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Review Article

Vaginal Cytology: An Historical Perspective on its Diagnostic Use

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Abstract | The knowledge of the reproductive physiology of estrus cycle is important for animal management and in order to determine the reproductive and productive potential of animals. The stage of the estrus cycle was predicted through the morphologic, endocrine and secretary changes occurring in the ovaries and the tubular genitalia during the estrous cycle, which had been associated with levels of steroid sex hormones. Vaginal cytology is a simple technique that can be used by practitioners to characterize stages of the reproductive cycle, disease conditions of genital tract and is a useful tool in determining optimum mating time (standing heat) in bitches. Many researchers studied the variations that occur in the vaginal mucosa at different phases of estrus cycle in bitch, sheep, goats, swine, cow etc. by using vaginal smears. The vaginal epithelial cells are classified into three cell types according to the differences in their sizes as a superficial squamous cells (40-65um diameter) with light cytoplasm, intermediate squamous cells (20-40um diameter) and parabasal cells (12-15um diameter or four type's viz. parabasal, intermediate, superficial intermediate and superficial cells. The relative proportion of different types of vaginal epithelial cells can be used as a marker of the endocrine environment as under the influence of estrogen, the epithelial cells accumulate large amount of glycogen and undergo rapid cell proliferation in the basal and parabasal layers and more number of superficial squamous cells are produced. Increased number of neutrophils and endometrial cells in vaginal smear is shown to be related with metritis in cow. A vaginal smear during pregnancy is characterized by few intermediate cells, small number of R.B.C. and moderate to large number of polymorphonuclear cells. The vaginal cytology may be used clinically to evaluate the stage of estrous cycle, hormonal status, disease condition of genital tract.

Keywords | Vaginal cytology, Cow, Sheep, Bitch, Reproductive cycle

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INTRODUCTION

Vaginal exfoliative cytology is reported to be a sensitive indicator of the stage of oestrus cycle in many species presumably reflecting the balance between the influence of oestrogens and progesterone (Papanicolau, 1954). Exfoliative vaginal cytology has been used in diagnosing various reproductive stages and in predicting certain abnormal conditions during periparturient and post-partum periods (Rao et al., 1979). The vaginal epithelial cells were classified according to their location in the vaginal mucosa as parabasal, intermediate, superficial intermediate and superficial cells (Mayor, 2005).

In some species *viz.* dog, cat, mouse and rabbit such changes are fairly consistent and can reliably be used for diagnosing stage of oestrus and optimum time for mating or artificial insemination. However, in cows these changes are not consistent (Hansel, 1950; Rao et al., 1979).

The use of vaginal smear for assessing sex hormone activity is a convenient method, based on basic principle of sensitivity of vaginal epithelium to stimulation by sex hormones to which it responds by altering its height (number of cell layers) and thickness (number of cell rows per layer) in a characteristic way (Wachtel, 1969). In mammals, estrogenic activity causes cornification and desquamation of vaginal epithelium (Robson, 1947). Different phases of sexual cycle as well as other physiological reproductive phase like pregnancy or post-partum quiescent period represent dif-

ferent progesterone and estrogen ratio and can be assessed through vaginal cytology. Moreover, when any inflammatory condition affects then genital tract undergoes characteristic change in vaginal cytology.

EXFOLIATIVE VAGINAL CYTOLOGY IN DOGS

The examination of exfoliative vaginal cells is now commonly used to monitor the oestrous cycle of dogs. The relative proportion of different types of epithelial cells can be used as a marker of the endocrine environment. Many authors have described the cyclical changes occurring in the picture of exfoliative vaginal cytology (Hancock and Rowlands, 1949; Schutte, 1967). Vaginal epithelial cells may be collected using either a moistened swab or by aspiration. For the ease of examination the vaginal smear is stained with simple stains like Leishman's, a modified Wright-Giemsa stain or a trichrome stain like Shorr's. At the onset of pro-oestrus large number of erythrocytes are present (Olsan et al., 1984). This occurs due to diapedesis through uterine capillaries due to oestrogen effect. However as the cycle enters the true oestrus phase the number of erythrocytes is reduced considerably and the smear mainly consists of superficial cell types from the stratified squamous epithelium, such as anuclear cells, cells with pyknotic nucleus and large intermediate cells, which could probably be due to rise in oestrogen concentration (Linde and Karlson, 1984). During pro-oestrus, oestrus and early metoestrus the epithelium and lamina propria are infiltrated with large numbers of neutrophils, which eventually escape into the vaginal lumen (Blendiger, 2013). Towards the end of oestrus, numbers of polymorphonuclear neutrophil leucocytes appear in the smear. During metoestrus these polymorphonuclear neutrophil leucocytes are the dominant cell type. In anoestrus the smear consists of nucleated basal and intermediate cells of the stratified squamous epithelium together with few neutrophils.

During pro-oestrus increased plasma oestrogen concentrations cause thickening of the vaginal mucosa, which becomes a keratinized squamous epithelium (Reddy et al., 2011). The fertile period can be predicted by calculating the percentage of epithelial cells, which appear cornified. Polymorphonuclear leucocytes are absent from the vaginal smear during oestrus because the keratinized epithelium is impervious to these cells (Kusritz, 2012). Their reappearance during the late oestrus reflects the breakdown of this epithelium (Evans and Cole, 1931). The return of polymorphonuclear leucocytes to the vaginal smear can be used as an indicator of the time of optimum fertility (Andersen, 1980). Feldman and Nelson (1996) suggested that breeding should be attempted throughout the period when more than 80% of epithelial cells are cornified in nature. Interestingly, in canines vaginal exfoliative cytology has been proposed as a good alternative to progesterone assay (Bonchard et al., 1991) or as a reliable indirect oestrogen assay (Feldman and Nelson, 1996) for timed mating in dogs.

EXFOLIATIVE VAGINAL CYTOLOGY IN CATTLE

Oestrous Cycle

In bovines the smear of exfoliative vaginal cells during oestrus shows enhanced cornification of epithelial cells (Rao et al., 1979). The cornified cells formed about 50% of the cell population in the smears of normal cycling animal at oestrum (Kurude et al., 1993). Subramanian and Pattabiraman (1988) observed an increase in number of superficial cells along with cornified cells in oestrum. In heifers, cornification of epithelial cells was found to be mild, moderate and intense at onset of oestrus, during late heat and after ovulation respectively (Rao and Rao, 1982).

The smear in dioestrus is characterized by an increased number neutrophils and intermediate cells (Subramanian and Pattabiraman, 1988). Kurude et al. (1993) reported an increased number of parabasal and intermediate cells under progesterone dominance.

Pregnancy

In pregnant cows, Hussain and Khan (1979) reported various vaginal epithelial cells during early, mid and advanced stage of pregnancy as 81.63, 85.9 and 88.23 percent of intermediate, 13.83, 5.97 and 4.53 percent of parabasal cells, 2.97, 5.5, 4.64 percent of superficial squamous and 1.57, 2.55 and 2.60 percent of cornified cells respectively. It is well evident that as in diestrum there is predominantly higher number of intermediate cells followed by parabasal cells during pregnancy. However, leucocytes and histiocytes were absent during most stages of pregnancy (Hussain and Khan, 1979; Hughes and Dodds, 1968). There occurs a characteristic change in percentage of different cell types (Small, intermediate, large with cornification and vacuolated) associated with the stage of pregnancy (Abdo, 1973). Subramanian and Pattabiraman (1988) have reported the presence of stripped nuclei and parabasal cells during pregnancy. Hussain (1979) stated that a smear showing lack of leucocytes along with predominance of intermediate cells with an increasing trend could be used as an aid in confirmation of pregnancy.

At around 250-260 days of pregnancy there is a significant increase in the number of superficial squamous cells. This could probably be due to estrogenic activity with continued strong progesterone influence. With advancement of pregnancy there is a non-significant decrease in their numbers but this is followed by a significant decline within

2-3 days of parturition. No changes have been recorded in the keratinized cells, which remain at around 1% level throughout the pregnancy.

An increase in the number of neutrophils is observed during 265-275 days of gestation and 2-3 days before parturition compared to other periods of gestation. This could possibly be due to the increase in vascularity associated with the influence of oestrogen.

Following parturition there occurs an increase in parabasal cells while the numbers of intermediate cells decline. This is ascribed to changes in progesterone estrogen ratio after parturition.

Disease Condition

Vaginal cytology has been utilized in diagnosing of many disease conditions of the genital tract. Gospodinov et al. (1987) reported the use of vaginal cytology in diagnosing disease conditions like hypofunction, persistent corpus luteum and metrorrhagia in bovine.

Repeat breeder cows showed an increase in the number of leucocytes and intermediate cells and a reduction in the number of cornified cells in the vaginal smears at oestrus (Kurude et al., 1993). Animals in anoestrus had an increased number of cornified cells in the vaginal smears (Hussain and khan, 1979; Kurude et al., 1993).

Subramanian and Pattabiraman (1988) observed stripped nuclei in anoestrum, neutrophils and endocervical cells in endometritis and cornified and partly cornified superficial intermediate cells in cystic ovarian conditions. They also reported presence of orange coloured, oval or round, large squamous cells with vacuolated cytoplasm and centrally placed large vesicular nuclei in the vaginal smear of cows and buffalo with prominent corpus luteum and were considered to indicate possible hormonal imbalance.

No much difference is observed in the vaginal cytology picture of metritis affected crossbred cattle in context of intermediate cells, parabasal cells keratinized and superficial squamous cells, when compared to the vaginal cytology of unaffected crossbred cattle. The number of neutrophils and endometrial cells in the vaginal smears is significantly higher in metritis affected crossbred cattle compared to normal crossbred cattle during the 3 weeks following parturition. However, by 28th day post-partum, the neutrophil population in vaginal smears showed significant increase in normal animals in comparison to metritis affected crossbred cattle.

Jubb et al. (1985) observed 1.93, 8.15, 10.5, 4.3 and 29.3 percent cornified cells in the vaginal smears of cattle with inactive ovaries, developing follicle, mature follicle, cyclic corpus luteum and follicular cyst respectively. This data reflects the influence of oestrogen on the cornification of cells.

EXFOLIATIVE VAGINAL CYTOLOGY IN SHEEP AND GOAT

Several workers have reported the role of vaginal cytology as a diagnostic tool in detecting reproductive stages in ewe (Cole and Miller, 1935; Sanger et al., 1958; Kraznicakova et al., 1992). However, other researchers have contested this claim stating that the characteristics of vaginal contents might not be constant to be used as a reliable diagnostic measure in detecting various reproductive stages (Grant, 1933; Radford and Watson, 1955). The changes occurring in the vaginal cytology picture during the course of oestrous cycle in ewes and does is more or less similar to the changes in cows. 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 (Zohara et al., 2014)

Pregnancy

A vaginal smear during pregnancy in goats is characterized by few intermediate cells, small number of R.B.C. and moderate to large number of polymorphonuclear cells (Lafi et al., 1997).

Grant (1933) had reported similar histological changes in vaginal mucosa during anoestrus and pregnancy. But this was contradicted by Ghannam and Bose (1972) as they reported that the vaginal smears of pregnancy, which consists of mostly small vaginal epithelial cells between 22nd and 83rd day of pregnancy, could be easily distinguished from the vaginal smears taken during sexual or anoestrous season.

In ewe Lafi et al. (1997) reported moderate number of RBC, parabasal cells, intermediate cells and superficial cells during puerperium while during same period in doe a lack of parabasal cells, with moderate number of small intermediate, superficial and cornified cells were observed.

The predominancy of vaginal epithelial cells differs from one stage to another may be due to the changes in physiological and hormonal status of the animal during the post parturient period. (Dudek, 2004; Lamond and Lang, 2005).

Disease

Vaginal smears taken from ewes suffering from pyometra had nil to few intermediate cells, moderate number of large intermediate cells and cornified cells, large number of superficial cells, few to moderate number of erythrocytes and

quite large number of polymorphonuclear cells (Lafi et al., 1997). However in doe, the condition was characterized by large number of both erythrocytes and polymorphonuclear cells with clusters of small and large intermediate cells and scattering of karyolytic cells all over the surface indicating a necrotic change of vaginal epithelium.

Smears from aborted ewes showed scanty parabasal cells, small intermediate cells, erythrocytes and polymorphonuclear cells but moderate number of large intermediate and superficial cells. However, smears from aborted doe showed scanty polymorphonuclear cells, moderate no of erythrocytes with clusters of parabasal cells, few granular cells showing vacuolation or cornification indicating degenerative changes along with necrosis and sloughing of epithelial tissue (Jubb et al., 1985).

Oestrogen treatment in ewes resulted in keratinization of the upper layers of vaginal epithelium by third day of oestrogen treatment (Green, 1959; Eddy and Walker, 1969). The oestrogenic effect on vaginal epithelium could be retarded or suppressed by combining it with progesterone (Green, 1959; Eddy and Walker, 1969).

VAGINAL BIOPSY

While in exfoliative vaginal cytology only the exfoliated cells are taken for examination, in vaginal biopsy histological sections are taken from the vaginal mucosa for clinical examination. The procedure of vaginal biopsy has been used for the diagnosis of stage of oestrous cycle and pregnancy in animal species like swine and ewe.

Histological assessment of the number of layers of the stratified squamous epithelium of the vaginal mucosa obtained by biopsy has been used for diagnosing of pregnancy in the sow (Morton and Rankin, 1969). The diagnosis depends on the number of layers of vaginal epithelial cells, which in turn reflect the endocrine state. During pro-oestrus, when oestrogen is dominant, a rapid proliferation of the stratum germinativum occurs so that at oestrus there are up to 20 layers. From the end of oestrus and throughout the luteal phase, when progesterone is dominant, the depth of vaginal epithelium falls and by day 11 or 12 there are only three or four irregularly arranged layers and only two or three layers in late dioestrous. During pregnancy the progesterone dominance continues, and by day 26 the histological picture shows two parallel rows of epithelial cells with condensed darkly staining nuclei. The pattern persists until the final 3 weeks of gestation. The best time for application of the test is between days 18 to 25 after mating. The accuracy of this method between day 30 and 90 days of pregnancy is over 90%. Between 18 and 22 days after service it is 97 and 94% for the diagnosis of pregnancy and non-pregnancy, respectively. The difference in the histological picture is greater between oestrus and pregnancy than dioestrous and pregnancy. Sections taken erroneously from the cervix or posterior vagina are unsatisfactory for diagnosis.

Vaginal biopsy has also been used for the diagnosis of pregnancy in ewes. The method is similar to that reported for the sow. Richardson (1972) found 81% accuracy in detecting barren ewes. The accuracy of detecting pregnancy after 40 days of gestation was 91% and it increased to 100% after 80 days of gestation. Errors in diagnosis may occur in ewes, which are in late dioestrous, and late anoestrous as the histological appearance of the sections is similar to that of pregnancy.

In sow Mota et al. (2002), reported the role of vaginal exfoliative cytology in detection of oestrus along with 17-β oestradiol and progesterone assay.

CONCLUSION

Vaginal exfoliative cytology is a good aid in the diagnosis of the stage of oestrus cycle in many species and it reflects the effect of interaction of various hormones, oestrogen and progesterone in particular, on the reproductive tract. Since the vaginal epithelium reflects the changes in hormone milieu, it follows that any abnormality in cycle either due to a direct hormonal involvement or disease condition would be reflected as changes in the cell types of vaginal epithelium. Exfoliative vaginal cytology has been used in diagnosing various reproductive stages and in predicting certain abnormal conditions during periparturient and post-partum periods. The procedure can reliably be used for diagnosing stage of oestrus and optimum time for mating in species in which the changes are fairly consistent. Exfoliative vaginal cytology has been most frequently used in the diagnosis of stage of oestrous in dogs. The technique has also found application in the small ruminants, but in cattle a lot of investigation needs to be done before the procedure can be used frequently and efficiently in diagnosing both the normal stages of oestrous cycle and the abnormal conditions of the genital tract.

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CONFLICT OF INTERST

There is no conflict of interest.

AUTHORS' CONTRIBUTION

All the authors have contributed equallyin terms of giving

their technical knowledge to frame the article.

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