The Indian Journal of Veterinary Sciences & Biotechnology ***(2018) Volume 13, Issue 4, 26-31***

ISSN (Print) : 2394-0247 : ISSN (Print and online) : 2395-1176, abbreviated as IJVSBT

10.21887/ijvsbt.v13i4.11554

Submitted : 16-01-2018 ***Accepted : 20-02-2018*** ***Published : 08-04-2018***

Endometrial Cytology and Cervical Mucus Characteristics of Repeat Breeding Crossbred Cows in Relation to Post-Treatment Fertility

S.R. Raval, M.T. Panchal, A.J. Dhami* and S.C. Parmar

Dept. of Animal Reproduction Gynaecology & Obstetrics

College of Veterinary science & Animal Husbandry, AAU, Anand-388 001, Gujarat

Corresponding Author: ajdhami@aau.in

Abstract

The present study was conducted on repeat breeder (RB, n=40) crossbred cows under field conditions to evaluate their endometrial cytology and cervico-vaginal mucus (CVM) characteristics in relation to post-treatment fertility. The endometrial cytology was obtained using cytobrush technique and CVM by aspiration technique from vaginal fornix using sterile pipette and syringe. The cows yielding bacterial growth in CVM were treated with sensitive antibiotics (i/m) for 3-5 days (Gr I, RBT: 28) and cows not yielding bacterial growth (control, Gr II, RBC: 12) were given 10 ml of normal saline (i/m). The cows were inseminated at subsequent estrus and followed for repeating to estrus and/or pregnancy. The subclinical endometritis was diagnosed using endometrial cytology (PMN%: $\geq$ 4%) in cows yielding bacterial isolates (n=28) with mean PMN% of 21.80±8.39, 32.18±3.26 and 29.25±6.63 for those having clear stringy consistency, turbid viscous, and watery white flaky CVM, respectively. The mean PMN% in RB cows with no bacterial isolates (n=12) and having clear stringy CVM was 1.50±0.23. The mean values of spinnbarkeit (cm), pH and Whiteside test scores of CVM were 14.60±0.51, 5.82±0.85 and 3.00±0.17; 7.41±0.15, 7.44±0.21 and 7.08±0.19; and 1.40±0.60, 1.91±0.28, and 1.75±0.25, respectively, for culturally positive cows having CVM of clear stringy, turbid viscous and watery with white flakes, respectively (Group I). The mean spinnbarkeit (cm), pH and Whiteside test scores in control cows not yielding any bacterial isolates and having clear stringy CVM (Group II) were 13.08±0.99, 7.37±0.10 and 0.33±0.19. The RB cows with clear stringy, turbid viscous and white flaky CVM and yielding bacterial isolates had mean inter-estrus intervals of 18.80 ±0.51, 15.91±0.48 and 13.83±0.41 days, respectively, the differences being significant (P<0.05), with respective post-treatment pregnancy rates of 80.00, 63.63 and 41.66 %, with an overall pregnancy rate of 57.15%. This interval was 19.42±0.45 days in RB control cows, wherein the pregnancy rate was 66.66% without any treatment. The cytobrush technique (PMN%) for endometrial cytology and Whiteside test of CVM were good tools for ruling out the subclinical genital infections in repeat breeding cows.

Key words: Crossbred cows, Repeat breeding, Cytobrush, Endometrial cytology, Whiteside test, Spinnbarkeit, Association with Fertility.

Introduction

Repeat breeding is the most commonly encountered but poorly understood subfertile conditions in cattle with prevalence rate of 10-14% (Yusuf *et al*., 2010). The nutritional and hormonal imbalance, anatomical defects, improper management and endometritis have been recognized to cause repeat breeding in bovines (Kumar *et al*., 2015). Cytological endometritis and purulent vaginal discharge are overlapping, but largely distinct conditions, and there are emerging data that cervicitis exists both concurrent with and separate from endometritis. Much remains to be learnt about what initiates and sustains harmful inflammation of the reproductive tract. Such information is necessary to develop effective treatments for the various forms of disease and more importantly, to develop means to prevent endometritis (Stephen *et al*., 2011). Endometrial cytology and physical characteristics of cervical mucus are considered as effective laboratory tools to predict the uterine health and infertility in repeat breeding cattle (Rangnekar *et al.,* 2002; Madoz *et al.,* 2013). Hence, this study was aimed to evaluate the endometrial cytology, as well as physical characteristics, pH, spinnbarkeit and Whiteside test score of genital discharge in relation to post-treatment fertility in repeat breeding cows under field conditions.

Materials and Methods

This investigation was carried out during August 2016 to February 2017 on 40 repeat breeding crossbred cows presented at AI Centres of Chikhodra and Bedva villages of Anand district in Gujarat.

Collection and Evaluation of Physical Properties of CVM

The cervico-vaginal mucus (CVM) samples were aspirated aseptically from the cervix/ vaginal fornix during estrus using sterilized glass pipette (10 ml capacity) with wide mouth end, employing recto-vaginal technique of Panangala *et al.* (1978). The pointed end of pipette was connected to a 20 ml syringe with rubber junction. After collection, the samples were brought to the College within 2 hrs and were cultured for bacterial isolation and antibiotics sensitivity spectrum. The CVM samples were assessed for its colour by direct examination and were categorized as clear stringy, turbid viscous and watery with white flakes (Bhat *et al.,* 2015). The pH of CVM was determined using digital pH meter. The spinnbarkeit measurements were made in centimeter as described by Verma *et al*. (2014). For Whiteside test, the CVM (1 ml) samples were heated with equal volume of 5% sodium hydroxide in glass test tubes up to boiling point and the intensity of colour changes were graded as colourless (normal), light yellow colour (mild infection), yellow colour (moderate infection) and dark yellow colour (severe infection) with 1 to 4 scores (Kumar *et al.,* 2015).

Collection of Endometrial Cytology using Cytobrush Assembly and its Evaluation

After proper restraining, back raking and cleaning of vulva and perineal area, the vulvar lips were pulled apart by an assistant and the cytobrush assembly (specially fabricated for collection of bovine endometrial cyctology comprising intrauterine catheter and a stylette attached with cytobrush) was introduced into vagina and then through the cervix, body of the uterus and up to the mid uterine horn. The stylette was pushed to expose cytobrush and then screwed gently in clockwise direction. Gentle pressure was applied on uterine horn by the left hand via the rectum to ensure proper contact of cytobrush with the endometrium. The inner stylette was then withdrawn into the outer catheter to its normal position and then the whole catheter was withdrawn from the reproductive tract. Immediately after removing the cytobrush from uterine horn, the cytobrush was rolled on clean glass slide. Slide was fixed with methyl alcohol, air dried and stained with Field stain (Eosin and Methylene blue). The slide was washed in running tap water, air dried and was observed under microscope with oil immersion to determine the percentage of neutrophils (PMN %). A total of 100 endometrial and PMN cells were counted in each specimen and the per cent PMN cells count was assessed. The subclinical endometritis was declared in repeat breeder cows on the basis of clear CVM and $\geq$ 4 % PMN cells (Singh *et al*., 2016).

Treatment and Follow up

The cows yielding bacterial isolates were treated with effective antibiotics based on *in vitro* ABST by i/m route for 3-5 days (RBT, n=28). They were inseminated at subsequent estrus and followed for 3 cycles and/or pregnancy. The cows not yielding any bacterial growth received 10 ml normal saline i/m and served as control (RBC, n=12), and were inseminated and followed without antibiotic treatment. The observations made on PMN%, Whiteside test, pH, spinnbarkeit, inter-estrus intervals and fertility results were analyzed statistically.

Results and Discussion

Characteristics of Cervico-Vaginal Mucus

Out of 40 CVM samples collected from repeat breeder cows, 28 (70%) samples yielded bacterial isolates and were put under antibiotics treatment group. The quality of the CVM in these cases was found to be clear stringy (17.86%; 5), turbid viscous (39.29%; 11) and watery mixed with white flakes (42.85%; 12). The CVM of rest of all 12 (30%) cows not yielding any bacterial isolates (control group) was clear stringy (Table 1).

(a) Spinnbarkeit Value

The mean spinnbarkeit values found in CVM of cows yielding bacterial isolates from clear stringy, turbid viscous, and watery white flaky mucus were 14.60±0.51, 5.82±0.85 and 3.00±0.17 cm, respectively, differing significantly ($P<0.05$). The post-treatment pregnancy rates in cows under these three mucus categories were 80.00, 63.63 and 41.66 % ($P<0.05$), respectively, with the overall pregnancy rate of 57.15 %. The mean spinnbarkeit value in clear stringy CVM of cows not yielding bacterial isolates was 13.08±0.99 cm, with the pregnancy rate of 66.66 % (Table 1). The mean spinnbarkeit value observed in present study varied significantly ($P<0.05$), being the highest (14.60±0.51 and 13.08±0.99 cm) in CVM having clear stringy colour-consistency in cows under both treatment and control groups, with significantly higher respective pregnancy rates (80.00%, 4/5; 66.66%, 8/12). These observations are indicative of importance of physical characteristics of CVM and resultant pregnancy with conducive uterine environment. These observations corroborated well with the report of Rangnekar *et al.* (2002) in repeat breeder crossbred cattle and Sharma *et al.* (2011) and Verma *et al.* (2014) in buffaloes. In contrast to above findings, the mean spinnbarkeit values (cm) were found to be significantly lower (5.82±0.85; 3.00±0.17 resp.) in repeat breeder cows yielding bacterial isolates (treatment group) with turbid and watery white flaky discharge as compared to the clear stringy (14.60±0.51) CVM samples, with corresponding pregnancy rates of 63.63 and 41.66 vs. 80.00 %. These observations of reduced spinnbarkeit values were indicative of unfavourable estrus and uterine environment as reflected by reduced rate of pregnancy. Sharma *et al.* (2011) also reported significantly ($P<0.01$) lower spinnbarkeit value of CVM in non-conceived buffaloes as compared to that of conceived buffaloes.

(b) pH of CVM

The mean pH values recorded for CVM having clear stringy, turbid, and watery white flaky characteristics and yielding bacterial isolates did not differ significantly, though the pregnancy rates were distinctly different ($P<0.05$). The mean pH value of CVM having clear stringy characteristic in cows not yielding bacterial isolates was 7.37±0.10, with considerably good pregnancy rate of 66.66 % (Table 1). It is worth to note that the mean pH values were found to be alkaline in both the groups indicative of subclinical bacterial infection in repeating cows. Samad *et al.* (2002) reported the mean pH of 7.39±0.35 in repeat breeder buffaloes. Hafez and Hafez (2000) and Sheldon *et al.* (2006) opined that high alkalinity of the CVM could be due to bacterial contamination and is not suitable for survival and penetration or mobilization of spermatozoa. Modi *et al.* (2011) however observed alkaline pH in normal breeders (8.39±0.17) as compared to repeat breeder (6.19±0.18) Kankrej cows.

Table 1: Relationship of endometrial cytology (PMN%), CVM characteristics and pregnancy rates in repeat breeder crossbred cows (n=40) under treatment and control groups

Treatment Group I: Cows yielding bacterial isolates (n=28)						
CVM characteristics	PMN (%)	Inter-estrus interval (days)	Spinbarkeit (cm)	pH	Whiteside test (Score)	Pregnancy rate* (%)
Clear stringy (17.86%; 5/28)	21.80±8.39^{b}	18.80±0.51^{c}	14.60±0.51^{c}	7.41±0.15	1.40±0.60^{b}	80.00 (4)
Turbid viscous (39.29%; 11/28)	32.18±3.26^{b}	15.91±0.48^{b}	5.82±0.85^{b}	7.44±0.21	1.91±0.28^{b}	63.63 (7)
Watery with white flakes (42.85%; 12/28)	29.25±6.63^{b}	13.83±0.41^{a}	3.00±0.17^{a}	7.08±0.19	1.75±0.25^{b}	41.66 (5)
Control Group II: Cows not yielding bacterial isolates (n=12)						
Clear stringy (100%; 12/12)	1.50±0.23^{a}	19.42±0.45^{c}	13.08±0.99^{c}	7.37±0.10	0.33±0.19^{a}	66.66 (8)

Means bearing different superscripts (a, b, c) within column differ significantly ($p<0.05$)

* Pregnancy rates differed significantly ($p<0.05$) within the column (÷2: 1.97×10^{-9}).

(c) Whiteside Test (WST) of CVM

The mean WST scores recorded for CVM having clear stringy, turbid, and watery white flaky characteristics in cows yielding bacterial isolates were 1.40±0.60, 1.91±0.28 and 1.75±0.25, respectively, with non-significant differences. The mean WST score was 0.33±0.19 in CVM having clear stringy consistency in cows not yielding bacterial isolates. The mean WST scores were found to be significantly ($P<0.05$) higher in CVM samples yielding bacterial growth than those not yielding any bacterial culture (Table 1).

Among the 40 cows screened for detection of subclinical endometritis using WST, irrespective of bacterial growth, the nil/colourless, light, moderate and intense yellow colour reactions (score 1 to 4) were found in 32.50 (13), 25.00 (10), 25.00 (10) and 17.50 (7) % CVM samples. These results obtained on WST scores of CVM corroborated well with the observations reported by Mohan *et al.* (2006), that the Whiteside test is a simple, quick, reliable and accurate test which is highly useful for veterinarians in field conditions to differentiate the normal healthy animal from subclinical and clinical case of endometritis. Present findings were also in line with the observations made by Bhattaharya *et al.* (2011), Gupta *et al.* (2012), Raja *et al.* (2012), Bhat *et al.* (2015) and Kumar *et al.* (2015).

Inter-Estrus Intervals

The mean inter-estrus intervals recorded in the repeat breeder cows yielding bacterial isolates with the CVM having clear stringy, turbid, and watery white flaky characteristics were 18.80±0.51, 15.91±0.48 and 13.83±0.41 days, respectively ($P<0.05$; Table 1). The mean inter-estrus interval recorded in cows not yielding bacterial isolates and having clear stringy CVM was 19.42±0.45 days, with the pregnancy rate of 66.66 %.

The repeat breeder cows yielding bacterial isolates, but having clear stringy CVM had normal mean inter-estrus interval (18.80±0.51 days) with the highest spinnbarkeit value (14.60±0.51 cm) and the least WST score (1.40±0.60), as compared to cows having apparently unhealthy mucus with the respective inter-estrus interval of 15.91±0.48 and 13.83±0.41 days, indicative of having their favourable effects on getting the animals pregnant with a good pregnancy rate of 80.00 %, far better

than cows not yielding bacterial isolates and having clear stingy CVM (Table 1). These results are indicative of positive impact of healthy uterine environment as reflected by the lower PMN (1.50±0.23 %) and WST score (0.33±0.19) and normal inter-estrus interval (19.42±0.45 days) and spinnbarkeit (13.08±0.99 cm) values along with nearly normal pH (7.37) in getting the animal easily pregnant.

Polymorphonuclear Cells (PMN%) of Endometrial Cytology

The mean per cent PMN cells recorded in endometrial cytology of cows having clear stringy, turbid viscous, and watery white flaky CVM and yielding bacterial isolates were 21.80±8.39, 32.18±3.26 and 29.25±6.63, respectively, the difference being non-significant. The mean PMN (%) obtained in the culturally sterile cows (Control Group) was significantly ($P<0.05$) lower (1.50±0.23) than those of cows yielding bacterial isolates (Treatment Group). Thus, endometrial cytology would be of immense importance in ruling out the genital infection to take a judicious and timely decision whether to treat or not the animal with antibiotics and thereby to bypass the undue use of antibiotics and to prevent the problem of antibiotics resistance. Further, there were 5 (17.86%) cows yielding bacterial growth from clear stringy mucus apparently non-indicative of genital infection, however turned out to be positive for subclinical genital bacterial infection on endometrial cytology criteria as evidenced by the mean PMN (%) of 21.80±8.39. These observations are indicative of prevalence of subclinical or occult genital infections, to the tune of 18 %, among repeating bovine females. Pothmann *et al.* (2015) also reported the prevalence of subclinical endometritis in repeat breeder cattle to the extent of 12.70 %. However, as compared to the present finding, Singh *et al.* (2016) reported higher incidence of subclinical (51.20%) and clinical (29.00%) endometritis in repeat breeder crossbred cows on the basis of cytobrush technique results.

The repeat breeder cows yielding bacterial isolates and treated with the antibiotics found sensitive on ABST gave pregnancy rates of 80.00, 63.63 and 41.66 % for those having clear stringy, turbid and watery white flaky CVM, respectively. In contrast to this overall 57.15 % pregnancy rate of treated cows, 66.66 % of control cows having clear mucus discharge and not yielding bacterial isolates conceived over three cycles. It was thus concluded that about 70 % of repeat breeding cows harbour bacterial genital infection and accordingly show varying qualities of CVM, and that endometrial cytology using cytobrush technique (PMN%) as well as spinnbarkeit, pH and Whiteside test of CVM are good tools for ruling out the subclinical endometritis in them with good fertility, if treated using antibiotics according to ABST of isolates.

Acknowledgements

We thank Dr. A.M. Thaker, Dean of the Faculty of Veterinary Science, AAU, Anand and Professor & Head, Dept. of Microbiology for infrastructure and lab facilities provided.

Conflict of Interest: All authors declare no conflict of interest.

References:

Bhat, F.A., Bhattacharyya, H.K., Fazili, M.R., Hussain, S.A. and Khan, M.Z. (2015). Studies on estrual cervical mucus of repeat breeding cows with special reference to ovulatory disturbances and genital infection. *Theriogenology,* 5(2): 113-23.

Bhattacharyya, H.K., Makhdoomi, D.M., Hafiz, A. and Fazil, M.R. (2011). Clinico-therapeutic management of subclinical metritis in cows. *Intas Polivet*, 12(1): 26-27.

Gupta, G.P., Shyam, K. ., Mohandas, A.C. and Snehraj, R.K. (2011). Use of whiteside test to study subclinical endometritis in crossbred cattle. *Bhartiya Krushi Anusandhan Patrika,* 26(3): 123-125.

Hafez, E.S.E. and Hafez, B. (2000). Transport and survival of gametes. In: *Reproduction in Farm Animals*. 7th Edition, Lippincott Williams and Wilkins, Philadelphia, pp. 82-95.

Kumar, S., Bhardwaz, A., Srivastava, A.K., Rao, M. and Kumar, N. (2015). Whiteside test: a field test on the cervical mucus of cows for diagnosis of endometritis. *Intas Polivet,* 16(2): 207-213.

Madoz, L.V., Giuliodori, M.Z., Jaureguiberry, M., Plontzk, J., Drillich, M. and De la Sota, R.L. (2013). The relationship between endometrial cytology during estrous cycle and cut off points for the diagnosis of subclinical endometritis in grazing dairy cows. *J. Dairy Sci.,* 96: 4333-4339.

Modi, L.C., Suthar, B.N., Nakhashi, H.C., Sharma, V.K. and Panchasara, H.H. (2011). Physical characteristics of estrual cervical mucus and conception rate in repeat breeder Kankrej cattle. *Int. J. Agri. Vet. Med. Sci.,* 5(4): 416-423.

Mohan Kumar, O.R., Prasuna, K. and Srinivasa Rao, T. (2006). Study on subclinical metritis in buffaloes using white side test. *Intas Polivet,* 7(1): 24-25.

Panangala, V.S., Fish, N.A. and Barnum, D.A. (1978). Microflora of cervico-vaginal mucus of repeat breeder cows. *Can. Vet. J.,* 19: 83-89.

Pothmann, H., Prunner, I., Wagener, K., Jaureguiberry, M., de la Sota, R.L., Erber, R., Aurich, C., Ehling-Schulz, M. and Drillich, M. (2015). The prevalence of subclinical endometritis and intrauterine infections in repeat breeder cows. *Theriogenology,* 83: 1249-1253.

Raja, S., Devanathan, T.G., Kulasekar, K., Pazhanivel, N. and Balachandran, C. (2012). Whiteside test endometrial biopsy for diagnosis of endometritis in repeat breeding cows. *Indian J. Anim. Reprod.,* 33(1): 56-58.

Rangnekar, M.M., Dhoble, R.L., Gacche, M.G., Ingawale, M.V., Sawale, A.G. and Jadav, J.M. (2002). Physical properties of estrual cervical mucus in repeat breeding HF crossbred cows with reference to fertility. *Indian J. Anim. Sci.,* 72(12): 1122-24.

Samad, H.A., Shah, S.M.I., Nazir, A. and Nafees, A. (2002). Physical characteristics of estrus mucus and conception rates in repeat breeder buffaloes. *Pakistan Vet. J.,* 22(1): 31-34.

Sharma, H.C., Dhami, A.J. and Kavani, F.S. (2011). Properties of estrual cervical mucus in relation to plasma progesterone and conception rates in buffaloes. *Indian J. Anim. Reprod.,* 32(3): 8-11.

Sheldon, I.M., Lewis G.S., LeBlanc, S. and Gilbert, R.O. (2006). Defining postpartum uterine disease in cattle. *Theriogenology,* 65: 1516-1530.

Singh, J., Honparkhe, M., Chandra, M., Kumar, A., Ghuman, S.P.S. and Dhindsa, S.S. (2016). Diagnostic efficacy of uterine cytobrush technique for subclinical endometritis in crossbred dairy cattle. *Indian Vet. J.,* 93(2): 11-13.

Stephen, J.L., Takeshi, O. and Jocelyn, D. (2011). Advances in bovine reproduction and embryo technology reproductive tract defence and disease in postpartum dairy cows. *Theriogenology,* 76: 1610-1618.

Verma, K.K., Prasad, S., Kumaresan, A., Mohanty, T.K., Layek, S.S., Patbandha, T.K. and Chand, S. (2014). Characterization of physio-chemical properties of cervical mucus in relation to parity and conception rate in Murrah buffaloes. *Vet. World.,* 7(7): 467-471.

Yusuf, M., Nakao, T., Ranasinghe, R. B. K., Gautam, G., Long, S. T. and Yoshida, C. (2010) Reproductive performance of repeat breeders in dairy herds. *Theriogenology,* 73: 1220-1229.

❑