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Modified cytobrush device for the detection of subclinical endometritis in crossbred dairy cows

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Abstract
Early diagnosis of uterine infection in postpartum dairy cows is a key factor of profitable dairying. Modified human cytobrush was economic and cost effective method equipment for the early detection of subclinical endometritis under field conditions. Endometrial cytobrush cytology helped in the diagnosis of subclinical endometritis in crossbred dairy cows during early postpartum period.

Keywords: Endometrial cytobrush, dairy cows, profitable dairying

Introduction
Postpartum period has a great influence on the reproductive efficiency of dairy cows and any systemic or reproductive tract infections during this period will adversely affect future production and reproduction of the animal. Mild inflammatory changes such as endometritis may interfere with conception, implantation and further development of conceptus. Hence, regulation of uterine microenvironment is vital for the establishment and maintenance of future fertility in dairy cows.
Superficial inflammation of endometrium not extending beyond stratum spongiosum is recognised as endometritis [3]. In the absence of vaginal discharge, endometrial inflammation based on cytology by determining the proportion of polymorphonuclear cells in the uterine flush [8, 7, 10] or cytobrush [11] sample is characterised as subclinical endometritis [8]. Endometritis has negative influence on days open [7].
Leucocytes migrate to the site of inflammation for the phagocytosis of invading organisms, its proportion within the uterus is indicative of an inflammatory reaction. Several techniques were used to determine the number of PMN cells in endometrial samples such as uterine flush [7, 2], endometrial cytology using cytobrush [11, 13, 6, 12, 15, 16, 9], endometrial cytology using cytotape [15] and uterine biopsy [4, 13, 9]. Hence, the determination of the proportion of PMN cells in EC samples collected by cytobrush technique have much predictive value and it has been considered as the gold standard method for the diagnosis SE in postpartum cows [11, 1].

Materials and methods
Detection of subclinical endometritis by EC
EC samples were collected from 137 postpartum crossbred dairy cows by cytobrush technique as per [10], with minor modifications.

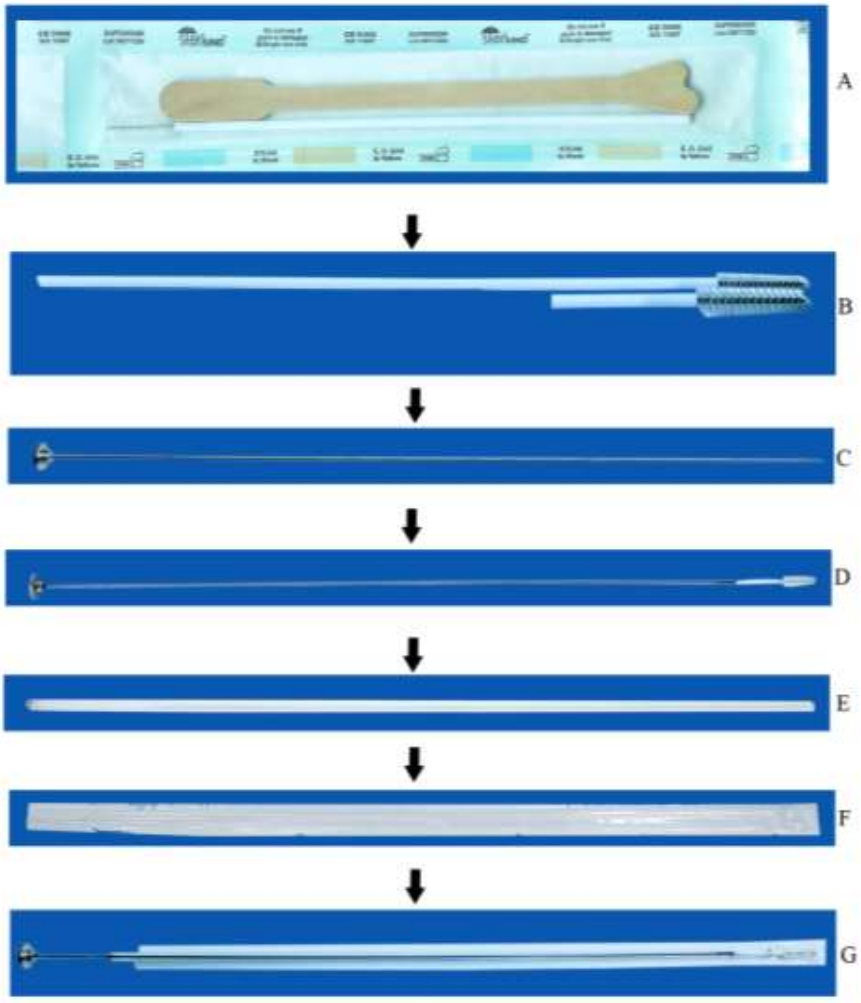
Assembly of cytobrush
Sterile endocervical cytobrush (Steri UNO®) used for preparation of cervical pap smear for diagnosing cervical cancer in humans was modified as endometrial cytobrush in bovines. The human cytobrush (SteriUNO®, 19 cm, India) was cut to a length of five centimeters and heat fixed on to a previously sharpened stylet of an artificial insemination (AI) gun. The assembly was inserted into an AI sheath (0.5cm) covered by a sanitary plastic sleeve for protection from vaginal contamination and sterilized by ultraviolet irradiation in a laminar air flow (Klenz Flo, India) for a period of 15 min (Plate 1).

Results and Discussion
Collection of endometrial samples
Before the collection of EC samples, the cow was properly restrained, the external genitalia was cleaned with paper towels and the cytobrush assembly was inserted in to the anterior vagina with all precautions to avoid contamination.

The sanitary sleeve was punctured at the level of external os of cervix and the instrument was advanced to reach the uterine body. The stylet was pushed forward to expose the cytobrush and EC samples collected by rotating it in a clockwise direction from the base of the horn. To ensure proper contact of cytobrush and endometrium, with gentle pressure applied on the uterus transrectally. After collection, the stylet was retracted back in to the outer sheath within the uterus itself and the whole assembly was withdrawn from the genital tract. The cytology slides were prepared by rolling the cytobrush onto a sterile microscope slides and fixed with 95 per cent methanol for 2-3 minutes. The slides were stained using modified Wright-Geimsa stain using standard staining procedure and were evaluated for the presence of PMN cells

The endometrial samples harvested for evaluating the presence of polymorphonuclear cells (PMN cells) for early diagnosis of subclinical endometritis in postpartum dairy cows. Endometrial cytology sample collected from 137 dairy cows during 29 and 35 dpp (mean 31.58 ± 0.13 dpp) using modified cytobrush technique to detect the presence of PMN cells. The threshold of PMN cells to classify cows as positive for subclinical endometritis in the present study was the detection of more than or equal to five per cent PMN cells in cytosmear [7] (Plate 2). The prepared cytological smears were examined for various cell populations. A modified cytobrush (Cytobrush plus GT, Medscand Medical, Sweden) for the collection of cytological samples, stained with modified Wright Giemsa stain, and evaluated by differential count of different cell populations (endometrial, PMNs and squamous cells) obtained a quantitative assessment of endometrial inflammation [1]. The cytobrush could yield significantly more cells than other endometrial cytological methods like low volume lavage and cotton swab [14, 5]. The cytobrush and low volume flushing were the minimal invasive methods for the diagnosis of SE without any detrimental effect on subsequent conception and the microscopic evaluation of endometrial cytology smears were a reliable and reproducible method for estimating the proportion of PMN cells.

In conclusion, modified cytobrush technique is economic and cost-effective, simple, easy to assemble under strict aseptic conditions that is more consistent and reliable under field conditions for the early detection of subclinical endometritis. Cytobush cytology could be suggested as a cow-side test under field condition for the early diagnosis of subclinical endometritis in postpartum dairy cows.



A. Human Cytobrush (SteriUNO®, 19cm, India)
B. Modified cytobrush
C. Sharpened stylet
D. Cytobrush fixed stylet
E. AI sheath
F. Plastic sleeve
G. Modified Cytobrush assembly

Plate 1: Modified cytobrush assembly

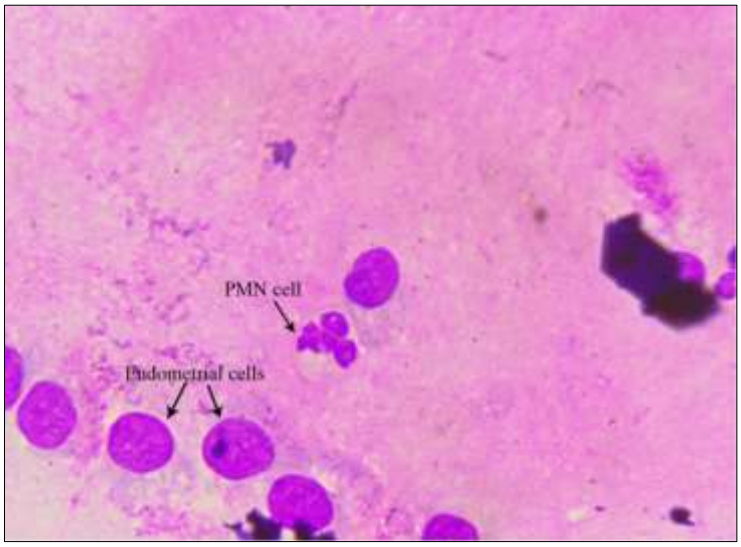


Plate 2: Endometrial cytosmear (both PMN cells) and endometrial cells) (400X, Modified Wright Giemsa stain)

References

1. Barlund CS, Carruthers TD, Waldner CL, Palmer CW. A comparison of diagnostic techniques for postpartum endometritis in dairy cattle. Theriogenology 2008;69:714-723.
2. Bicalho MLS, Lima FS, Machado VS, Meira Jr EB, Ganda EK, Foditsch C *et al*. Associations among Trueperella pyogenes, endometritis diagnosis and pregnancy outcomes in dairy cows. Theriogenology 2016;85:267-274.
3. Bon Durant RH. Inflammation in the bovine reproductive tract. Journal of Dairy Science 1999;82(2):101-10.
4. Bonnett BN, Martin SW, Gannon VP, Miller RB. Etherington WG. Endometrial biopsy in Holstein-Friesian dairy cows, bacterial analysis and correlations with histological findings. Canadian Journal of Veterinary Research 1991;55:168-73.
5. Cocchia N, Paciello O, Auletta, L, Uccello V, Silvestro L, Mallardo K, Paraggio G. Comparison of the cytobrush, cotton swab and low-volume uterine flush techniques to evaluate endometrial cytology for diagnosing endometritis in chronically infertile mares. Theriogenology 2012;77:89-98.
6. Denis-Robichaud J, Dubuc J. Randomized clinical trial of intrauterine cephapirin infusion in dairy cows for the treatment of purulent vaginal discharge and cytological endometritis. Journal of Dairy Science 2015b;98:6856-6864. doi:10.3168/jds.2014- 9129.
7. Gilbert RO, Shin ST, Guard CL, Erb HN, Frajblat M. Prevalence of endometritis and its effects on reproductive performance of dairy cows. Theriogenology 2005;64:1879-1888.
8. Gilbert RO, Shin ST, Guard CL, Erb HN. Incidence of endometritis and effects on reproductive performance of dairy cows. Theriogenology 1998;1:251.
9. Hartmann D, Rohkohl J, Merbach S, Heilkenbrinker T, Klindworth HP, Schoon HA. Prevalence of cervicitis in dairy cows and its effect on reproduction. Theriogenology 2016;85:247-253.
10. Kasimanickam RTF, Duffield R., Gartley CJ, Leslie KE, Walton JS, Johnson WH. A comparison of the cytobrush and uterine lavage techniques to evaluate endometrial cytology in clinically normal postpartum dairy cows. Canadian Veterinary Journal 2005;46:255-259.
11. Kasimanickam RTF, Duffield RA, Foster CJ, Leslie KE, Walton JS, Johnson WH. Endometrial cytology and ultrasonography for the detection of subclinical endometritis in postpartum dairy cows. Theriogenology. 2004;62:9-23.
12. Ledgard AM, Smolenski GA, Henderson H, Lee RF. Influence of pathogenic bacteria species present in the postpartum bovine uterus on proteome profiles. Reproduction Fertility and Development 2015;27:395-406.
13. Madoz LV, Giuliodori MJ, Migliorisi AL, Jaureguiberry M, De la Sota RL. Endometrial cytology, biopsy, and bacteriology for the diagnosis of subclinical endometritis in grazing dairy cows. J. Dairy Sci 2014;97:195-201.
14. Overbeck W, Witte TS, Heuwieser W. Comparison of three diagnostic methods to identify subclinical endometritis in mares. Theriogenology 2011;75:1311-1318.
15. Pascottini OB, Dini P, Hostens M, Ducatelle R, Opsomer G. A novel cytologic sampling technique to diagnose subclinical endometritis and comparison of staining methods for endometrial cytology samples in dairy cows. Theriogenology 2015;84:1438-1446.
16. Polat B, Cengiz M, Cannazik O, Colak A, Oruc E, Altun S. Endometrial echotexture variables in postpartum cows with subclinical endometritis. Animal Reproduction Science 2015;155:50-55.