Journal of Animal Research: v.9 n.2, p. 359-361. April 2019

DOI: 10.30954/2277-940X.02.2019.22

Vaginal Bacterial Profile in Buffaloes Following Treatment with Progesterone Insert

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Received: 02 Jan., 2019 **Revised:** 15 Feb, 2019 **Accepted:** 19 Feb., 2019

ABSTRACT

The objective of this study was to characterize the vaginal bacterial flora and subsequent conception rates after AI in buffaloes subjected to 3 different estrous induction regimes-the use of CIDR and use of two intravaginal sponges (Polyurethane sponges with micronized progesterone and Polyurethane sponges with micronized progesterone with Carboxy methyl cellulose). The estrus induction was 100% in Group I and II followed by 91.67 % in group III. The pregnancy rates were Group I, II and III were 50.00, 66.67 and 54.55% respectively. All the vaginal swabs in all three groups yielded growth of bacteria and the predominance of mixed isolates over single isolates 81.94% *vs* 18.06% was indicative of dominance of mixed culture over single isolates. *E. coli*, *Staphylococcus, Proteus* and *Klebsiela* spp. were the commonest isolates obtained prior to insertion and after removal of implants in postpartum anestrus buffaloes. The gram positive bacteria were *Staphylococcus*, *Streptococcus* and *Bacillus* spp. while, gram negative bacteria were *E. coli*, *Proteus* and *Klebsiella* spp. These organisms could be considered as a part of the normal bacterial flora of the buffalo.

Keywords: Buffaloes, CIDR, Polyurathane sponge, vaginal bacterial flora

Different methods are employed to reduce the postpartum anestrus and subsequent inter-calving period in order to increase the fertility during the low breeding season. Intravaginal devices impregnated with progestagens being the most commonly used. The CIDR-B is the most common intravaginal progesterone available and contains 1.38 g of progesterone and is extensively used in various protocols for estrus synchronization, fixed-time AI, fixed-time embryo transfer, and superstimulation programs. Polyurethane sponges impregnated with varying amounts of progesterone and of various lenghts, diametres and densities have been used in cattle. In India, at present no locally made commercial progesterone- releasing intravaginal device is available for treatment of post-partum anestrus in buffaloes. These device are generally manufactured from different materials that can generate changes in the vaginal environment. These changes may be attributed to the physical action and / or the constant absorption and retention of the vaginal secretions by the intravaginal sponge during the time of insertion (Al-Hamedawi *et al*., 2003). Suarez *et al.* (2006) reported the presence of a foreign body in the vagina (sponge) to stimulate bacterial growth, localized inflammation with accumulation of mucus secretion and foul smelling fluid.

The aim of this study was to provide information regarding the effect of the foreign body (two different intravaginal devices mad from two different materials) for a period of 9 days on (i) to evaluate the bacterial effect on subsequent fertility and (ii) variation in the bacterial population at device withdrawal.

MATERIALS AND METHODS

The experiment was conducted in village of R. S. Pura, Jammu. Thirty six postpartum buffaloes were included to this study. All the buffaloes were randomly allocated to

three treatment groups. In buffaloes from Group I (n=12) were treated with with CIDR (Controlled Intravaginal Drug Release, Pfizer Ltd.) for 9 days. In the buffaloes of Group II (n=12) treated with Polyurethane sponge, a vaginal implant (containing 1.5 gm of Natural Micronized Progesterone) for 9 days and in the buffaloes of group III were treated with Polyurethane sponge, a vaginal implant (containing 1.5 gm of Natural Micronized Progesterone along with 1% carboxymethylcelulose) for 9 days. At the device withdrawal, each buffaloes was treated i.m. with 600 IU of PMSG. Estrus was recorded and AI was done accordingly. Mucus sample were collected from the posterior vaginal region of each buffalo using sterile swab and care was taken not to rub it against the vaginal wall, and then transported to the laboratory. Samples were collected immediately prior to the introduction of the device, at the time of the withdrawal. The swabs were dipped into nutrient broth and were incubated at 37˚C for 24 hours. Subculture was carried on MacConkey and Blood agar plates. Plates were incubated at 37˚C for 24 hours. Based on colony morphology, the bacteria were identified. For further confirmation, Gram staining and biochemical tests such as catalase test, oxidase, IMVIC (Indole, Methylred, Vogas Proskauser, and Citrate utilization test) were done

RESULTS AND DISCUSSION

Overall 97.22 % (35/36) of the buffaloes exhibited estrus in response to different treatments. The intervals between device withdrawal and estrus (37.07±1.58, 39.33±2.24 and 40.33±2.41 h for Groups I, II and III, respectively) and the conception rates (50.00, 66.67 and 54.55% for Groups I, II and III, respectively) recorded no significant differences between groups.

All the vaginal swabs (insertion/removal) in all three groups yielded growth of bacteria. These observations are supported by Kavyashree (2013) who reported 100% bacterial culture from vaginal swabs. The total number of bacterial isolates obtained in each group of postpartum anestrus buffaloes at the time of implant insertion were 22, 23 and 22 in group I (CIDR), II (Intra-vaginal sponge) and III (Intra-vaginal sponge with CMC) respectively. While, total number of bacterial isolates obtained in each group of postpartum anestrus buffaloes at the time of implant removal were 23, 25 and 26 in group I, II and III, respectively. The frequency of single and mixed isolates observed in different groups of buffaloes at the time of insertion and removal of implants in the present study revealed predominance of mixed isolates over single isolates 59 (81.94%) *vs* 13 13 (18.06%), indicating dominance of mixed culture over single isolates. Similar findings were reported by Panangala *et al*. (1978) who reported 93.10 % and 6.90% of mixed and single bacterial isolates, respectively. The isolates were also classified on basis of gram reaction and majority of isolates were gram negative at time of implant insertion and removal. Out of 141 bacterial isolates, gram positive bacteria isolates were 58(41.13%) and gram negative bacteria were 83(58.87%), indicating higher number gram negative bacteria and the increase in gram negative bacteria was also observed at time of implant removal. This finding have been supported by Jayachandran *et al.* (2013). This may be due to Lysozyme present in vaginal mucus which degrades peptidoglycans in the cell wall of gram positive bacteria (Nash *et al*., 2006). *E. coli*, *Staphylococcus, Proteus* and *Klebsiela* spp. were the commonest isolates obtained prior to insertion and after removal of implants in postpartum anestrus buffaloes. The gram positive bacteria were *Staphylococcus*, *Streptococcus* and *Bacillus* spp. while, gram negative bacteria were *E. coli*, *Proteus* and *Klebsiella* spp. These organisms could be considered as a part of the normal bacterial flora of the buffalo. These findings were supported by several authors (Williams *et al.,* 2005; El-Jakee *et al.,* 2008). They isolated *Escherichia coli, Klebsiella spp., Proteus spp.,* environmental streptococci and staphylococci, and other gram-positive, rod shaped organisms from the vagina of apparently healthy buffaloes. In the present study *E.Coli* was the predominant isolates in vagina of post-partum buffaloes. This finding coincides with those reported by many authors who concluded that *E. coli* was the most predominant pathogens in genital tract of buffaloes (Ahmed *et al.*, 2007) and in cattle (Irino *et al*., 2005; Kuhnert *et al*., 2005; Yilmaz *et al*., 2005).

In group II and III, some post-partum buffaloes treated with intravaginal sponge showed mucopurulent, foul smelling discharge at time of sponge removal. This may be due to CMC which induces bacterial growth and inflammation **(**Swidsinki, *et al*., 2009) and reported that the presence of a foreign body, such as sponge in the vagina stimulates bacterial growth and local mucus secretion during sponge treatment and these changes generated a localized inflammation in ewes Suarez *et al.* (2006). However, in

dairy cows, bacterial culture of swabs of the vagina after treatment with PRID for 7 days period revealed moderate growth of coliforms, environmental *Streptococcus spp.* and *Staphylococcus spp.* and other gram-positive, rod-shaped organisms (Walsh *et al.,* 2008).

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