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Research Article

CYTOLOGICAL AND MICROBIOLOGICAL EVALUATION OF UTERINE FLUSH OF FERTILE AND REPEAT BREEDER MARES AND POST-TREATMENT FERTILITY

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ABSTRACT

Cytological and microbiological examination of uterine flush was conducted on fertile (n=5) and repeat breeder mares (n=5) and post-treatment fertility was recorded in repeat breeder mares. Three repeat breeder mares yielded single uterine bacterial pathogen (*Escherichia coli, Klebsiella spp. and Micrococcus spp.*, respectively) and remaining two were positive for single fungal pathogen (*Aspergillus spp. and Candida spp.*, respectively). *In-vitro* antibiogram showed susceptibility of *Escherichia coli, Klebsiella* spp. and *Micrococcus* spp. to amoxycillin, chloramphenicol, doxycycline and gentamicin and resistance to cloxacillin, metronidazole, penicillin and sulphadiazine. Fungal uterine pathogens (*Aspergillus* spp. and *Candida* spp.) were sensitive to mycostatin and resistant to ketoconazole. Positive correlation (100%) was observed between uterine cytological and microbiological examinations in fertile and infertile mares. Three of the five repeat breeder mares conceived following intra-uterine treatment with chloramphenicol (n = 2/3) and mycostatin (n =1/2).

Key words: Rrepeat breeder mare, uterine culture, uterine cytology, infertility

Uterine infection is the major cause of conception failure in mares. Young cyclic mares resolve uterine infection but infection persists in pleuriparous non-cyclic mares due to inadequate uterine humoral and cellular defense mechanism (Allen and Newcombe, 1979). Further, intra-uterine antibiotic treatment without *in-vitro* sensitivity of uterine pathogens in infertile mares leads to establishment of fungal pathogen invaded during foaling or service (Blue, 1983). Diagnosis of uterine infection in mares is a much difficult task than its therapeutic management (Moreno *et al.*, 1995). Thus, uterine cytology is useful in detection of uterine infection in false positive and false negative mares on culture. The present paper describes the diagnostic utility of uterine culture and uterine cytology in detection of uterine infection in fertile and repeat breeder mares and correlation between the two diagnostic techniques to establish predictive value of both the diagnostic techniques for fertility in mares.

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MATERIALS AND METHODS

Experimental mares: Fertile (n=5) and repeat breeder mares (n=5) were used for cytological and microbiological investigations of uterine flush during breeding season (May to October, 1996). Standardized ration consisting of green fodder (maize and berseem), oat hay and concentrate mixture was fed to the mares. Drinking water was provided *ad libitum*.

Collection of uterine flush and isolation of bacteria and fungi: Uterine flush samples were collected from fertile and repeat breeder mares during oestrus before breeding using two way Foley's catheter and sterile phosphate buffer saline (PBS, pH 7.2). Each uterine flush sample was divided into two aliquots of 30 to 40 ml and processed for microbiological and cytological examination, respectively as per the method described by Ball *et al.* (1988). The aliquots of uterine flush were subjected to bacterial and fungal isolation using standard methods (Ajello *et al.*, 1966, Cowan and Steel, 1974).

***In-vitro* sensitivity:** The sensitivity of bacterial isolates to antibiotics was studied by single disc

diffusion technique (Ellner, 1978) using various antibiotics discs of Himedia Lab., Bombay, India. *In- vitro* sensitivity of fungal isolates to ketoconazole and mycostatin was studied using the technique described by Narwal *et al.* (1994).

Uterine cytology: Uterine flush samples were processed for cytological examination as per the method described by Digby (1978).

RESULTS AND DISCUSSION

Pre-service uterine flush samples collected from fertile mares were negative for both bacterial and fungal isolates. It could be due to adequate humoral and cellular resistance in fertile mares as reported by Allen and Newcombe (1979). However, non-specific single uterine pathogen was recovered from 2 out of 20 fertile mares (*Staphylococcus aureus* and *Staphylococcus epidermidis,* respectively) on uterine culture possibly due to vaginal and perineal contamination of the swabs and these organisms did not interfere with the fertility in mares (Narwal *et al.*, 1994). The observations in fertile mares indicate significance of microbiological evaluation of pre-service uterine flush in prediction of fertility. *Escherichia coli, Klebsiella* spp. and *Micrococcus* spp. recovered from the repeat breeder mares during this study were also reported earlier in infertile mares (Allen and Newcombe, 1979). *Klebsiella* spp. infection occurs through breeding (Crouch *et al.*, 1972) whereas faecal contamination during foaling and breeding causes *E. coli* infection (Collins, 1964). Both *Aspergillus* spp. and *Candida* spp. were found to be the most common cause of conception failure among mycotic uterine infections in mares (Blue, 1983) as also observed in this study in repeat breeder mares. Uterine infection of *Candida parapsillosis* was also reported to cause embryonic death in mares (Ball *et al.*, 1988).

Pre-service uterine flush samples were cytological negative for uterine infection in fertile mares and positive for repeat breeder mares (Figs 1, 2). Further, positive correlation between uterine culture and cytology in fertile and repeat breeder mares indicated that both the diagnostic techniques were equally reliable in detection of uterine infection in infertile mares as also opined by Digby (1978).

In-vitro sensitivity of *Escherichia coli* to gentamicin and chloramphenicol (Table 1) concurs with the findings of Shin *et al.* (1979). *In-vitro* sensitivity of *Klebsiella* spp. to gentamicin and chloramphenicol compares well with the earlier report in mares (Moreno *et al.,* 1995). Maximum *in-vitro* activity of gentamicin, chloramphenicol and nitrofurazone against *Micrococcus* spp. was consistent with reported sensitivity pattern in infertile mares (Shin *et al.*, 1979). The observed *in-vitro* resistance of E. coli and *Klebsiella* spp. to penicillin and cloxacillin in repeat breeder mares may be due to prolonged intra-uterine treatment with these antibiotics without *in-vitro* sensitivity tests as reported by Narwal *et al.* (1994). Further, post-partum uterus harbors bacterial pathogens producing penicillinase, rendering penicillin ineffective locally following intra-uterine antibiotic treatment (Shin *et al.*, 1979). Maximum *in-vitro* sensitivity of *Aspergillus* spp. and *Candida* spp. to mycostatin and ketoconazole in repeat breeder mares was in accordance with the observations

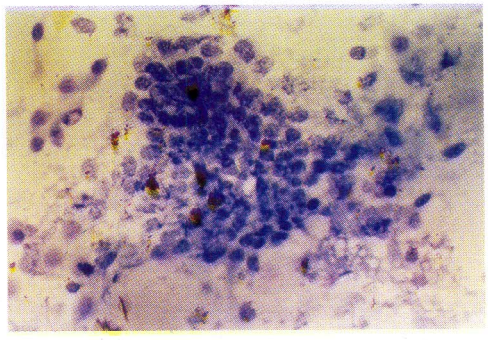

Fig 1. Endometrial cytological smear from a fertile mare during oestrus showing only epithelial cells. (Giemsa x 120)

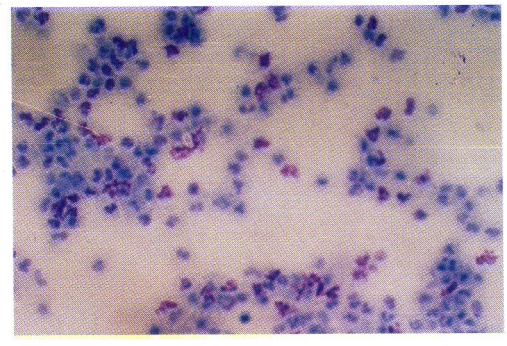

Fig 2. Endometrial cytological smear from a repeat breeder mare during oestrus showing numerous neutrophils. (Giemsa x 120)

Table 1

***In-vitro* sensitivity of uterine bacterial and fungal isolates from repeat breeder mares and post-treatment fertility**

Uterine pathogens	Reproductive status		*In-vitro* sensitivity of uterine pathogens		Intra-uterine treatment and post-treatment fertility
	Fertile (n=5)	Repeat breeder (n=5)	Susceptible	Resistant	
a) Bacteria					
i) E. coli	0	1	AM, C, DO, G, S, TR	A, CX, M, NF, P, SZ	Chloramphenicol 3 gm/day for 5 days, (n=1/1)
ii) Klebsiella spp.	0	1	A, AM, C, DO NF	CX, G, M, P, S, SZ, TR	Chloramphenicol 3 gm/day for 5 days, (n=0/1)
iii) Micrococcus spp.	0	1	AM, C, DO, G, NF, S	A, CX, M, P, SZ, TR	Chloramphenicol 3 gm/day for 5 days, (n=1/1)
b) Fungi					
i) Aspergillus spp.	0	1	Mycostatin	Ketoconazole	Mycostatin 2.5 millions units/day for 5 days, (n=1/1)
ii) Candida spp.	0	1	Mycostatin	Ketoconazole	Mycostatin 2.5 millions units/day for 5 days, (n=0/1)

A- ampicillin, AM- amoxycillin, C- chloramphenicol, CX- cloxacillin, DO- doxycycline, G- gentamicin, M- metronidazole, NF- nitrofurazone, P- penicillin, S- streptomicin, SZ- sulphadiazine, TR- trimethoprim.

of Narwal *et al.* (1994). Further, positive *in-vivo* response to mycostatin was reported in infertile mares harboring *Candida* spp. infection of the uterus (Zarfracas, 1975). Total conception rate at 1st and 2nd post-treatment oestrus with chloramphenicol (2/3 mares) and at 2nd post-treatment oestrus with mycostatin treatment (1/2 mares) in repeat breeder mares (Table 1) observed in the present study, concurs with reported fertility with chloramphenicol (Uppal *et al.*, 1994) and mycostatin treatment (Zarfracas, 1975), respectively in infertile mares.

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