Nature of Genital Discharge and pH of Cervical Mucus and Uterine Flushing Before and After Treatment in Endometritis Affected Cows

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Abstract

The nature of cervical mucus and the change in the pH of cervical mucus and uterine flushings in 72 crossbred cows were studied. Six groups (12 cows in each group) received treatment viz. 30 ml of 2 per cent Lugol's iodine (Group I), 30 ml PBS containing 100 µg of E.coli LPS (Group II), 2 mg of lysozyme (Group III), 500 mg of oyster glycogen (Group IV), 25 mg $PGF_2\alpha$ (Group V) and 30 ml PBS intrauterine on the day of oestrus (Group VI, control). The mean pH value of the cervical mucus and uterine fluid before treatment was ranged from 8.47 ± 0.02 to 8.62 ± 0.02 and 7.87 ± 0.04 to 8.30 ± 0.02 and after the treatment it ranged from 7.01 ± 0.02 to 8.17 ± 0.04 and 7.61 ± 0.01 to 7.97 ± 0.01 in all the treated and control groups, respectively with a significant (P≤0.01) reduction in the pH value. The cows were inseminated with good quality frozen semen after giving sexual rest for one cycle. The highest conception rate was obtained in E. coli LPS group (83.33 per cent), followed by lysozyme group (66.67 percent) and oyster glycogen group (66.67 per cent), respectively.

Key words: Endometritis, Immunomodulators, Ph, Cervical Mucus, Conception Rate

Introduction

A timely diagnosis and an efficacious treatment are essential for successful management of endometritis in bovines. The nature of vaginal discharge is commonly used for the diagnosis of the clinical endometritis in cows (Williams et al., 2005) which is present in only 43 per cent of affected cows (Dohmen et al., 1995). Alternatively vaginal speculum or vaginoscopy can be used to diagnose endometritis (Erin et al., 2005). The cervical mucus shows changes in pH which can be used as a useful diagnostic tool for endometritis (Krishnakumar et al., 2003). The present study is attempted to know the

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efficacy immunomodulators, $PGF_2\alpha$ and Lugol's iodine on the nature of vaginal discharge and also on the pH of cervical and uterine secretions in the endometritis affected cows.

Materials and Methods

The study was conducted in crossbred dairy cows presented for artificial insemination at Teaching Veterinary Clinical Complex (TVCC) and Veterinary Dispensaries in Namakkal District, Tamil Nadu State. The cows were screened thoroughly for their genital health and reproductive status through history and gynaecological examination and used for the study. Among all, 72 crossbred cows affected with endometritis were utilized for the study. The selected crossbred cows were randomly divided into five treatment (Group I to V) and one control (Group VI) groups and were marked as Group I (LI group;

30 ml of 2% Lugol's iodine solution intrauterine for three consecutive days from the day of oestrus), Group II (LPS group; 100 µg/cow in 30 ml of PBS intrauterine), Group III (LYZ group; 2 mg/cow in 30 ml of PBS intrauterine), Group IV (OG group; 500 mg/cow in 30 ml of PBS intrauterine), Group V ($PGF_2\alpha$ group; 25 mg of Dinoprost Tromethamine, Lutalyse®, Pfizer India Private Limited on day 10 of the estrous cycle) and Group VI (control group; sterile 30 ml PBS intrauterine during oestrus).
All the cows were examined with vaginal speculum for the presence of any purulent or mucopurulent material on the walls of vagina and the characteristics of secretions in the anterior vagina and external os of the cervix just before the start of treatment (day 0) and during subsequent oestrus as per the technique described by Azawi et al. (2008). Similarly, the vaginal and cervical mucus were observed with Olympus Vaginoscope as per Oral et al. (2009) with slight modifications.

In all the cows, the cervical mucus was collected at the time of oestrus just before the start of treatment and also in the subsequent oestrus following treatment as per technique of Raju et al. (2009). In all the selected cows, after induction of epidural anesthesia, the uterine flushing was done just before the start of treatment (0 hour) and at 24, 48 and 72 hours after treatment. The sterile Rusch catheter (18'') was inserted into the body of the uterus and the cuff was inflated with 10-12 ml of air. Sterile phosphate buffered saline (PBS, 30 ml, pH 7.4) solution was infused into the uterus by using a 50 ml disposable syringe. After 3-5 minutes, the uterine fluid was recovered by gentle massage and back racking (Singh et al., 2000). The collected flushings were kept in sterile polypropylene tubes and stored in ice. The pH of the cervical mucus and uterine fluid was estimated using a Systronic Digital pH meter, model - 335.

All the control and treated cows were given sexual rest for one oestrus cycle following treatment. During the next oestrus all (i.e 2^{nd} oestrus following the treatment) the cows were inseminated artificially using good quality frozen thawed semen. The efficacy of different treatment regimens was assessed on the basis

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of negative reaction to white side test with cervical mucus collected in the subsequent oestrum following treatment. All the cows were examined rectally 60 days post AI to diagnose pregnancy.

All the collected data were analyzed as per the standard procedures described by Snedecor and Cochran (1989).

Results and Discussion

During speculum examination, the percentage of clear mucus discharge before treatment was 9.70 which increased to 76.40 per cent following treatment. Further, the percentage of mucopurulent (48.60) and purulent discharge (41.70) was greatly reduced to 15.30 and 8.30 per cent, respectively. These positive changes were in accordance with the report of Oral et al. (2009). Similarly, all the selected cows were also subjected to vaginoscopic examination before and after treatment to study the nature of genital discharges. The effectiveness of the treatment was studied by a drastic increase in the percentage of clear mucus discharge (70.80 per cent) after treatment when compared to 12.50 per cent clear discharge before treatment. Further, percentages of mucopurulent (48.70) and purulent (38.80) discharges got reduced to 23.60 and 5.60 per cent, respectively.

In the present study mild variation in the percentages were observed in the nature of estrual discharge during speculum and vaginoscopic examination in the endometritis affected cows which concurred with results of Williams et al. (2005). Erin et al. (2005) and Oral et al. (2009) opined that the sensitivity and accuracy of vaginoscopy was higher than vaginal speculum in identification of endometritis whereas Williams et al. (2005) recommended both vaginal speculum examination and vaginoscopy for the identification and examination of oestrual mucus in the endometritis cows. Elimination of purulent and mucopurulent discharge with an increasing trend in clear mucus indicates the rejuvenation of uterine defense mechanism with respect to the treatment given.

The pH of cervical mucus and uterine flushing before and after treatment is given in Table 1. The mean (±SE) pH value of the cervical mucus before treatment ranged from 8.47 ± 0.02 to 8.62 ± 0.02 and after the treatment it ranged from 7.01 ± 0.02 to 8.17 ± 0.04 in all the treated and control groups. In the present study, there was a significant ($P \leq 0.01$) reduction in the mean (±SE) pH value of cervical mucus in all the animals after the treatment. However, only a minor fall in the pH value was noticed in the control group, which differed significantly with that of treatment groups. Among the various treatment groups, the groups of immunomodulators (Group II, III and IV) did not show any significant difference in pH among them, but these groups had high significant difference ($P \leq 0.01$) with the other groups (I, V and VI).

The mean (±SE) pH of uterine fluid in this study, before treatment ranged from 7.87 ± 0.04 to 8.30 ± 0.02 and after the treatment it ranged from 7.61 ± 0.01 to 7.97 ± 0.01 in all the treated and control groups

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which concurred with the results of Sahadev et al. (2007). Statistical analysis revealed that the mean pH values of uterine fluid before treatment in all the cows of treated and control groups did not have any significant difference. But after treatment, all the treatment groups showed drastic reduction in mean pH of uterine fluid, when compared to the values before treatment and the differences were statistically highly significant ($P \leq 0.01$).

Alteration in the pH of cervical mucus and uterine fluid to alkaline side was attributed to inflammation due to infection of endometrium, metabolites of bacteria and inflammatory exudates which resulted in conception failure due to death of spermatozoa (Singla et al., 1991). The drastic reduction in pH values following treatment in this study correlated to the declining levels of metabolites of bacteria and inflammatory exudates as reported by Salphale et al. (1993).

Table 1: Mean (± SE) pH values of cervical mucus and uterine fluid before and after treatment in endometritis affected cows

S.No	Treatment groups	Cervical mucus		Uterine flushing	
		Before treatment	After treatment	Before treatment	After treatment
1	I (LI)	$8.47^{a} \pm 0.02$	$7.22^{by} \pm 0.02$	$8.15^{a} \pm 0.01$	$7.66^{bx} \pm 0.01$
2	II (LPS)	$8.62^{a} \pm 0.02$	$7.01^{bx} \pm 0.02$	$8.21^{a} \pm 0.02$	$7.70^{bx} \pm 0.02$
3	III (LYZ)	$8.53^{a} \pm 0.02$	$7.05^{bx} \pm 0.02$	$7.87^{a} \pm 0.04$	$7.61^{bx} \pm 0.01$
4	IV (OG)	$8.50^{a} \pm 0.03$	$7.11^{bx} \pm 0.03$	$8.12^{a} \pm 0.01$	$7.68^{bx} \pm 0.02$
5	V ($PGF_2\alpha$)	$8.53^{a} \pm 0.03$	$7.22^{by} \pm 0.03$	$8.30^{a} \pm 0.02$	$7.72^{bx} \pm 0.03$
6	VI (Control)	$8.52^{a} \pm 0.03$	$8.17^{bz} \pm 0.04$	$8.01^{a} \pm 0.02$	$7.97^{ay} \pm 0.01$

Mean values bearing different superscripts between columns (a, b) and among rows (x, y, z) differ significantly ($P \leq 0.01$).

In the present study the pregnancy rate of 33.33, 83.33, 75.00, 66.67, 50.00 and 8.33 per cent was recorded in group Group I, II, III, IV, V and VI, respectively. Specifically all immunomodulators had improved the conception rate following administration in endometritis cows. Among the various immunomodulators used, the highest conception rate was obtained in E. coli LPS group (Group II, 83.33 per cent), followed by lysozyme group (Group III, 66.67 percent) and oyster glycogen group (Group IV, 66.67 per cent), respectively. $PGF_2\alpha$ also considerably improved the conception rate (Group IV, 50 per cent) when compared to LI (Group I, 33.33 per cent) and control (Group I, 8.33 per cent). The results of this study concurred with Saini et al. (1999) and Sahadev et al. (2007). The highest conception rate in the immunomodulators group might be due to corrected uterine defense mechanism, significant change in the immunoglobulin level in the uterine fluid and efficiency in the elimination of bacterial contamination (Raju et al., 2009).

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From this study it was found that pH of genital discharge could be a useful diagnostic tool for diagnosis of endometritis in dairy cows (Krishnakumar et al., 2003 and Ravikumar, 2004) and the intra immunomodulators were highly efficient in eliminating bacterial contamination from in the uterus and enhancing the conception rate.

Conclusion

A total of 72 crossbred cows divided equally in to six groups and received 1). 30 ml of 2 per cent Lugol's iodine (Group I), 30 ml PBS containing 2). 100 µg of E.coli LPS (Group II), 3). 2 mg of lysozymes (Group III), 4). 500 mg of oyster glycogen (Group IV), 5) 25 mg $PGF_2\alpha$ (Group V) and 6) 30 ml PBS intrauterine on the day of oestrus (Group VI, control). The increasing percentage of clear mucus and reduction in mucopurulent and purulent discharge noticed in all treated cows during vaginal speculum and vaginoscopic examination. Mean pH decreased in all the treated groups compared to its initial level. After insemination, the pregnancy rate of 33.33, 83.33, 75.00, 66.67, 50.00 and 8.33 per cent was recorded in groups I, II, III, IV, V and VI (control), respectively.

References

1. Azwai OI, Omran SN and Hadad JJ 2008. A study on repeat breeding of Iraqi buffalo cows. Buffalo Bulletin, 27: 274.
2. Dohmen MJW, Lohuis JACM, Huszenicza GY, Nagy P and Gacs M 1995. The relationship between bacteriological and clinical findings in cows with subacute/ chronic endometritis. Theriogenology, 43:1379.
3. Erin JW, Deborah PF, Dirk UP, Gary CWE, David EN, Hilary D and Sheldon IM. 2005. Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the immune response in cattle Theriogenology, 63: 102.
4. Gilbert RO. 2003. Reproductive opportunities and challenges. Cited in: www.google.com. pp: 75.
5. Krishnakumar K, Amarnath M, Rajasundaram RC and Chandrahasan C. 2003. Efficacy of white side test for subclinical endometritis in crossbred cows. Indian Journal of Dairy Science, 56: 119.
6. Oral H, Sozmen M, Serin G and Kaya S. 2009. Comparison of the cytobrush technique, vaginoscopy and transrectal ultrasonography methods for the diagnosis of postpartum endometritis in cows. Journal of Animal and Veterinary Advances, 8: 1252.
7. Raju KGS, Venugopal Naidu K. and Sadasiva Rao K. 2009. Efficacy of intrauterine infusion of immunomodulators and $PGF_2\alpha$ in the treatment of endometritis in buffaloes. Indian Journal of Animal Reproduction, 30: 60.
8. Rangnekar MN, Dhoble RL, Gacchi MG, Ingawale MV, Sawale AG and Jadhav JM 2002. Physical properties of estrual cervical mucus in repeat breeding crossbred (Holstein-Friesian) cows with reference to fertility. Indian Journal of Animal Science., 72: 1122.
9. Ravikumar K. 2004. Studies on efficacy of certain therapeutic protocols in treatment of endometritis in crossbred cows. M.V.Sc., thesis. Submitted to the University of Agricultural Science, Bangalore.
10. Sahadev A, Devaraj M, Honappa TG and Chandrasekara Murthy V. 2007. Effect of immunomodulators on endometritis in cows. Indian Journal of Animal Reproduction, 28: 66.
11. Saini PS, Nanda AS, Grewal AS and Singh J. 1999. Uterine defense modulation for the treatment of repeat breeding due to infectious endometritis in bovines. Indian J. Anim. Sci., 69: 307-309.

DOI 10.5455/ijlr.20141006071857

12. Salphale GV, Kadu M, Fasihuddin M and Kadu MS. 1993. Study of some physical properties of estrual cervical mucus in synchronised normal and repeat breeder crossbred cows with reference to fertility. Indian Journal of Animal Research, 14: 77.
13. Singh J, Sidhu SS, Dhaliwal GS, Pangaonkar GR, Nanda AS and Grewal AS. 2000. Effectiveness of lipopolysaccharide as an intrauterine immunomodulators in curing bacterial endometritis in repeat breeding crossbred cows. Animal Reproduction Science, 59:159.
14. Singla VK, Verma HK, Dwivedi PN and Gandotra VK. 1991. Bacteriological isolates in repeat breeder cows. Indian Journal of Animal Science, 61: 181.
15. Snedecor M and Cochran CH. 1986. Statistical Methods. 2nd Eds., Iowa State University, USA.
16. Williams EJ, Fischer DP, England GCW, Dobson H, Pfeiffer DU and Sheldon IM. 2005. Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the inflammatory response to endometritis in cattle. Theriogenology, 63:102.

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