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Comparison of the Jackson Uterine Biopsy Forceps with a Fabricated Model of Human Bronchoscopy Biopsy Device for Uterine Biopsy in Bovines

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ABSTRACT

Background: Uterine biopsy is considered as the most conclusive and efficient method for diagnosis of subclinical endometritis especially to evaluate further reproductive efficiency.

Methods: The present study was conducted at ULF & FRDS, Mannuthy to compare the efficacy of two devices in obtaining histopathological samples for the diagnosis of subclinical endometritis (SCE) in crossbred dairy cows at 30 and 40 days postpartum. The two devices used were the Jacksons uterine biopsy forceps (Jorvet, USA) and a slimmer stainless-steel biopsy forceps with a circular cupped jaw, fabricated similar to human bronchoscopy biopsy device.

Result: The Jacksons uterine biopsy forceps was used in 35 cows to collect the samples. Manoeuvring this forceps through the cervix was found to be difficult and only in 16 out of the 35 animals (45.71%) collection for both days was successful, while in 14 animals (40%) samples could be collected only on day 30 of observation and in remaining 5 animals (14.29%) no samples could be collected on both the days of observation. In those animals where samples could be procured, bigger jaw of the Jacksons forceps resulted in larger sample size which may slightly delay healing. The fabricated biopsy forceps was used in 10 animals and because of its slim structure, it was possible to collect samples in all of them (100%). The smaller jaw ensured that the sample obtained was smaller and thus minimalizing tissue damage, contamination with blood and possibly promoting a swifter healing. Tissue sections were examined for pathological changes in the endometrium involving surface epithelium, lamina propria, endometrial glands and vascular inflammatory status. The samples obtained using the modified forceps provided sufficient data for the diagnosis of SCE similar to the traditional device. Thus, the fabricated uterine biopsy forceps, which is comparatively much cheaper, can be used with more ease in indigenous/crossbred cows and heifers which have smaller cervices.

Key words: Endometrial histopathology, Subclinical endometritis, Uterine biopsy forceps,

INTRODUCTION

In cows the postpartum period is a time when the uterine health and overall immunity is compromised. Following parturition uterus tolerates the proliferation of a wide range of bacterial organisms, which are normally eliminated during the process of uterine involution. However, in immuno-compromised animals the persistence of the microorganisms can lead to an infection and development of metritis (within 21 days) or clinical endometritis which could be aggravated by post parturient complications. This can further result in pyometra, chronic endometritis and subclinical endometritis (SCE) which could result in poor reproductive performance (Sheldon *et al*., 2006).

One of the main contributing factors of repeat breeder syndrome in cows is thought to be SCE which is defined as the inflammation of superficial layer of uterus where there is no outward expression of any disease condition (Sheldon *et al*., 2006). It could delay resumption of post parturient ovarian cyclicity and reduce the conception rates (Kasimanickam *et al*., 2004; Gilbert *et al*., 2005).

The diagnosis of SCE is difficult by mere observation of the animal due to the absence of overt clinical signs and hence alternatives have to be sought in the critical diagnosis of the condition. Most common and easy method is counting polymorphoneutrophils (PMN cells) in the endometrial smears collected using uterine cytobrush technique (Gilbert *et al*., 2005). They are the first cells to respond to an invasion in the uterus, hence an elevation in its number in the uterine

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lumen is indicative of the inflammatory response of the endometrium (Butt *et al*., 1993).

However, the most conclusive method for diagnosis of endometritis is by histological examination of endometrial biopsy samples obtained using uterine biopsy forceps. The structures observed are the surface epithelium (SE), lamina propria (LP), endometrial glands and inflammatory status of blood vessels. In addition to the effectiveness of biopsy technique in estimating the inflammatory status of both superficial and deeper layers of endometrium it allows prediction of subsequent fertility of the animal (Bonnett *et al*., 1993). The technique has been widely used in mares as a common diagnostic tool to determine the causes of infertility. But in cows, this method has some disadvantages such as the high cost and time-consuming nature of the procedure besides being a method with difficulty in obtaining samples and additionally there are possibilities of reduced fertility (Sheldon *et al*., 2006).

On comparison of biopsy and cytology findings, the two diagnostic methods showed poor or average agreement. The advantage of histopathological examination of biopsy samples over the cytology samples was that it gave a detailed idea about the degree of inflammation, its distribution and extent of the lesions, whereas cytology could only assess the superficial layer of the endometrium (Pascottini *et al*., 2016). Ramirez-Garzone *et al*. (2017) investigated the effectiveness of using human bronchoscopy biopsy (Karl Storz® 10366L) device instead of the usual bovine uterine biopsy forceps in *Bos indicus* cross - beef heifers and noted its higher success rate in obtaining histopathological samples.

MATERIALS AND METHODS

The study was conducted at University Livestock Farm, Fodder Research and Development Scheme (ULF & FRDS), Mannuthy, Thrissur, Kerala from September 2018 to June 2019 with an objective to compare the efficacy of two devices in obtaining histopathological samples for the diagnosis of SCE in crossbred dairy cows at 30 and 40 days postpartum. The study was conducted in 45 crossbred dairy cows of three to 12 years of age in their first to seventh parity, which were clinically normal and without any postpartum complications and reared in uniform housing, feeding and other managemental conditions. A balanced feeding protocol was followed consisting of green and dry fodder along with concentrates and mineral mixture according to the Nutrient Requirements of Cattle and Buffalo (Singh, 2013).

The two devices used in the study were the Jacksons uterine biopsy forceps (Jorvet, USA) (Fig 1) and a slimmer stainless-steel biopsy forceps with a circular cupped jaw, fabricated similar to human bronchoscopy biopsy device (Fig 2). The modified device was fabricated because of difficulty in using and procuring samples with the conventional forceps in smaller sized crossbred cows of Kerala. The Jacksons uterine biopsy forceps which had a shaft length of 60 cm and a cutting area of 4 mm × 28 mm was used in 35 cows to collect the samples. The fabricated biopsy forceps with a length of 50 cm and circular cutting edge 2.5 mm was used in 10 animals.

The animals were restrained in a trevis and the vulva and nearby areas were cleansed to free of any dung and dirt. The biopsy forceps was protected from contamination by means of a stainless-steel cover and directed through the vulva and vagina to the external os of the cervix and further the biopsy forceps alone was guided through the cervix in to the uterus by per rectal manipulation of the cervix. The biopsy samples were collected from dorsolateral aspect of the uterine body behind the uterine bifurcation in all cows. Once at the preferred site, the jaws of the forceps were opened and a portion of the endometrium was placed within the jaws which were then closed, rotated at 90° and the forceps was withdrawn gently out of the reproductive tract with the jaws kept closed.

The samples collected were examined for its size, kept in 10 per cent neutral buffered formalin and sent for histopathological slide preparation. Tissue sections were stained with hematoxylin and eosin (Bancroft and Gamble, 2002) and were examined for any pathological changes of the endometrium involving S.E, L.P, endometrial glands and vascular inflammatory status. The scoring was given according to the score card developed by Meira *et al.* (2012).

RESULTS AND DISCUSSION

The samples collections were attempted using the Jacksons uterine biopsy forceps in 35 cows, out of which only in 16 animals (45.71%) collection for both the days (at 30 and 40 days postpartum) was successful using this instrument due to the difficulty in manoeuvring this forceps through the cervix. In 14 animals (40%) samples could be collected only on day 30 of observation and in remaining five animals (14.29%) no samples could be collected on either of these days of observation and had to be

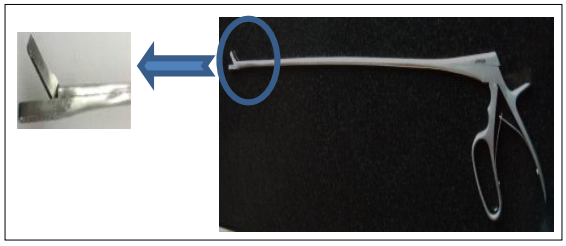


Fig 1: Jacksons uterine biopsy (Jorvet, USA).

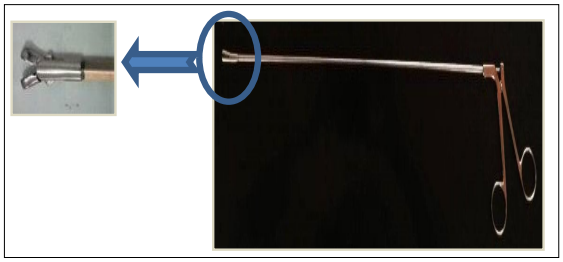


Fig 2: Fabricated slimmer stainless-steel biopsy forceps with a circular cupped jaw similar to human bronchoscopy biopsy device.

discarded. In those animals where samples could be procured correctly, bigger jaw size of the device resulted in larger sample size, more tissue damage and possibly more bleeding, which probably could delay healing. The size of the samples was non uniform ranging from about 2.5 mm to almost 1cm with an average size of around 5 mm (Fig 3). Thus, the difficulty in obtaining samples using regular biopsy forceps (Jackson forceps) was clearly demonstrated in this study.

The present study was conducted in crossbred cattle which had a notably smaller uterine and cervical size when compared to the purebred cows. This could be the reason for the traditional Jackson forceps being unsuccessful in obtaining samples from all the 35 animals it was used.

Chapwanya *et al*. (2010) stated that biopsy of uterus could provide with decent amount of good quality tissue necessary for conduction of wide array of tests thereby allowing a thorough assessment of endometrial health (both deeper and superficial layers), particularly during the postpartum period in cows. Routine performance of uterine biopsy was done in mares since 1960 as a tool for evaluating the fertility aspects. However, its adaptation and application in farm animals were under debate for fear of its negative effect on subsequent fertility. The procedure was considered risky, requiring expertise and the cons outweighed the pros.

Keeping these difficulties in mind the possibility for obtaining tissue sample of good size (to conduct necessary histologic, cellular and molecular assessment) with minimum invasion of the uterus was pondered. In this context the importance of a fabricated slim uterine biopsy forceps which had almost the same width as that of an artificial insemination gun was considered.

The fabricated biopsy forceps was used in 10 animals and because of its slim nature, it was possible to collect samples in all of them (100%). The jaw size and circular cup ensured that the sample obtained was smaller and the quantity was ideal for histopathological studies. On an average, size of the samples was about 2.5 mm (ranging from 1.25 mm to 5 mm) (Fig 4), thus minimalizing tissue damage, contamination with blood and possibly promoting a faster healing. Its manipulation was considerably easier when compared to the traditional uterine biopsy forceps used in cattle like Jackson forceps, Yeoman Hauptner biopsy instrument *etc*. This was in agreement with the observations by Ramirez-Garzone *et al*. (2017) where they had a successful extraction in 86 per cent of the animals on day 4 or day 7 post-oestrus and had highlighted the advantage of having circular cup which helped in obtaining smaller sample size and concluded that this forceps was ideal for minimising tissue damage and hastening the healing process.

Histopathological examination of the samples procured using the two biopsy forceps during both days of sampling was done. It could be inferred that the samples collected, regardless of the instrument used, provided detailed information and allowed the proper diagnosis of SCE in the animals under study. All the layers of the endometrium based on which the interpretations are done are clearly visible in both sample groups.

Cows with SCE had a damaged SE along with high infiltration of PMN cells in both SE and LP indicating acute infection. Also, in SCE positive animals there was damage, desquamation and atrophy of endometrial glands along with its fibrosis. The endometrial vessels too had undergone degeneration, haemorrhage, perivascular fibrosis and even deposition of hemosiderin (Fig 5 and Fig 6). The cows that did not have SCE had very little infiltration of PMN cells in the endometrial layers with an intact SE, endometrial glands and vessels.

Chethan *et al.* (2015) on conduction of histological examination observed that in cytological endometritis (SCE) epithelial lining of endometrium was disrupted and

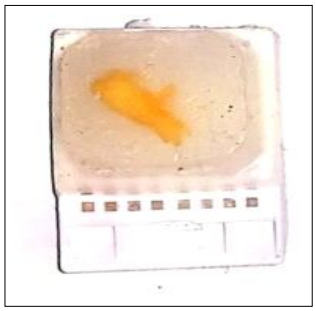
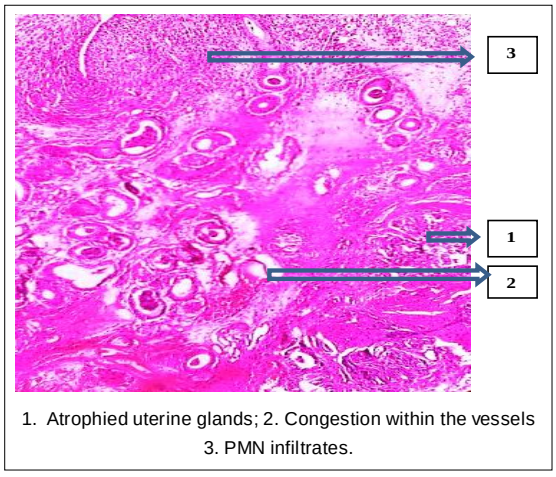


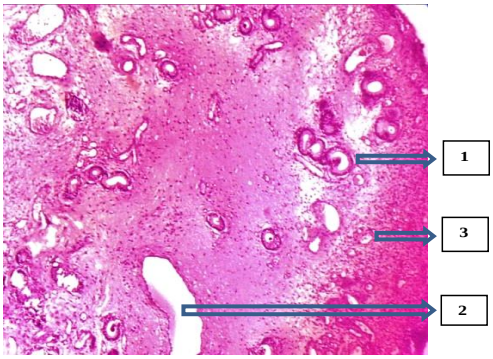
Fig 3: Uterine biopsy sample obtained using Jacksons biopsy forceps.

Fig 4: Uterine biopsy sample obtained using fabricated forceps.



1. Atrophied uterine glands; 2. Congestion within the vessels 3. PMN infiltrates.

Fig 5: Biopsy sample obtained using fabricated forceps.



1. Normal uterine glands; 2. Congestion within the vessels
3. PMN infiltrates.

Fig 6: Biopsy sample obtained using conventional forceps**.**

hyperplastic changes were noted at some areas. There was also infiltration of inflammatory cells in subepithelial layer, stratum compactum along with perivascular and periglandular infiltration. Vascular changes such as dilatation, haemorrhage in lamina propria and degeneration of endometrial glands were also observed.

Ahmadi *et al.* (2005) noted that in subacute endometritis, lymphocytes (either focal or dispersed), haemosiderin-laden neutrophils and regenerative activity of glands in the absence of fibrosis were seen and in endometritis lasting over a period of time, infiltration of mononuclear inflammatory cells accompanied by cystic dilatation of endometrial glands and periglandular fibrosis were observed.

CONCLUSION

In Indian scenario, the crossbred animals are smaller in size when compared to those breeds of animals in which the previous studies were conducted. The reproductive tract, especially the cervix of these animals was smaller in girth. Hence, the use of traditional equine and bovine uterine biopsy forceps was found to be too cumbersome. Also, it had to be performed by an expert to obtain a smaller sample size to avoid unnecessary pain, bleeding and stress to the animals. The use of the fabricated model of the human bronchoscopy biopsy device made the procedure far easier to perform and the instrument was ideal for use in the smaller sized crossbred cows. The smaller jaw size ensured a smaller wound area and a probable faster healing. Also, the samples obtained using the slender forceps was on par with the samples obtained using the conventional uterine biopsy forceps. To conclude the use of modified or fabricated human bronchoscopy biopsy device for bovine uterine biopsy in crossbred or small sized cows and heifers should be considered for quick and easy sample collection.

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Conflict of interest

All authors declared that there is no conflict of interest.

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