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Fertility following intrauterine administration of Tinidazole and Ciprofloxacin combination in repeat breeding crossbred cows

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ABSTRACT

It is well established that bovine uterus provides an environment, which is conducive for the growth of both aerobes and anaerobes that act synergistically to cause bacterial endometritis. Therefore, treatment strategies demand that drugs effective against both aerobes and anaerobes should be used. In the present study 15 repeat breeding cross bred cows having uterine infection were selected on the basis of aerobic and /or anaerobic bacterial growth in the uterine swabs. All these cows were treated with tinidazole and ciprofloxacin combination for 3 consecutive days. At subsequent oestrus uterine swabs were again taken and cultured for both aerobic and anaerobic bacteria and artificial insemination was done at the same oestrus. Both aerobic and anaerobic infection were cleared completely in 9 cows while the clearance was partial in 5 cows i.e. elimination of either aerobes or anaerobes after treatment. One cow did not respond to treatment in terms of clearance of infection. Overall, six (40%) cows became pregnant within 3 inseminations following the treatment, of them 5 cows belonged to totally cleared group. First service pregnancy was achieved in only 2 cows that had sterile uterine environment after the treatment. Bacteriological cure rate after treatment was better in case of obligate anaerobes (90%) as compared to aerobes (67%). Also treatment was not effective in clearing *E. coli* infection. To conclude, though ciprofloxacin and tinidazole combination was effective against mixed infection of aerobes and anaerobes *in vitro*, results in terms of pregnancy establishment were not encouraging.

Keywords: Tinidazole, ciprofloxacin, gram-negative anaerobes, repeat breeding cows

Repeat breeding especially due to bacterial endometritis is the most prominent reproductive problem in cross bred cows in Punjab. Administration of antibiotics and antiseptics via intrauterine route is the most widely used line of treatment, and it has shown varying degree of success (Shukla and Pandit 1989, Verma *et al.*, 1994).

However most of the research done so far in India has emphasized on the isolation of different aerobic bacteria and using antibiotics effective *in vitro* against those aerobes which may be the reason for partial success rates reported. Recent research has shown that the environment within the bovine uterine lumen is conducive for the growth of anaerobic organisms (Cohen *et al.*, 1996), which inturn promote the growth of certain other pathogenic aerobes such as *Arcanobacter pyogenes* and these two pathogens act synergistically in causing bacterial endometritis in post partum cows (Olson *et al.*, 1984; Dohmen *et al.*, 1995; Huszenicza *et al.*, 1999). Certain antibiotics especially of aminoglycosides group, are completely ineffective against uterine obligate anaerobes (Olson, 1985). On the contrary, aerobic bacteria are often resistant to antibiotics effective against the obligate anaerobic bacteria (Sokolova, 1986). Intrauterine administration of tinidazole combination improves the conception rate in treated repeat breeding cows harbouring uterine infections (Chakrabarti and Lodhi, 1991), which needs authentication using both aerobic and anaerobic bacteriological cultural techniques.

MATERIALS AND METHODS

Animals : Fifteen repeat breeding cross-bred cows from PAU Dairy farm, having uterine infection were selected on the basis of aerobic and/or anaerobic bacterial growth in the uterine swabs collected during estrus. All these cows were treated with 50 ml of intrauterine preparation containing tinidazole 15 mg/ml and ciprofloxacin 12.5 mg/ml for 3 consecutive days during estrus. At subsequent oestrus uterine swabs were again taken before insemination. Pregnancy diagnosis was carried out per

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rectum after 10 weeks of insemination to know conception rate following treatment.

All the swabs (collected before and after treatment) were put either in Cary Blair and/or Thioglycollate broth to prevent their dehydration. Later on swabs were inoculated on blood agar with 5-10% sterile defebrinated sheep blood and Maconkey's lactose agar and then incubated aerobically upto 48 h at 37°C. Colonies were identified as per standard bacteriological culture techniques (Quinn *et al.*, 1999). For anaerobic culturing Anaerobic Brewer's agar was prepared fresh and was supplemented with Vit-K (10 mg/ml), Haemin 5mg/ml, Kanamycin (100 mg/ml) and Vancomycin (7.5 mg/ml) for the selective growth of gram negative obligate anaerobes. The inoculated plates were then immediately placed in Mac-Intosh anaerobic jar having Hi-media gas pack and indicator tablet system for anaerobiosis. True anaerobes were identified on the basis of cultural and morphological characteristics as per Lennette *et al* (1985) and Holt *et al* (1994).

RESULTS AND DISCUSSION

Bacteriological cure rate following treatment with tinidazole and ciprofloxacin combination was found to be significantly better against obligate anaerobes (90%) than aerobes (73.3%) The number of isolates decreased from 10 to 1 in the anaerobic group while the number decreased from 15 to 4 in the aerobic group. Of the aerobic bacteria *E. coli* did not respond very well to the treatment as 4 of the 7 isolates were still present at subsequent oestrus after treatment. The treatment was highly effective in clearing aerobic bacteria such as *Arcanobacter pyogenes, Pseudomonas* spp., *Staphylococcus* spp. as well as gram negative anaerobic bacteria such as *Bacteroides* spp. with number of isolates falling from 1, 5, 2 and 10 before treatment to 0, 1, 0 and 1 respectively respectively. Out of a total 15 treated repeat breeding cross bred cows infection was totally cleared in 9 cows i.e. samples collected at subsequent estrus following treatment yielded no growth under both aerobic and anaerobic conditions. In 5 cows infection was partially cleared while in 1 infection persisted. Overall, 6 cows became pregnant within 3 inseminations after treatment, of which 2 became pregnant with first insemination i.e. at subsequent estrus after treatment. So overall conception rate following intrauterine treatment of ciprofloxacin + tinidazole was only 40%. Of 6 pregnant cows, 5 were those in which infection was totally cleared after the treatment.

The treatment comprising of Nitroimidazole (Tinidazole) and Fluroquinolones (Ciprofloxacin) combination was found effective in terms of clearing mixed infection of aerobes and anaerobes, though actual conception rates were low. Earlier *in vitro* studies (Cohen *et al* 1996), also showed that combination of metronidazole and ciprofloxacin was highly effective in clearing mixed infection of aerobes and anaerobes. In cow in which infection was cleared completely following treatment, overall conception rate was good (55%) with 2 cow conceiving to the first insemination. However, clinical trial with tinidazole plus amoxycillin against bacterial endometritis in repeat breeding cows resulted in 85% conception rate in repeat breeding cows (Chakrabarti and Lodh 1991). In pyometra cases Stephens and Sleek (1987) achieved conception rate of 48% following intrauterine infusion of metronidazole plus ampicillin.

The treatment adopted in the present study was quite effective interms of bacteriological cure rates, especially with regard to anaerobic infections. Bacteriological cure rate similar to the present study was obtained with intrauterine infusion of metronidazole and ampicillin (Stephens and Sleek 1987). Of aerobes, it was mainly *E. coli* organism which were again isolated from the uterus at subsequent oestrus following treatment. It is difficult to prove whether these are the same organisms of the previous cycle or are the new entrants. *E. coli* is well known opportunistic organism. Several factors could be responsible for the low conception rate in the present study. Firstly, the dose to tinidazole plus ciprofloxacin used may not be appropriate, because of that therapeutic concentration of drug might not have been achieved in deeper layers of myometrium and infection persisted. Alternatively, epithelial layer of endometrium in these cases might have been damaged extensively, which could not be healed by the time of first insemination.

Intrauterine infusion of tinidazole and ciprofloxacin in repeat breeding cows proved to be very effective in eliminating both aerobic and anaerobic infection, subsequent fertility however, did not improve substantially.

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