

Prevalence of uterine infections in the post partum period of dystocia affected buffaloes and its effect on fertility

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ABSTRACT

The objective of the study was to find the prevalence of different types of bacterial infections, especially anaerobes in the uterine lumen and its impact on subsequent fertility. Out of 19 dystocia affected buffaloes studied during days 24-60 postpartum, 11 (58%) had uterine infections with 8 (42%) animals having mixed infection of *Arcanobacter pyogenes* and gram negative obligate anaerobes such as *Fusobacterium* spp. and *Bacteroides* spp. and voided whitish thick pus and none conceived subsequently. While the other three were infected with aerobes only (2 infected with *Pseudomonas aureginosa* and 1 with *A. pyogenes*); they came into estrus and conceived by day 109 postpartum. The uterus of remaining eight animals was sterile and all conceived by day 89 postpartum. These observations suggest that a pathogenic synergism exists between *A. pyogenes* and gram-negative obligate anaerobes, which might have increased severity of uterine infections, and consequently affected conception.

Key words : Buffaloes, dystocia, uterine infections, gram-negative obligate anaerobes, fertility

The uterus is known to harbor a wide panorama of microbes even after normal parturition (Arthur *et al.*, 2001). These microbes, however, are not deleterious because of intact genital investment as well as effective local and general defense mechanism (Bostedt, 1984), and in most of the cases the infection remain hidden with no clinical evidence of inflammation. The chances of bacterial infections become many fold in dystocia cases; obstetrical interventions provide a vehicle for ascending opportunistic pathogens while trauma to the endometrium assists in flaring up and colonization of pathogenic microbes. A definite negative effect on the fertility has been corroborated when the infection was caused by *Corynebacterium pyogenes* (Hartigen *et al.*, 1974). *Corynebacterium pyogenes* (now called as *arcanobacter pyogenes*) are frequently accompanied by gram negative obligate anaerobes such as *Fusobacterium necrophorum* and *bacteroides* spp, and they act synergistically by enhancing the growth and/or pathogenicity of each other (Bekana *et al.*, 1994). These infections appear frequently in cows with dystocia or retained fetal membranes (Dohmen *et al.*, 2000), and often result in causation of endometritis, and significantly delay the post

partum return of reproductive functions (Ruder *et al.*, 1981b), and thereby reducing fertility of animals. Buffaloes are highly prone to dystocia, especially uterine torsion, which in turn make them more susceptible to ascending infections associated with post partum endometritis. The objective of the present study was to investigate the prevalence of aerobic and anaerobic infections during the post partum period in buffaloes, which experienced obstetrical interventions, and its effect on subsequent fertility.

The study involved 19 dystocia affected buffaloes presented to Veterinary Clinics, Punjab Agricultural University, Ludhiana during the period from June 2002 to April 2003, which survived after obstetrical interventions. All the buffaloes had completed their gestation period, and were suffering from the maternal or fetal dystocia. Deliveries were made effective using obstetrical operation such as mutation, fetotomy or caesarian section. All the buffaloes were followed by visiting the farmer's door steps for collection of samples and data related to first postpartum estrus and subsequent pregnancy status. Single uterine swab was collected from each dystocia affected buffalo anywhere between days 24-60 postpartum using Neilson's swab catheter (Jadon *et al.*, 2004). The uterine catheter was opened in front of flame, and the swab was put into Carry Blair transport media with the help of sterilized forceps. All the swabs were cultured for both aerobic and

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anaerobic bacteria within 2 to 3 hours of collection. Isolation and identification of the bacteria was based on morphology, culture characteristics and biochemical tests (Quin *et al.*, 1999).

All the dystocia affected animals were examined per rectum to study the involution of uterus and post partum ovarian activity. The animals detected in oestrus were bred by natural service, and were palpated per rectum for pregnancy diagnosis around 45 days post breeding. Buffaloes conceiving within 110 days post partum were identified as fertile group (n = 11), while the non conceiving buffaloes were classified as infertile group (n = 8).

A total of 19 buffaloes, which survived after obstetrical interventions were followed for assessing the presence of uterine infections and the type of cervico vaginal discharge during the 24-60 day postpartum period. A total of 11 buffaloes conceived subsequently and were placed in fertile group. In the fertile group, the uterus of 3 buffaloes had bacterial growth; 1 was infected with *Arcanobacter pyogenes* and 2 had *Pseudomonas aeruginosa* infection. Cervico vaginal mucus (CVM) discharge in these 3 buffaloes was cloudy, and these buffaloes showed first sign of oestrus by 77 ± 2 days postpartum and got conceived by 109 ± 2 days postpartum. The remaining 8 animals in the fertile group were free from uterine infection and the CVM discharge was clear, and they showed first sign of oestrus by day 65 ± 1 postpartum and got conceived by day 89 ± 1 days postpartum. In the infertile group, the uterus of all the 8 animals had combined infections of *A. pyogenes* and gram negative obligate anaerobes, and showed bloody thick/white, foul odor purulent cervico vaginal discharge.

In the present study, it seems that animal infected with *A. pyogenes* and gram negative obligate anaerobes (*Fusobacterium* spp, *Bacteroides* spp.) did not conceive during the study period, whereas those infected with aerobic bacteria viz. *Pseudomonas aeruginosa* or *A. pyogenes* conceived. These observations indicate that a pathogenic synergism exist between *A. pyogenes* and gram negative obligate anaerobes, which might have caused increase severity of uterine infections in the post partum period of dystocia affected buffaloes and affected fertility adversely. Further, the clinical signs of uterine infections, characterized by thick pussy discharge were more severe in animals infected with *A. pyogenes* and gram negative obligate anaerobes than those infected with *A. pyogenes* or *Pseudomonas aeruginosa* alone.

Several studies have indicated that mixed infections of aerobes and gram negative obligate anaerobes are found in the post partum endometritis in cows (Noakes *et al.*, 1991; Bekana *et al.*, 1994). A possible synergistic effect of *A. pyogenes* and *Fusobacterium necrophorum* was recorded by Ruder *et al.* (1981b). They reported that the cows infected with *A. pyogenes* and *Fusobacterium necrophorum* did not conceive while those infected with either *A. pyogenes* or *Fusobacterium necrophorum* alone conceived; these findings fully endorse our observations.

It has been shown in experimental models that anaerobes are able to produce a variety of toxins, enzymes and other virulence determinants, which are responsible for pathogenicity (Hofstad, 1989; Ruder *et al.*, 1981b). Tissue destruction, a prominent feature of many anaerobic infections results from the action of bacterial toxins and enzymes, particularly those of *Fusobacterium* and *Bacteroides* spp., which were found to be the most predominating pathogen in the present study. The former is known to produce a potent endotoxin, which destroys leukocytes (Ruder *et al.*, 1981a&b; Hofstad, 1989). The nature of leukotoxins and their role in pathogenesis is not fully understood (Hofstad, 1989). It has been suggested that these toxins facilitate tissue invasion by *A. pyogenes*, which in turn produces growth enhancement factor for *Fusobacterium*, and possible growth stimulating factor for the *Bacteroides* spp., which seems to have unusual potent lipopolysaccharides molecules that may, in part, account for some of the clinical signs (Price and McMillan, 1986; Markusfeld, 1993).

Bacteriological surveys on endometritis in buffaloes in the past have totally neglected the importance of obligate anaerobes. This study has clearly shown that gram negative obligate anaerobes coupled with *A. Pyogenes* are frequently found in dystocia induced endometritis, and are responsible for infertility. Therefore, a strategic treatment effective against both aerobes and anaerobes is warranted for effective treatment of endometritis.

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