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**Nishi Pande**
Division of VGO, FVSc. & A.H, SKUAST-J, Jammu, Jammu & Kashmir, India

**Rajesh Agrawal**
Division of Veterinary Medicine, FVSc. & A.H, SKUAST-J, Jammu, Jammu & Kashmir, India

**OP Shrivastava**
Retired Professor and Head, Department of Veterinary Gynaecology and Obstetrics, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India

**RG Agrawal**
Retired Professor and Head, Department of Veterinary Gynaecology and Obstetrics, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India

**Corresponding Author**
**Nishi Pande**
Division of VGO, FVSc. & A.H, SKUAST-J, Jammu, Jammu & Kashmir, India

# Uterine lavage for endometrial cytological evaluation in postpartum buffaloes

**Nishi Pande, Rajesh Agrawal, OP Shrivastava and RG Agrawal**

**Abstract**
Uterine lavage was conducted in 22 buffaloes at 30±2 days in milk to define sub-clinical endometritis by endometrial cytologic evaluation. The study reports a high failure rate of fluid recovery in buffaloes. Satisfactory uterine lavage samples could be obtained only from seven (~32%) buffaloes. Wrights stained endometrial cytological smears revealed 2-5% distorted neutrophils. Due to high rate of non-recovery of lavage fluid (36.36%) or recovery of little fluid with copious mucus (31.80%), sub-clinical endometritis based on reduction of subsequent reproductive performance could not be determined.

**Keywords:** Buffalo, endometrial cytology, post partum and subclinical endometritis

## Introduction
Uterine diseases, postpartum endometritis in particular, reduce the reproductive efficiency of cows by extending the calving to conception interval, increasing the number of inseminations per conception and increasing culling rates (LeBlanc *et al*., 2002 and Gilbert *et al*., 2005) [8, 4]. Clinical and subclinical health during puerperium is associated with uterine health and subsequent reproductive performance. Endometritis in dairy cows is one of the most controversial topics among practitioners due to the lack of a diagnostic gold standard (Kasimanickam *et al.*, 2005) [7]. Subclinical endometritis is defined as an inflammation of the endometrium without systemic illness or signs, and it is associated with delayed uterine involution (Kasimanickam *et al.*, 2004) [6]. Polymorphonuclear cells (PMNs) are the predominant inflammatory cell type found in intrauterine fluid accumulations and the determination of the relative proportion of PMNs has been shown to be predictive of reproductive performance in the postpartum cow (Kasimanickam *et al.*, 2004 and Gilbert *et al.*, 2005) [6, 4]. Investigators have reported differing findings about the percentage of neutrophils that indicates subclinical endometritis in cows. Hammon *et al.* (2006) established subclinical endometritis-positive as those cows with counts of PMN >25% performed at 28±3 days pp, while others established percentages greater than 18% or 10% in endometrial samples collected between days 21 to 33 or 34 to 47 after calving, respectively (Kasimanickam *et al.*, 2004 and Sheldon *et al.*, 2006) [6, 9]. On the other hand, it has been reported that >5% of PMNs is a significant cut-off point for the endometrial inflammatory response in cows sampled between day 40-60 pp (Gilbert *et al.* 2005) [4]. Dubec *et al.* (2010) [2] defined cytological endometritis as the presence of ≥6% PMN's at 35±3 DIM. Barlund *et al.* (2008) [1] used 8% PMN as cut-off to classify endometritis positive cows as it was the lowest percentage of PMNs assessed between day 28-41 pp that was associated with pregnancy at 150 days pp. Due to paucity of such reports on endometrial cytologic evaluation and per cent PMN cells in buffaloes, the present study was conducted for defining sub-clinical endometritis in post partum buffaloes.

## Materials and Methods
A total of 36 freshly calved buffaloes were enrolled for the study. Fourteen buffaloes which showed evidence of either clinical metritis or clinical endometritis as per Sheldon *et al.* (2006) [9] till day 28 postpartum were excluded from the study. Animals that were not systemically ill, but had an abnormally enlarged uterus and a purulent uterine discharge detectable in the vagina, within 21 days postpartum were classified as having clinical metritis. Presence of purulent (>50% pus) or mucuopurulent (approximately 50% pus, 50% mucus) discharge detectable in the vagina 21 days or more after parturition, was classified under clinical endometritis.

Uterine lavage was conducted in 22 buffaloes at day 30±2 postpartum as per Kasimanickam *et al.* (2005) [7] with some modifications. Briefly, the uterine body was lavaged by infusing 50 ml of sterile normal saline solution into the uterine body with a 50-ml syringe attached to uterine catheter. The uterus was massaged and then retracted to recover the fluid. As much fluid as possible was recovered by aspiration into the syringe and transferred to a centrifuge tube and centrifuged at 1000 rpm for 10 min within 2 hours. A drop of sediment was streaked on to a clean microscopic slide and air dried. Slides were fixed with methyl alcohol and stained with Wrights and Giemsa stain separately. Each slide was examined at 400x magnification to perform a differential cell count of 100 cells (endometrial cells and polymorphonuclear [PMN] cells or neutrophils).

## Results and Discussion

Satisfactory fluid could be recovered only from seven out of twenty two (~32%) buffaloes. On cytological examination of smears, two to five per cent neutrophils were seen (Figure). In others, either there was no recovery of lavage fluid (8/22, ~36%) or little fluid with a lot of mucus was recovered (7/22, 32%) which when centrifuged did not yield pellet. Subclinical endometritis could not be determined.

Cytologic examination by uterine lavage with low volume of saline to recover neutrophils has been studied in dairy cattle as a method to define sub-clinical endometritis (Gilbert *et al.*, 1998) [3]. Since the materials required to perform a uterine lavage are available in most veterinary practices, this technique was evaluated. However, due to high rate of non-recovery of lavage fluid (36.36%) or recovery of little fluid with copious mucus (31.80%), the presence of sub-clinical endometritis, which was to be based on reduction of subsequent reproductive performance, could not be determined in the present study. Kasimanickam *et al.* (2005) [7] reported 17% failure in attempts to recover lavage fluid in cows, and increased distortion of cells caused by saline in cells harvested by uterine lavage as compared to cytobrush technique.

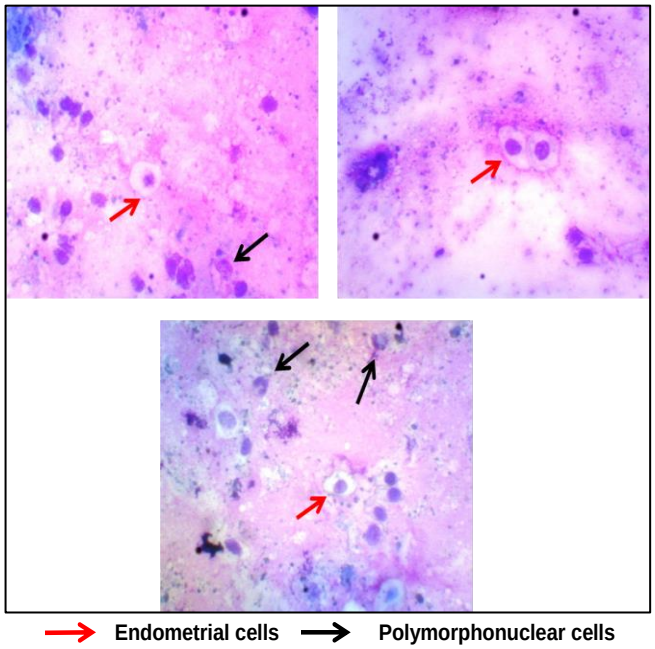


**Fig 1:** Cytological preparation of uterine lavage from 30 day postpartum buffaloes showing endometrial cells and polymorphonuclear cells (PMN's) (Wright stain 400x)

The present study reports high rate non-recovery of satisfactory uterine lavage fluid in postpartum buffaloes and suggests that uterine lavage may not be a method of choice for conducting endometrial cytological examination in buffaloes.

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