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Short Communication

DIFFERENTIAL STAINING TECHNIQUES TO ASSESS THE STAINING CHARACTERISTICS OF UTERINE EPITHELIAL CELLS FROM UTERINE FLUSHING OF ENDOMETRITIS AFFECTED BUFFALOES

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SUMMARY

The present study was conducted in buffaloes which were brought to the Department of Veterinary Gynaecology and Obstetrics, NTR College of Veterinary Science, Gannavaram. The present study was designed to evaluate the efficacy of differential staining techniques (Modified PAP staining, Eosin and methylene blue staining, Field stain and Leishman's) for assessing the staining characteristics of uterine epithelial cells and polymorphonuclear cell from uterine flushing of endometritis affected buffaloes. In conclusion, Leishman's stain and Field's stain could be effectively used under field conditions for staining of uterine discharge smears with ease in staining procedure and interpretation.

Keywords: Buffalo, Endometritis, Field Stain, Modified PAP staining, Polymorphonuclear cell, Uterine flushing cytology

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Endometritis is defined as the inflammation of mucosal layer of the uterus and underlying glandular tissue with infiltration of lymphocytes and plasma cells in the upper endothelial layers without systemic signs (Reddy *et al.*, 2017; Kavith *et al.*, 2019 and Thangamani *et al.*, 2023). Different diagnostic methods are available for diagnosis of subclinical and clinical endometritis in bovines. Absolute diagnosis of subclinical and clinical endometritis was based on endometrial cytology along with the histopathological changes in the endometrium. Commercially available stains like Leishman's and Giemsa's were used to stain the cytological smears, but in the present study utilized differential staining techniques for staining of uterine flushing smear of endometritis affected buffaloes.

Uterine discharge cytology

The present study was designed to evaluate the efficacy of differential staining techniques for assessing the staining characteristics of uterine epithelial cells (UEC) and polymorphonuclear cell (PMN) from uterine flushing of endometritis affected buffaloes (n=30). The uterine flushing was collected from endometritis affected buffaloes and evaluated at the time of estrus to assess the endometrial cytology from the smears as per the procedure described by Babu (2010) and Gahlot *et al.* (2016) Briefly mentioned in the flow diagram 1.

Staining and evaluation of smears

Smears from uterine flushing sample (sediment) from each buffalo was made in duplicates for cytological evaluation (Flow diagram 2). Three different combination of staining methods were used for evaluate and compare the staining characteristics of UEC and PMN cells in the cytological smears. Further, routine stain like Leishman's stain was also used to evaluate the uterine flushing smear in buffaloes as described by Babu (2010).

Modified PAP staining method

Modified PAP stain composition: Papanicolaou stain (Cytological solution 1a, Merck), Papanicolaou stain (Cytological solution 2b, Merck). Smears were air dried after fixation with methanol (99%) for 30 seconds. Smears stained with PAP solution (1a) for 5 minutes, washed with distilled water, stained with PAP solution (2b) for 30 seconds, washed with distilled water, air dried and evaluated at 10X; 40X magnification under a microscope (Flow diagram 2).

Eosin and methylene blue staining method

Eosin and methylene blue stain composition: Eosin (EA-50, Himedia), Methylene blue (Alakline-Loeffler's solution, 1% aqueous solution, Merck). Smears fixed with methanol (99%) for 30 seconds. Smears were stained with EA-50 solution for 30-45 seconds, washed with distilled water, stained with methylene blue solution for 15-20 seconds, washed with distilled water, washed, air dried and evaluated (Flow diagram 2).

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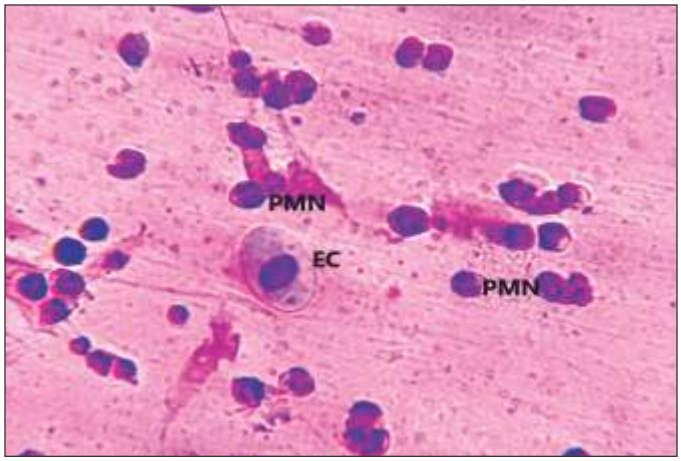


Fig. 1. Photomicrograph showing endometrial cells and PMN cells in uterine flushing cytology. Modified PAP stain X 400

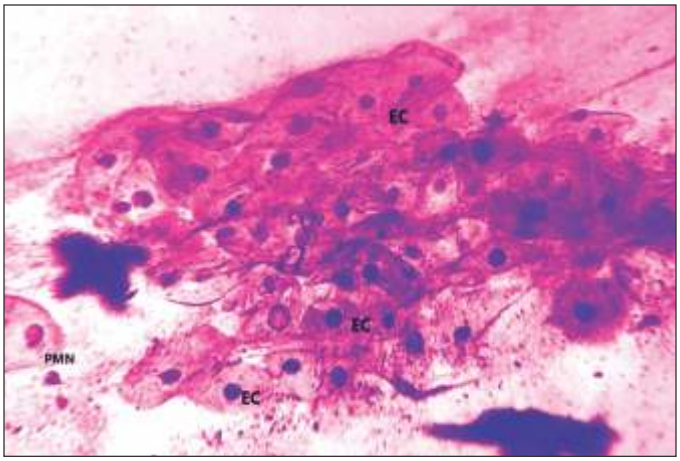


Fig. 2. Photomicrograph showing endometrial cells and PMN cells in uterine flushing cytology. Eosin & Methylene blue stain X 400

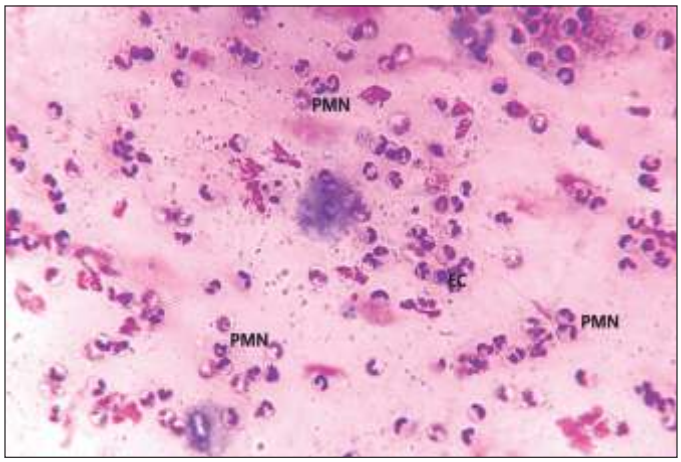


Fig. 3. Photomicrograph showing endometrial cells and PMN cells in uterine flushing cytology. Field's stain X 100

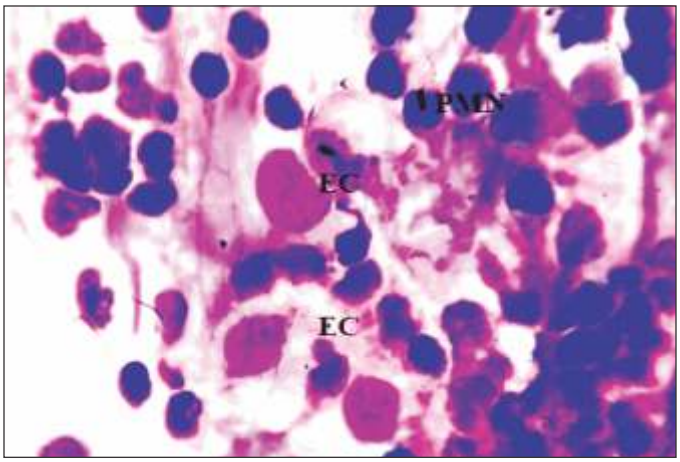


Fig. 4. Photomicrograph showing endometrial cells and PMN cells in uterine flushing cytology. Leishman's stain X 1000

Field's stain method

Field's stain composition (Himedia): Field stain A (Buffer, Methylene blue 0.5% & Preservatives), Field stain B (Buffer, Eosin 0.4% & Preservatives). Smears were air dried after fixation with methanol (99%) for 30 seconds. Smears stained with Field Stain B for 15-20 seconds, washed with distilled water, stained with Field Stain A for 10 seconds, washed, air dried and evaluated (Flow diagram 2).

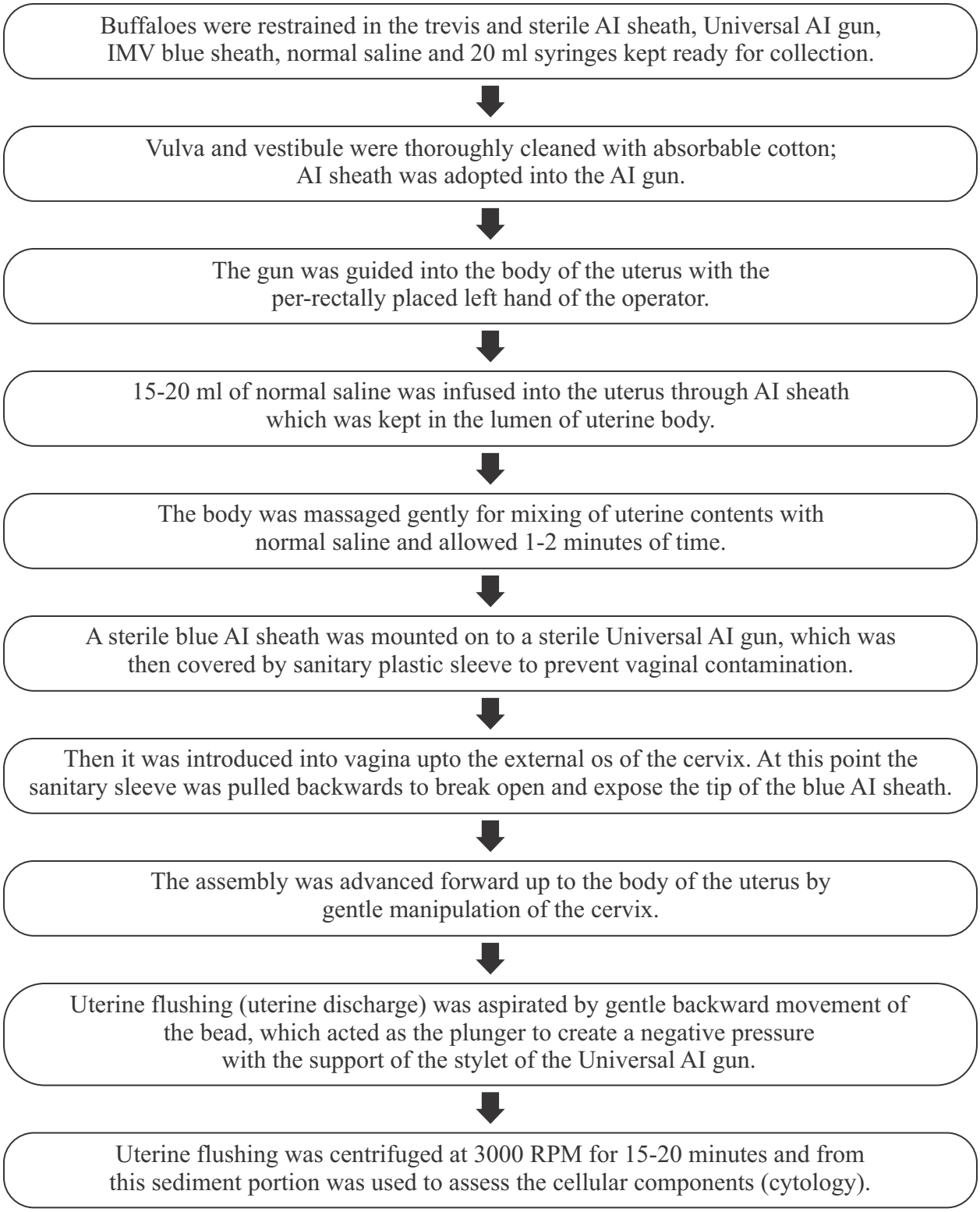
Uterine epithelial cells could be readily demonstrated as oval to round and slightly polyhedral cells with basophilic cytoplasm and presence of distinct nucleus. Polymorphonuclear cells had the characteristics of round cells with multi lobed nucleus and were stained neutral pink.

Smears stained by modified PAP stain had a clearly stained deep purple nucleus and non-distinctly stained the PMN cells with faded purple colour (Fig. 1). Smears stained by eosin and methylene blue clearly showed endometrial cells having a nucleus with purple to pink colour and distinctly stained dark purple PMN cells. However, methylene blue did not stain either the cell wall or nucleus of uterine epithelial cells, PMN cells and background (Fig. 2). Smears stained with Field's stain had shown a clearly stained purple blue coloured nucleus in uterine epithelial cells and PMN cells. Detailed outline between cell wall, cytoplasm and nucleus could be visualized as the concentration of methylene blue which was present in the staining solution acted (Field's stain A) as a counter stain and improved the characteristic appearance of cells which are present in the uterine discharge samples with ease in counting of cells (Fig. 3).

Smears stained with Leishman's stain had shown a purple coloured nucleus of uterine epithelial cells and violet coloured nucleus in PMN cells. Detailed contrast between cell wall, cytoplasm and nucleus was significantly improved showing all the characteristic appearance of cells, which are present in the uterine discharge samples ease in counting of different cell types (Fig. 4).

Collectively, among the different staining techniques

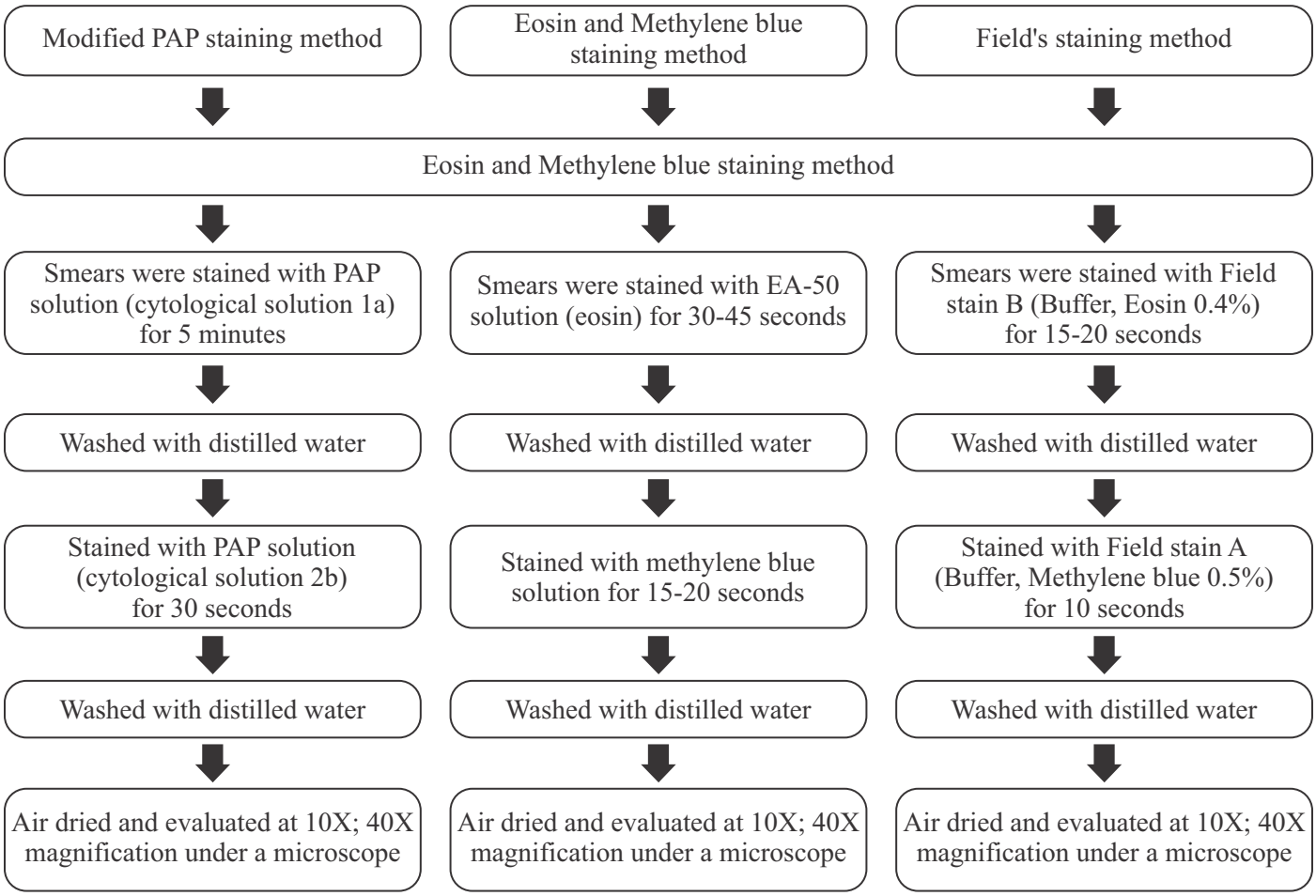
Flow diagram 1.Methodology of collection of uterine flushing in buffaloes



compared for assessment of uterine discharge cytology routine staining methods like Leishman's stain and recently introduced staining method like Field's stain improved the characteristic appearance of different cells which were present in the uterine discharge samples with ease in making a count.

Staining characteristic of uterine epithelial cells and polymorphonuclear cells from the cytological smears observed in the present study were in conformity with the cytological studies of Gahlot *et al.* (2016) and Hussaini (2021) who reported that Field's stain could be used for staining of uterine discharge smears. Similarly,

Flow diagram 2. Different staining methods for cytological evaluation of uterine discharge (flushing technique) smears



Leishman'sstain was also used to evaluate the uterine discharge smear in cows and buffaloes as stated by Babu (2010) and Reddy *et al.* (2017). In the present study also suggested that Field's staincost effective and high reducibilityand best stain to stain and evaluate the uterine flushing smears.

CONCLUSION

The present study concluded that Field's stain could be effectively used under field conditions for staining of uterine discharge smears with ease in staining procedure and interpretation.

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