±0.07 ng/ml. The length of diestrus was 5～10 days with a range of mean progesterone level of 1.6±0.07 to 2.8±0.11 ng/ml. Progesterone levels increased significantly (p<0.01) after Day 5 and maximum level was 2.8±0.11 ng/ml observed on Day 10 of the estrous cycle. Thereafter it dropped rapidly to basal level of 0.11±0.04 ng/ml on Day 0 (p<0.01). These results indicate that the pattern of exfoliation of vaginal cells along with progesterone concentration could be used to determine the reproductive stages of indigenous ewe.

(Key words: estrus cycle, vaginal cytology, serum progesterone, ewes)

INTRODUCTION

The knowledge of the reproductive physiology of estrus cycle is important in flock management and in order to determine the reproductive and productive potential of the indigenous sheep. The stage of the estrus cycle was predicted through the morphologic, endocrine and secretory changes occurring in the ovaries and the tubular genitalia during the estrous cycle of the ewes which have been associated with levels of steroid sex hormones. Vaginal cytology changes during estrous cycle have been studied in sheep (Hounzangbe-Adote, 1994), goats (Ola *et al.*, 2006), swine (Valerie *et al.*, 2003) and in boars (Mayor *et al.*, 2005). The morphology of exfoliated cells has been found very useful to determine the physiological and pathological status of the female animal as well as a tool for hormonal bioassay in several animal species (Perez-Martinez *et al.*, 1999, Lafi *et al.*, 1997). Many researchers have been studied the variations which occur in the vaginal mucosa at different phases of estrus cycle by using vaginal smears (Reddy *et al.*, 2011; Dudek, 2004). These variations occur under the effect of estrogen and progesterone hormones that secreted from the ovary (Lamond and Lang, 2005). The relative proportion of different types of vaginal epithelial cells can be used as a marker of the endocrine environment (Lamond and Lang, 2005; Arthur *et al.*, 1996). Moreover the vaginal cytology may be used clinically to evaluate the hormonal status, or/and to characterize the reproductive stages in the ewes. However, there has been no systemic study of reproductive patterns and vaginal cytology of indigenous sheep in Bangladesh. Thus, the purpose of the present work was to evaluate morphologic characteristics of the epithelial cells from the vagina along with the measurement of serum progesterone concentration during estrous cycle that not being studied before in indigenous ewes in Bangladesh.

MATREIALS AND METHODS

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1. Study Area and Animals

The study was carried out at the Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh. The research units are located on N 24.73 and E 90.44 latitude and longitude, respectively and elevated 9 m above sea level. The area receives on average 174 mm of rainfall. Mean annual minimum and maximum temperatures experienced at the site are 16.46 and 29.13℃, respectively.

The study was conducted between January to December, 2011. A total of 16 clinically healthy indigenous ewes of 2～3 months old and 6～8kg body weight were used for this study. Two grown up rams were vasectomized in the laboratory to be used as teasure. The ewes were maintained on natural grazing along with concentrate mixture (25% crushed maize, 50% wheat bran, 20% soybean meal, 1% fish meal, 2% DCP powder, 1.5 % salt and 0.5% vitamin- mineral premix) during the experimental period. Water was supplied *ad labium*.

2. Detection of Estrus and Estrus Cycle Length

Estrous detection in ewes was carried out twice a day for 1 h period of observation using vasectomized ram. Estrus duration was defined as the time interval between the onset of estrus and when a ewe no longer stood to be mounted (Godfrey *et al.*, 2001) while estrous cycle length was defined as the number of days between the onsets of two consecutive estrous periods (Chemineau *et al.*, 1992a).

3. Vaginal Smear and Cytology

For determining the stages vaginal smear was collected from the ewes with the aid of vaginal swabs which consisted of clean, soft and gentle pure cotton buds. The vulva and perineum were rinsed with clean water and gently wiped with a clean towel. Each ewe was well restrained in standing position by an assistant and the swab was gently inserted into the anterior vaginal with the right hand while the left thumb and forefinger were used to expose the vulva lips. At the anterior vagina, the swab was gently and briskly rolled against the vaginal mucosa and carefully withdrawn. The swab was immediately smeared on a warm gland slide, air dried and immediately fixed with 100% ethanol. The smears were stained with stained with Giemsa stain (Hewitt, 1984). The cells encountered in the vaginal smear were categorized as percentage of superficial, parabasal, intermediate and leucocytes. Identification of vaginal epithelial cells was performed by microscopic observation (G×100), based on their morphological and stained characteristics (Hounzangbe-Adote, 1994; Valerie *et al.*, 2003). The percentage of vaginal cells was calculated as the number of each type of cell divided by the total number of cells seen within 2 microscopic fields.

4. Measurement of Serum Progesterone

Ovulation response and cyclical stages was further confirmed by the measurement of serum progesterone concentration. Blood sample (5 ml) was collected from each ewe of both groups on every 5th day in order to determineluteal activity. Serum was recovered by centrifugation (15 minutes at 3,000 rpm) and stored at −20℃ until assayed for serum progesterone concentrations using commercially available sheep progesterone (PROG) ELISA kit (Cusabio Biotech Co., Ltd., Wuhan, Hubei Province 430206, P.R. China).

5. Statistical Analysis

Data were analyzed using a statistical program (SPSS, version 17.0). Vaginal cells percentages among the stages of estrus cycle were compared by Chi-square test. The mean progesterone concentrations between groups were analyzed by ANOVA-repeated measures. Significance was assigned at $p<0.05$.

RESULTS

1. Estrus Duration and Estrus Cycle Length

The frequency distribution of estrous cycle length throughout the experimental period is shown Fig. 1. Total 128 estrous cycles were recorded where length of 16 days had higher (83) and 13 and 20 days lower number (1), respectively. The mean estrous cycle length and duration of estrus were 15.8±0.12

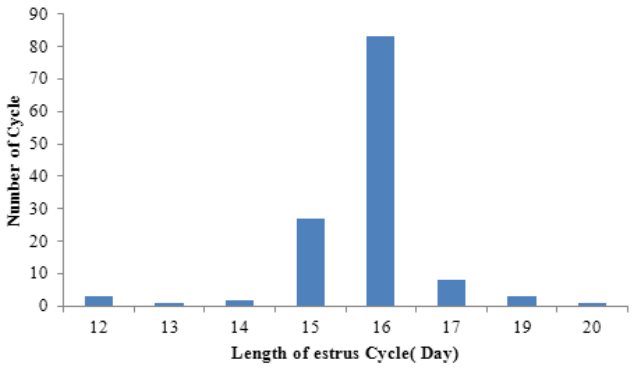

Fig. 1. Frequency distribution of estrous cycle length in indigenous ewes.

days and 31.1±0.57 h respectively.

2. Vaginal Cytology

During the study a total of 5 types of vaginal cells were identified and illustrated in figures (Fig. 2A, 2B, 2C, 2D). Parabasal (p) cells were small, round or nearly round and had a high nuclear to cytoplasmic ratio, oval shape with large, prominent nuclei or polygonal shape with a small nuclear/cytoplasmic ratio were intermediate (i) cells. The Neutrophils (n) were the little ones with segmented nuclei. Polygonal in shape and distinctly flat, with pyknotic nuclei (very small and dark) were superficial (s) cells. Keratinized (k) or cornified cells were without nuclei, seen in large sheets or strings (Fig. 2F). Normal bacterial flora was present and organisms often were attached to the superficial cells. Immediately after mating, large number of spermatozoa was detec-ted among the epithelial cells in all smears improves the accuracy of predicting ewes for mating (Fig. 2E).

Cell counts increased sharply after standing oestrus and clumping of the cells increased in metestrus (Fig. 2C). The intermediate cell dominated the majority of the smears, however, superficial cells were more frequently observed and they appeared to be associated with the proestrus, oestrus and early metoestrus phases of the cycle. The keratinization and the appearance of quantities of material of a cheesy consistency within 3 days following oestrus and usually persisted for 3 or more days. In diestrus, smear contained highly keratinized, generally anucleated cells and seen in large sheets or strings

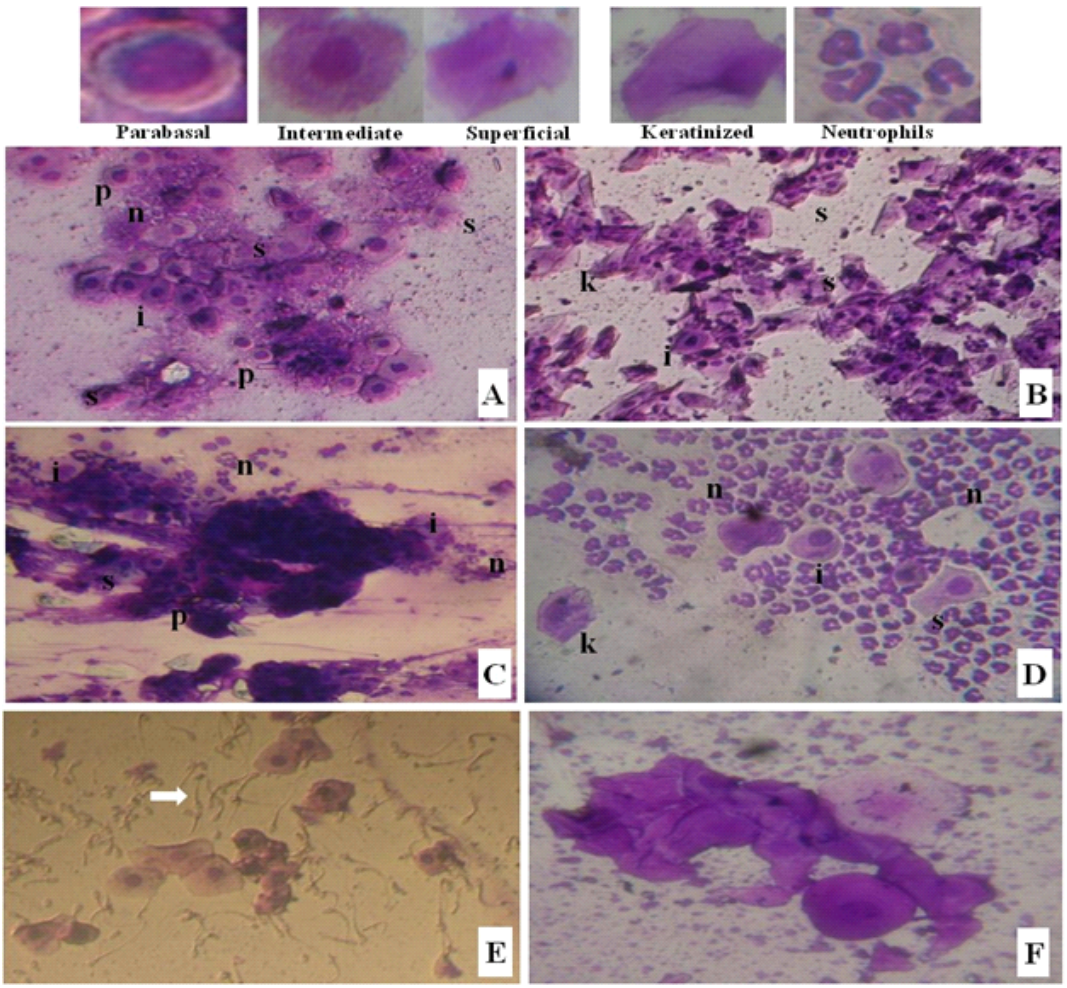

Fig. 2. Photomicrographs of exfoliative vagina cells during various stages of the estrus cycle of indigenous ewes (A: pro-oestrus, B: estrus, C: metoestrus, D: diestrus, E: estrus after mating and F: early diestrus). Name of the vaginal epithelial cells shown as parabasal (p), intermediate (i), superficial (s) and neutrophils (n).

in some smears (Fig. 2F).

The percentages of vaginal epithelial cells according to stages of estrous cycle are presented in Table 1. In proestrus, the percentages of parabasal, intermediate and superficial cell type were similar. During estrous phase, superficial and keratinized superficial cells reached their significantly (p<0.01) higher rate (61.7% and 31.9%, respectively). Polymorphonuclear neutrophils were generally absent during this stage of the cycle but only one vaginal smear showed little number of neutrophils (2.1%). In metestrus, the cells chiefly observed included parabasal (3.5 %), intermediate (15.8%), superficial (26.3%), keratinized (26.8 %) and neutrophils (27.5%). Neutrophils (40.2%) were commonly observed in the diestrus smear together with cornified cells (27.2%), intermediate (4.8%) and superficial cells (11.5%). The differences between the percentage values for each stage of the study were significant (p<0.01).

3. Serum Progesterone

The Mean±S.E serum progesterone concentration in different days and stages of estrus cycle of indigenous ewes is presented in Fig. 3. The length of diestrus phase was 5～10 days with a range of mean progesterone level of 1.6±0.07 to 2.8±0.11 ng/ml. Maximum level of progesterone during this phase was 2.8±0.11 ng/ml and was observed on Day 10 of the estrous cycle. The estrus stage lasted 24～36 hours, with a mean progesterone level of 0.12±0.02 ng/ml. On days 0 to 5 of the cycle the progesterone concentration was 0.09 to 1.6±0.07 ng/ml. Progesterone levels increased significantly (p<0.01) after Day 5 and maximum level was 2.8±0.11 ng/ml observed on Day 10 of the estrous cycle. Thereafter it dropped rapidly to basal level of 0.11±0.04 ng/ml on Day 0 (p<0.01).

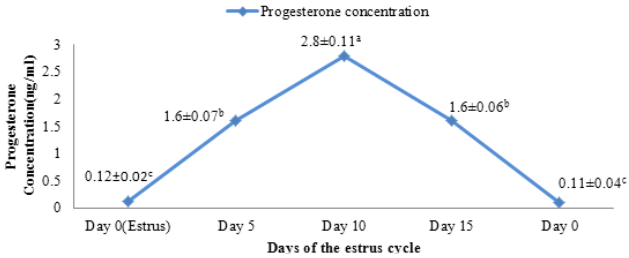

Fig. 3. Mean±S.E serum progesterone concentration (ng/ml) in different days of estrus cycle of indigenous ewes in different stages of the estrous cycle. [a~c] Values with different super scripts are significantly different (p<0.01) according to ANOVA-repeated measures.

DISCUSSION

The average length of the estrous cycle was comparable with observations made on other breeds (Talafha and Ababneh, 2011; Naqvi, 2001). The mean duration of estrus was within the range reported in previous studies (Talafha and Ababneh, 2011; Evans *et al.*, 2004; Elias *et al.*, 1985).

Vaginal cytology is an important aid for the diagnosis of sexual cycle. The typifying of morphologic and morphometric characteristics of the epithelial cells from the vagina was accordance with the study of other researchers (Dudek, 2004; Yamada and Kozicki, 1998) that the vaginal epithelial cells thrown into four type's viz. parabasal, intermediate, superficial and superficial keratinized cells and polymorphonuclear neutrophils. The mean cell counts of the epithelial cells and neutrophils were also compared between the days of cycle. Cell counts increased sharply on days 1 and 2 after standing oestrus. This results recorded in this study is similar to the study of Ola *et al.* (2006). Ghannam *et al.* (1972) observed that during the estrogenic phase of the estrous cycle of sheep, there is an increase in superficial and intermediary cell numbers.

Vaginal epithelial cells proportions trend recorded during

Table 1. Percentage of different exfoliative cells of vaginal smears in stages of estrus cycle in indigenous ewes

Stages of the estrus cycle	Percentages of vaginal cells				
	Parabasal	Intermediate	Superficial	Keratinized	Neutrophils
Proestrus	22.1^{Aa}	28.4^{Aa}	29.0^{Ba}	12.0^{Cb}	8.5^{Cb}
Estrus	0.0	4.3^{Cc}	61.7^{Aa}	31.9^{Ab}	2.1^{Dc}
Metestrus	3.5^{Bc}	15.8^{Bb}	26.3^{Ba}	26.8^{Ba}	27.5^{Ba}
Diestrus	0.5^{Ce}	4.8^{Cd}	11.5^{Cc}	27.2^{Bb}	40.2^{Aa}

[a~e] Different lower-case superscripts in a row indicate a statistically significant difference (p<0.01) according to Chi-squared test.
[A~D] Different upper-case superscripts in a column indicate a statistically significant difference (p<0.01) according to Chi-squared test.

estrous phases is similar to what was previously reported by Clemente Ovando (2013) and Hounzangbe-Adote (1994) in sheep; Ola *et al.* (2006) in goats, although variations are observed in their rate, which could be due to genetic factors in relation with each specie and environmental conditions. The rate of superficial cells we recorded (61.7%) on oestrous day was within the range of those obtained on zebu cows (Kilekoung Mingoas and Lalaud Ngayam, 2009), on mouse by Harold (2005), on sow by Rodgers *et al.* (1993), Valerie *et al.* (2003) and on boar by Mayor *et al.* (2005), which ranged from 60 to 90% on estrous day.

Day by day vaginal cells changes monitoring during the estrous cycle is also in accordance with what was observed in the mouse vagina by Harold (2005) and on brush tailed porcupine by Mayor *et al.* (2003) who concluded that this could be used as predictors of estrous cyclicity.

In this experiment, the progesterone level in estrous day was below 1.0 ng/ml. The average progesterone level and cyclic pattern reported in this experiment correspond with findings reported by Braun *et al.* (1988). Lafi *et al.* (1997) reported that the vaginal epithelium is greatly affected by ovarian hormones. Dudek (2004) mentioned that under the influence of estrogen, the epithelial cells accumulate large amount of glycogen and undergo cell proliferation in the basal and parabasal layers. During metestrus and diestrus in ewes, the corpus luteum becomes fully developed, and the effects of its progesterone, on the vaginal smear are marked. Numerous intermediate, superficial cells and neutrophils infiltration accompanied with few number of keratinized cells were observed, when the serum progesterone level was elevated during the diestrus phase of indigenous ewes (Ghannam, *et al.*, 1972). The number of larger cells was sharply reduced when the progesterone was predominant during metestrus and diestrus. Contrary to the current study, El-Sayed and Abdel-Ghaffar (1998) found that the neutrophils infiltration was decreased, when the plasma progesterone level was elevating in diestrus ewes.

The present results on vaginal epithelial cells and serum progesterone concentration changes during estrous cycle could also be a useful tool for detection of stages of estrus cycle and ovulation in indigenous ewes in Bangladesh.

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