ANNEXURE - 1

BOVINE ENDOMETRIUM CYTOTAPING CATHETER

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INTRODUCTION

Endometritis refers to the inflammation of endometrial layer of uterusleading to infertility problems like conception failure and early embryonic death. Endometritisis considered as the most common cause of infertility in cows and buffaloes. Depending on the severity, endometritis can be categorized into clinical endometritis (CE) and subclinical endometritis (SCE). The CE is characterized by the presence of mucopurulent discharge in the vagina originating from uterus in the absence of any systemic signs. On the other hand, SCE is characterized by the inflammation of endometrium in the absence of any clinical sign and therefore goes undetectable on the basis of physical appearance of genital discharge. The prevalence of CE and SCE ranges from 20-30% and 9-76%, respectively (Sheldon et al., 2009).

There are several methods to diagnose different grades of endometritis. The characteristics of genital discharge (appearance) happens to be most feasible and of practical use to diagnose CE. However, abnormal genital discharge can also be due to physiological elimination of uterine microbiome (Roberts, 1971), thereby questioning the diagnostic ability of the latter method. Contrarily, SCE is practically impossible to be diagnosed by evaluating genital secretions, which appear to be normal. Nonetheless, SCE is equally detrimental as CE in causing conception failure. Accordingly, advancements in assessment of uterine infection were made and relied on the detection of polymorphonuclear cells in the endometrial impression cytology. The earliest method used *per se* was cytobrush technique (Figure 1) (Kasimanickam et al., 2004). The disadvantage of using cytobrush was its partial invasiveness as well as contamination of the endometrial smears with RBCs, thereby rendering the smears to be bloody and of low quality. Moreover, every time a new cytobrush (Figure 1 marked as ‘a’) has to be used for diagnosing endometritis. The aforesaid limitation of RBC contamination was overcome by using cytotape method of endometrial assessment (Pascottini et al., 2015). The major drawback of the Pascotinni’s model of cytotape technique probably lies in the material configuring the assembly (Figure 2 and 3). The probable drawbacks of the latter method

included that a part of Sani-Shield Rod® covering the pipette can disintegrate (Figure 3 marked as 'c') and remain in the uterus as a foreign material that could act as a potential source of inflammation. Moreover, there is also a great likelihood of loss of endometrial cells while the pipette (having a rolled over paper tape) is retracted back into the Sani-Shield Rod® inside the uterus after sampling is completed (Figure 3, marked as 'b'). In addition, the latter assembly alike cytobrush is also for a single use thereby adding to the cost and influencing the economic perspective of the equipment.

Hence, considering the limitations of cytobrush and Pascottini's cytotape assembly, the 'Bovine Endometrium Cytotaping Catheter' was developed.

COMPONENTS AND ADJUNCTS OF THE BOVINE ENDOMETRIUM CYTOTAPING CATHETER

Components

1) Bovine Endometrium Cytotaping Catheter: It is a 47.2 cm long and 0.4 cm thick stainless steel barrel having stylette (Figure 4a). The stylette is a 47.2 cm long and 0.29 cm thick stainless steel rod with 2.0 cm long threaded portion (Figure 4b marked as '†') at the cranial part before it ends in a 1.4 cm long, elongated and elliptical blob at its terminal end (Figure 4b marked as '‡'). The thickness of elongated blob (apical portion-0.32 cm; middle portion-0.32 cm; base gradually decreasing to 0.30 cm) is such that more than half of it (0.8 cm) remains snuggly fitted inside the barrel (Figure 4d) so that it is guarded from any vaginal and / or cervical contamination while it traverses into the uterine body. The other end of catheter has three circular metallic rings, two of which (Figure 4a marked as '#') garner support while the catheter is being inserted into the uterus (fixed on to the barrel), while the one at the centre (Figure 4a marked as '$') is in continuation to the stylette and is used to roll the stylette in the uterus.

Adjuncts

2) Cyto tape- A 2.5 cm wide paper tape was trimmed to a size of 2.0 cm before use (Figure 5a) (M'PORE, Medicare Hygiene Limited, Gujarat, India). Three complete rolls of the cyto tape are applied over the threaded portion of the catheter (Figure 4c).
3) Customized slides- The commonly used slides were customized in a manner (1 mm thick, 1.8 cm wide and 7.6 cm long) so that the cyto tape (over the threaded portion) can be conveniently rolled over the customized slide (Figure 5b).

PREPARATORY WORK

The development of the proposed standard was stepwise. The initial model of 'Bovine Endomerium Cytotaping Catheter' planned by us was although effective in endometritis diagnosis, but had few limitations. One, being relatively thicker than the proposed standard (0.65 cm vs. 0.40 cm), its use was limited in cow heifers with a narrow cervical canal. Two, the threaded portion of the initial model was shorter (1.7 cm vs. 2.0 cm) and yielded lower cell count in the endometrial smears. Three, the threaded portion was likely to be contaminated with vaginal and cervical areas, which had to be prevented by covering the entire device with a sterile sanitary sheath. To the contrary, the proposed standard is thinner, has a wider base for endometrial impression and has been incorporated with a snuggly fitted blob, thereby overcoming all the limitations encountered in the preliminary model.

PROCEDURE OF CYTOTAPING

- The threaded portion of the catheter is given three complete rolls of paper tape (Figure 4c). Thereafter, the closed catheter is subjected to autoclaving at 15 psi and 121^{o}C for 15 minutes.
- A complete drying of the catheter is ensured prior to use.
- The perineal region of the cow is thoroughly cleaned with fresh water initially and thereafter with 70% isopropyl alcohol.
- Under rectal guidance, the catheter is inserted into the vagina and manipulated into the external-os of the cervix.
- Once the tip of the catheter reaches the uterine body, the stylette is pushed to expose the threaded portion of the stylette overlaid with cyto tape.
- The cyto tape portion is now rotated twice in clockwise direction while in close contact with the endometrial surface of the dorsal uterine wall.
- Thereafter the threaded portion is retrieved back into the barrel and the catheter is taken out.
- Immediately after sampling, the cyto tape is gently rolled over the clean customized glass slide to have the endometrial impression smears. The slides are dried and stained to be finally subjected to cell scoring.

FINDINGS

The proposed catheter was evaluated for its use during 58 estrus periods in repeat breeder / endometritis (n=53) and normal (n=5) cows. While the normal cows revealed no polymorphonuclear cells, the endometrial cytology expressions revealed varying percentage (1 to 5%) of polymorphonuclear cells, thereby signifying presence of varying grades (clinical and sub-clinical) of endometritis in the infertile cows. The findings were used in instituting a suitable treatment to overcome endometritis in the affected infertile cows. Irrespective of the reproductive status, the endometrial impressions were devoid of RBC contamination.

CONCLUSIONS

The proposed 'Bovine Endometrium Cytotaping Catheter' is an effective exponent highly feasible for use, is accurate and economical to yield reliable endometrium cytology impressions. As against the single use cytobrush and Pascotinni's cytotape model, the proposed catheter is autoclavable and reusable without any effect on its subsequent / repeated use. Accordingly the proposed catheter holds the potential of a widespread application with a high degree of reliability and repeatability.

REFERENCES

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Figures

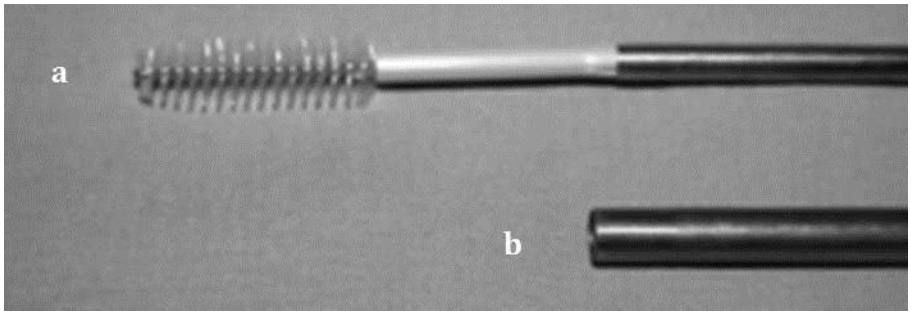

Figure 1. a) Cytobrush (Note the bristles in the cytobrush), b) rod. (Kasimanickam et al., 2005)

Figure 2. a) Equine infusion pipette, b) a 1.5 cm piece of paper tape rolled on the top of pipette, c) Sani-Shield Rod® which covers the pipette finally. (Pascotinni et al., 2015)

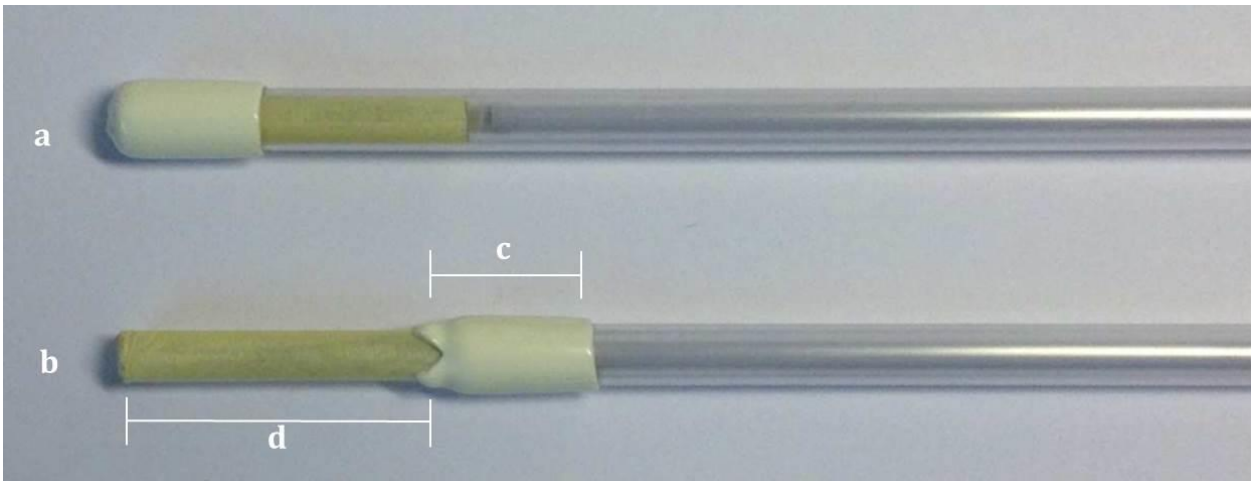

Figure 3.a) Sani-Shield Rod® covered equine infusion pipette with paper tape, b) released cyto tape from Sani-Shield Rod®, c) rubber top, d) paper tape rolled on equine infusion pipette.(Pascotinni et al., 2015)

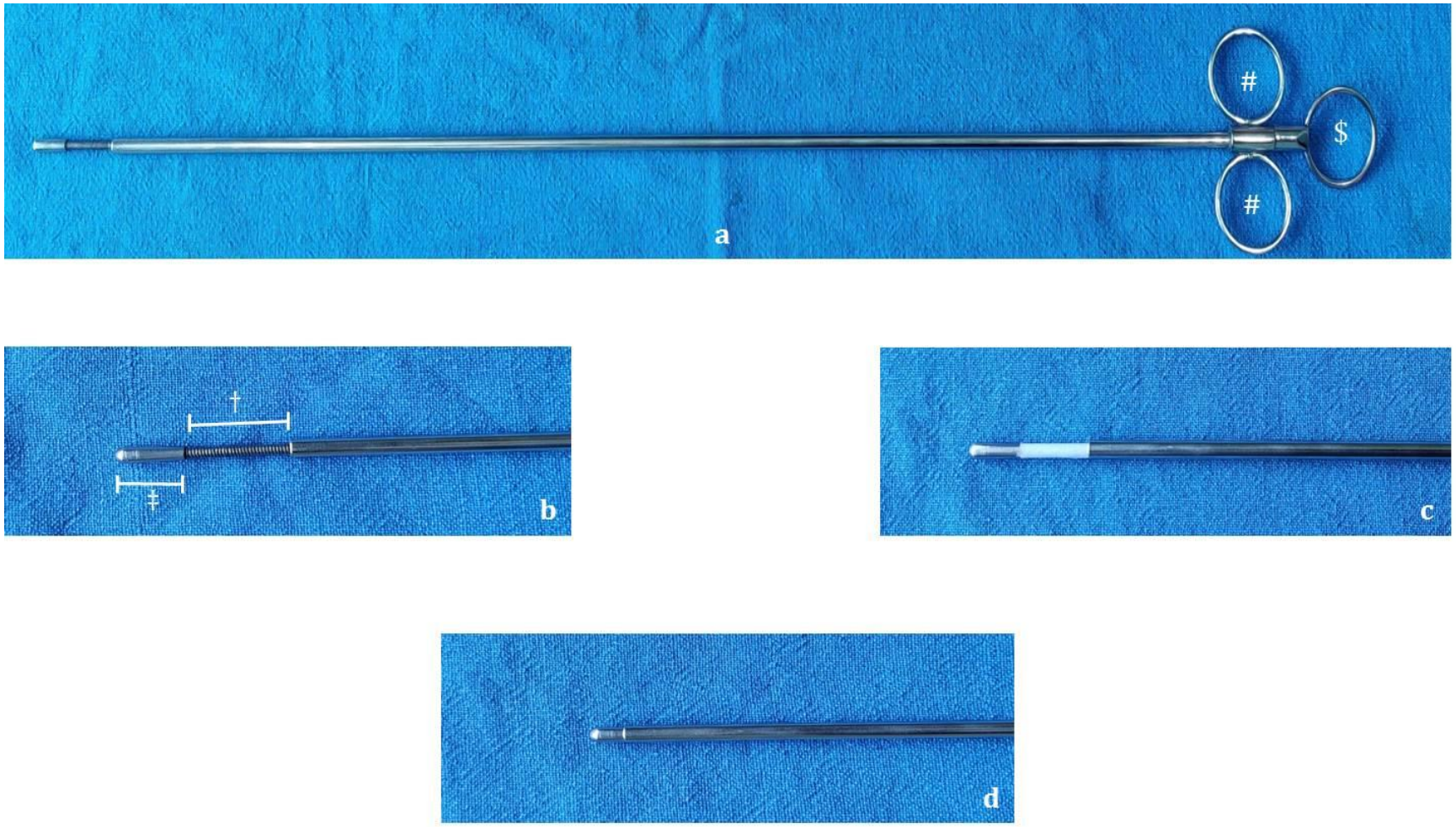

Figure 4.a) Bovine Endometrial Cytotaping Catheter, circular metallic rings are marked as '# and $' a) open catheter with threaded portion of the stylette marked as '†', elongated and elliptical blob marked as '‡', c) threaded portion of the catheter rolled with cytotape, d) closed catheter.

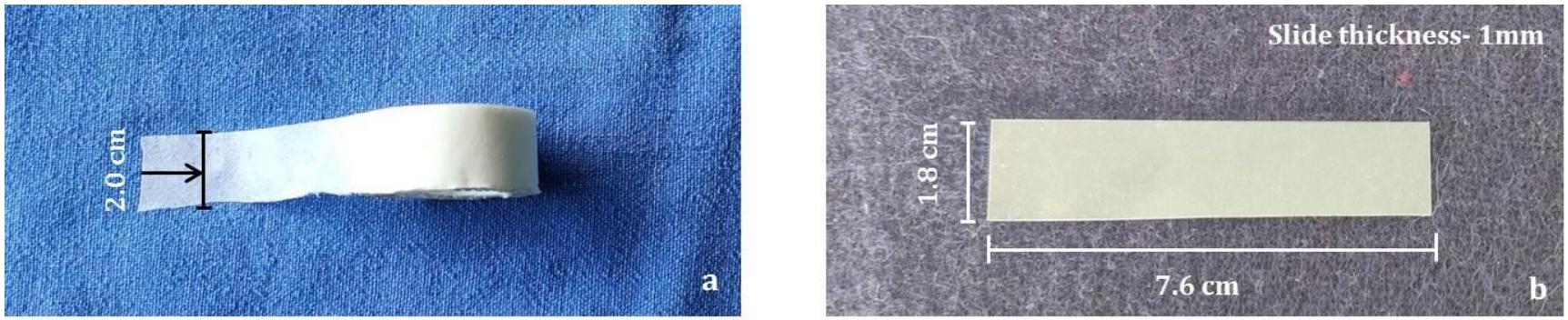

Figure 5.a) Cyto tape (2.0 cm wide), b) customized glass slide.