The incidence of subclinical endometritis in repeat breeding dairy cows and the comparative efficacy of different antibiotics and herbal intrauterine therapy

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ABSTRACT

The present research work was conducted to investigate the incidence of subclinical endometritis (SE) in repeat breeder dairy cattle, and to study the comparative efficacy of different antimicrobial and polyherbal intrauterine therapies. Repeat breeding crossbred cows (>90 days in milking) with clear cervico-vaginal mucous discharge in spontaneous estrus were examined for uterine cytology by the cytobrush technique. The cows with $\geq$ 4% polymorphonuclear cells (PMNs) in uterine cytology were considered to be affected with SE. These cows were randomly and equally divided (n=12/group) into 5 groups and treated with the intrauterine medicine Vodine-IU (T1), Metricare-IU (T2), Utriguard-IU (T3), Uraksha (polyherbal preparation) (T4), or left untreated (control/C). In the subsequent estrus, uterine cytology was repeated and the cows were inseminated. The cows that failed to conceive but showed estrus were re-inseminated. The pregnancy status was checked. The incidence of SE was observed to be 36.80% (60/163). The PMNs percentage significantly declined in all the treatment group cows in response to treatment. The highest conception rate was observed in the T4 group (Uraksha treated) cows. It was concluded that the uterine cytobrush technique can be used efficiently for diagnosis of SE in dairy cattle, and polyherbal intrauterine drugs may be a good alternative in intrauterine antimicrobial therapy.

Key words: cattle; repeat breeding; sub clinical endometritis; cytobrush; uterine cytology; polyherbal drugs

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Introduction

A healthy uterine environment facilitates the implantation and development of the embryo (MORAES et al., 2018). After parturition, the physiological barrier of the genital system becomes weak. Opportunistic pathogens enter the uterus and cause infection (DOBOS et al., 2022). These pathogens create a bio-film that protects them and helps them to survive continuously in the uterus (FERRIS et al., 2016). Endometritis is caused by the long term persistence of potential

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pathogens in the uterus or reproductive tract of cows, and results in repeat breeding (DOBOS et al., 2022). Endometritis alters the healthy uterine environment and increases the number of days open after parturition (ĐURIČIĆ et al., 2014). The endometritis may be clinical (the presence of a purulent/mucopurulent uterine discharge) or subclinical (inflammation of the endometrium in the absence of purulent material). Subclinical endometritis (SE) is also known as cytological endometritis (PASCOTTINI et al., 2017). It is the most prevalent of all uterine diseases. It affects approximately 30% of lactating dairy cows, with the prevalence ranging from 11 to >70% in some herds (PASCOTTINI et al., 2017). The prevalence of SE in repeat breeder cows has been reported to be 12.7% (POTHMANN et al., 2015) to 40.2% (JANOWSKI et al., 2013). Repeat breeder cows are those who have failed to conceive after three or more services and the regular return to estrus at normal intervals, without detectable anomalies of the genital tract (PARKINSON, 2019). Among the different breeds, crossbred cows are more susceptible to repeat breeding syndrome (ZOBEL et al., 2011).

Early diagnosis of endometritis often reduces the chances of the occurrence of complications in affected animals. Studies have revealed that uterine cytological examination using the cytobrush technique is considered a consistent and reliable method for SE diagnosis in dairy cattle (POTHMANN et al., 2015; SCHYNDEL et al., 2018). After proper diagnosis, treatment of endometritis usually requires the use of antibiotic therapy. Apart from the high cost of antibiotics, the residue of intrauterine or systemic antibiotics in food animal products and the development of microbial resistance are other concerns. Alternatives to antibiotics are being developed to treat puerperal disorders in domestic ruminants, and have given promising results (SAMARDŽIJA et al., 2017). Plant-based remedies have served as a source of valuable medication due to their antimicrobial and anti-inflammatory properties, immunomodulatory activities, precious therapeutic properties, for maintenance of general health and healing potential (WU et al., 2018). *Saracaasoca*, *Aloe barbadensis*,

Gossypium herbaceum, *Plumbago zeylanica* and *Azadirachta indica* have scientifically proven anti-inflammatory, antimicrobial activity (FERRO et al., 2003) and immunoregulatory activity (QIU et al., 2000). *Acacia catechu* leaf extract acts as an antioxidant and free-radical scavenger (SAHA et al. 2016; HAZRA et al. 2017); various parts of *Bambusaarundinacea* possess anti-inflammatory, anti-ulcer, antioxidant, anthelmintic, and astringent activity (KHARE, 2007, WRÓBLEWSKA et al., 2018). *Curcuma longa* (Turmeric) has proven anti-inflammatory, antioxidant, antimutagenic, and antibacterial pharmacological activities (KHARE, 2007; VERMA et al., 2018). Considering all the above, this study was conducted to investigate the incidence of SE in repeat breeding crossbred dairy cattle using the cytobrush technique, and to study the comparative efficacy of different antibiotics and herbal-based intrauterine therapy.

Materials and methods

Ethical approval. The study was conducted after approval of the experimental protocol (No.082-17) by the Ethical Committee of Rajasthan University of Veterinary and Animal Sciences (RAJUVAS), Bikaner, Rajasthan, India.

Study location and animals. The study was conducted at the Veterinary Gynecology and Obstetrics clinic, RAJUVAS and on nearby private cattle dairy farms in Bikaner city. Repeat breeding crossbred dairy cows with a history of a regular inter-estrus interval (21-24 days) but that had failed to conceive after three or more consecutive breeding attempts (natural/artificial) were used. Animals between 4 to 9 years of age with more than 90 days in milking, with body condition scores between 2.5-3.25 were included in the study.

Experimental grouping. The cows were examined by rectal palpation for the status of genitalia and cervico-vaginal mucous (CVM) discharge in spontaneous estrus. The animals with apparently normal genitalia and clear CVM discharge were further examined for uterine cytology (PMNs count) using the cytobrush technique. The cows (n=60) with ≥ 4% PMNs (SINGH et al., 2016) were diagnosed with SE. These animals were

randomly and equally (n= 12) divided into 5 groups (4 treatment and 1 control, i.e. T1, T2, T3, T4 and C). The cows in different treatment groups were subjected to different antibiotic and polyherbal intrauterine infusions, while the cows in the control group were left untreated (Table 1). In the subsequent estrus, uterine cytology was repeated to observe the reduction in PMNs count, and the cows were inseminated using AI. Later, the pregnancy status of the cows was tested by estimating the serum progesterone (P4) concentration (on day 24 post AI), and by rectal palpation (on day 60 post AI) for further confirmation. The cows that failed to conceive and showed heat symptoms were re-inseminated and their pregnancy status was also diagnosed in the same manner.

Table 1. Different intrauterine infusions used in the experimental groups

Groups	Medicine used	Dose rate	Trade name (Manufacturer)
T1	(Levofloxacin hemihydrate 20 mg, Ornidazole 25 mg and Alpha Tocopherol acetate 5 mg	30 ml/IU X 3 days	Vodine-IU (Vetoquinol)
T2	(Povidine iodine 5% and Metronidazole 1%)	30 ml/IU X 3 days	Metricare-IU (Zydus)
T3	(Cephalexin 1.5 g and Serratiopeptidase 10mg)	4 g dissolved in 60 ml of sterile water IU X 3 days	Utriguard-IU (Zydus)
T4	(*Azadirachta indica* Lf. 0.040 g, *Aloe barbadensis* Lx. 0.010 g, *Acacia catechu* Ht. Wd. 0.10 g, *Bambusaarundinacea* Lf. 0.30 g, *Curcuma longa* Rz. 0.010 g, *Gossypium herbaceum* Rt. 0.30 g, *Plumbago zeylanica* Rt. 0.30 g, *Saraca indica* St. Bk. 0.40 g, Excipients q.s) /10 ml	25 ml/IU X 3 days	Uraksha (Ayurvet)
C	No treatment was given		

Procedures. For the uterine cytology, the procedure followed by SINGH et al. (2016) was used in the present study. In brief, after proper restraining and following all hygiene measures, the vulvar lips of the cow were pulled apart by an assistant, the cytobrush assembly was introduced into vagina and then through the cervix into body of the uterus. After assuring its position, the stylette was pushed to expose the cytobrush and then turned gently in both directions (clockwise and anticlockwise). Gentle pressure was applied to its tip against the uterine body per rectum for proper contact of the brush with the endometrium. The inner stylette was then withdrawn into the outer catheter to its normal position and the whole catheter was withdrawn from the reproductive tract. Immediately after removal from reproductive tract, the cytobrush was rolled on a clean glass slide and the slide was brought to the laboratory as soon as possible. The smear was stained with Geimsa stain. After drying the slide, 100 cells (endometrial cells + PMNs) were counted under a microscope under oil immersion and the percentage of PMNs was calculated.

For serum P4 assay, a blood sample was collected from each animal aseptically by jugular vein puncture in a plain sterile glass tube on day 24 post AI (excluding the animals which showed heat symptoms). Serum was separated by centrifuging the sample at 3000 rpm for 10 minutes, and stored at -20 °C until the P4 assay. The serum P4 assay was conducted using a solid-phase enzyme immunoassay (EIA) as per the protocol given in the kit manufactured by XEMA Co. Ltd., Moscow,

Russia. The minimum serum progesterone level set for pregnancy diagnosis in the present study was 1.0 ng/ml, as described by ADEYEMO (1989).

Statistical analysis. Data obtained were analyzed statistically by one-way or two-way analysis of variance, using the SPSS computer program (version 20.0). The means were compared using Duncan's multiple range test (DMRT).

Results

Incidence of SE and uterine cytology. The incidence of SE in repeat breeding cross bred cows was observed to be 36.80% (60/163). The results of the first and second uterine cytology in terms of percentage of PMNs are presented in Table 2. A significant (P<0.05) decline in mean PMNs (%) was observed in all the treatment group cows, but not in the control cows on the day of the second uterine cytology (in the subsequent estrus).

Conception rate. The conception rates in the different experimental groups on day 24 (post AI) and on day 60 (post AI) are shown in Table 3. The highest and lowest conception rates were observed in T4 (75%) and control (25%) group cows, respectively (Table 3). The numbers of cows that were pregnant after the first and second AI in the different groups are presented in Table 4. The serum P4 concentration range (on day 24 post AI) was 2.24 to 5.96 ng/ml and 2.49 to 2.51 ng/ml in pregnant cows and non-pregnant cows, respectively. The serum P4 concentrations in different groups are presented in Table 3. The overall accuracy of the P4 assay for pregnancy diagnosis was 91.89%, in comparison to rectal palpation (Table 3).

Table 2. Polymorphonuclear cells (PMNs) (%) (Mean+SE) before and after treatment in different groups

Groups	PMNs (%)	
	Before treatment (I UC)*	After treatment (II UC)*
T1	5.66±0.35[aA]	2.41±0.19[cB]
T2	5.33±0.28[abA]	2.91±0.14[cB]
T3	6.08±0.49[aA]	2.25±0.21[cB]
T4	5.41±0.31[aA]	2.5±0.15[cB]
C	5.33±0.30[abA]	4.58±0.12[bA]

*I UC: First uterine cytology; II UC: Second uterine cytology.

Note: The values with different superscripts in upper case differ significantly (P<0.05) within groups (between the columns) and those with different superscripts in lower case differ significantly (P<0.05) between the groups (between the rows).

Table 3. Overall conception rates (%) in different experimental groups (n=12/group)

Groups	On day 24 post AI (by P4 assay)	Range of P_4 concentration (ng/ml) of pregnant animals (by P4 assay)	On day 60 post AI (by rectal palpation)	Accuracy of P4 assay for pregnancy diagnosis (%)
T1	66.66 (8/12)	2.94 - 5.27	66.66 (8/12)	100 (8/8)
T2	58.33 (7/12)	2.24-5.45	50 (6/12)	85.71 (6/7)
T3	75 (9/12)	3.0 - 5.96	66.66 (8/12)	88.88 (8/9)
T4	83.33 (10/12)	2.78 - 5.90	75 (9/12)	90 (9/10)
C	25 (3/12)	4.10 - 5.93	25 (3/12)	100 (3/3)
Overall	61.66 (37/60)	2.24 -5.96	56.66 (34/60)	91.89 (34/37)

Table 4. Numbers of cows pregnant after 1st and 2nd AI in different groups (n=12/group)

Groups	Number of cows pregnant after 1st AI	Number of cows pregnant after 2nd AI	Total number of pregnant cows (%)
Group 1	5	3	8/12 (66.66)
Group 2	3	3	6/12 (50)
Group 3	6	2	8/12 (66.66)
Group 4	7	2	9/12 (75)
Control	1	2	3/12 (25)

Discussion

Incidence of SE. One of the major reproductive problems in dairy cattle that is prevalent at field level is the existence of a large number of repeat breeder cows. Under field conditions, repeat breeding is mainly associated with SE, delayed ovulation and corpus luteum deficiency, and results in fertilization failure or embryonic mortality (CENARIU and JOSPE, 2017). In the present study, the prevalence of SE in repeat breeding cows was lower than in earlier studies by SALASEL et al. (2010) and JANOWSKI et al. (2013) as they observed the prevalence of SE in repeat breeding cows to be 52.7% and 40.2%, respectively. However, POTHMANN et al. (2015), SINGH et al. (2016) and PASCOTTINI et al. (2017) reported a lower percentage of repeat breeding cows with this disorder, 12.7%, 29.4% and 27.8%, respectively. The differences in prevalence between the studies may be associated with different study populations, management practices, feeding practices and environmental factors. Also, most of the animal farms in Bikaner city are unorganized and unhygienic.

The range of mean PMNs (%) was 5.33±0.28 to 6.08±0.49 on the day of the 1st uterine cytology (spontaneous estrus) which was in accordance with the finding of SINGH et al. (2016) (4.4±0.27 to 5.5±0.78). In another study, this range was recorded as 5.60±3.82 to 12.50±2.96 on the day of selection (D=0) (ALAGAR et al., 2017). The lower PMNs count in the present study may be due to the fact that uterine cytology was performed late postpartum (>90 days postpartum). There was a significant (P<0.05) decline in PMNs (%) at the subsequent estrus post treatment in all the treatment groups, indicating recovery from infection. In the control group no significant change was observed as no treatment was given.

Conception rate. The accuracy of P4 assay for pregnancy diagnosis in the present study {91.89% (34/37)} was in accordance with a previous study by BUTTERFIELD and LISHMAN (1988) who observed milk P4 values on days 20 and 24 post AI, and found P4 accuracy to be 89.1 and 91.4%, respectively. In other studies, MUHAMMD et al. (2000) and OTAVĂ et al. (2007) reported the accuracy of P4 assay for pregnancy diagnosis as 83.3 and 82% on days 25 and 19 post AI, respectively. The incorrect diagnosis of early pregnancy by P4 may be attributed to embryonic mortality occurring after day 16, followed by late luteolysis. Early pregnancy losses are common up to day 55 in cows (CHAUDHARY and PUROHIT, 2012).

In the present study, the conception rate was highest in the cows treated with Uraksha (polyherbal drug) (T4 group). KHILLARE et al. (2010) reported a 70% (14/20) conception rate in endometritis, metritis and repeat breeding cows treated with AV/RMI/45 (a polyherbal drug). This observation suggests that our herbal remedies are effective to combat uterine infection and may be a good alternative of antibiotics. The highest conception rate in this group (T4 group) may be due to the fact that different herbs contain different phytochemical properties, such as antibacterial, anti-inflammatory, immunoregulatory, anti-oxidant etc. (MUKHERJEE et al., 1996; QIU et al., 2000;

FERRO et al., 2003; SAHA et al., 2016; HAZRA et al., 2017; WRÓBLEWSKA et al., 2018). Moreover, the herbs have the potential to break the bio-film made by the pathogens (TSUKATANI et al., 2020) and thus provide a healthier uterine environment for conception. The lowest conception rate in the control group indicates that treating SE with antimicrobial or polyherbal drugs cleared the infection and improved conception rates in the treatment groups. Higher pregnancy rates in treatment groups (antimicrobial or polyherbal drugs) compared to the control (untreated) were also observed in previous studies (KHILLARE et al., 2010; HAJIBEMANI et al., 2016; TISON et al., 2017).

Conclusions

It was concluded that the uterine cytobrush technique can be used efficiently for diagnosing subclinical endometritis in dairy cattle. Polyherbal intrauterine drugs may be a good alternative to intrauterine antibiotic therapy to combat uterine infections. However, further studies are warranted to test the use of polyherbal intrauterine drugs on a larger cattle population.

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SAŽETAK

Rad je proveden kako bi se istražila učestalost supkliničkog endometritisa (SE) u mliječnih krava koje se pregone. Također, usporedila se učinkovitost različitih antimikrobnih i poliherbalnih intrauterinih terapijskih postupaka. U krava koje se pregone (više od 90 dana u mužnji), s čistim cerviko-vaginalnim iscjetkom iz rodnice u spontanom estrusu, tehnikom citočetkice učinjena je citološka pretraga maternice. Za krave kod kojih je utvrđeno 4% polimorfonuklearnih stanica (PMN) smatralo se da imaju supklinički endometritis. Životinje su nasumično podijeljene u pet skupina s jednakim brojem jedinki (n=12 po skupini) i liječene intrauterino sljedećim lijekovima: Vodine (skupina T1), Metricare (skupina T2), Utriguard (skupina T3), Uraksha (poliherbalni pripravak; skupina T4), dok je peta skupina bila kontrolna skupina (C). U sljedećem estrusu ponovljena je citološka analiza maternice i krave su osjemenjene. Krave nisu ostale gravidne te su u sljedećem estrusu ponovno osjemenjene. Provjeren je status gravidnosti. Incidencija SE-a bila je 36,80 % (60/163). Postotak PMN-a znatno se smanjio u svih krava u tretiranim skupinama kao odgovor na liječenje. Najveća stopa koncepcije zabilježena je u krava u skupini T4 (liječene Urakshom). Zaključeno je da tehnika citočetkica može učinkovito poslužiti u dijagnostici SE-a u mliječnih goveda te da poliherbalni intrauterini lijekovi mogu biti dobra alternativa antimikrobnoj intrauterinoj terapiji.

Ključne riječi: goveda; pregon; supklinički endometritis; citočetkica; citologija maternice; poliherbalni lijekovi