

Vaginal, Cervical and Uterine Bacterial Flora at the Different Stages of the Reproductive Cycle in Ovariectomized Bitches

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Abstract: The aim of the present study was to determine vaginal, cervical and uterine bacterial flora at the different stages of the reproductive cycle in mixed-bred bitches. Vaginal, cervical and uterine swab specimens were obtained from 55 bitches (Anoestrus = 11, Prooestrus = 16, Oestrus = 13, Metoestrus = 9 and Pregnant = 6) at ovariectomy. A total of 61 bacteria were isolated from vagina. These organisms include Yeast (45.4%) and *E. coli* (30.9%), *Proteus* sp. (20%), *Streptococcus* sp. (12.7%) and *Enterococcus* sp. (1.8%) have been isolated. The mean number of isolates from vagina was 1.2. High bacterial counts in the vagina were found proestrus and oestrus. The results indicated that no bacteria were isolated from the cervix and the uterus and no mycoplasmas were cultured in vagina, cervix and uterus. The uterine microflora did not reflect the vaginal and cervical microflora.

Key words: Bitch, bacterial flora, vagina, uterus, oestrus cycle, Turkey

INTRODUCTION

Many different bacterial organisms have been isolated from genital tract of healthy bitches (Holzmann *et al.*, 1979; Allen and Dagnall, 1982; Doig *et al.*, 1991; Janowski *et al.*, 2008b). The microorganisms most commonly isolated are *E. coli*, *Streptococcus* sp., *Staphylococcus* sp., *Pasteurella* sp., *Proteus* sp., *Bacillus* sp., *Corynebacterium* sp., *Pseudomonas* sp. and *Micrococcus* sp., *Nisseria* sp., *Klebsiella* sp. and *Moraxella* sp. (Hirsh and Wiger, 1977; Olson and Mather, 1978; Allen and Dagnall, 1982; Olson, 1986). Some of these organisms have been cultured from healthy as well as infertile bitches (Feldman and Nelson, 2004). All normal bitches have bacterial flora present in the anterior vagina and similar types of aerobic bacteria are present in the vaginal vaults of infertile bitches (Bjurström and Linde-Forsberg, 1992). The canine vaginal bacteria flora is not sterile and a large of contaminant or normal flora area routinely from caudal vagina. The vaginal flora contains a large variety of bacteria species, including both aerobic and anaerobic organisms (Zunin *et al.*, 1981). The canine vaginal bacterial flora varies relation to breed, stage of oestrus cycle and season (Holzmann *et al.*, 1979; Doig *et al.*, 1991; Bjurström and Linde-Forsberg, 1992).

Anaerobic bacteria and mycoplasmas were not considered normal flora in the canine uterus (Watts *et al.*,

1996). But *Mycoplasma* and *Ureaplasma* are organisms commonly cultured from the vaginal tract of normal bitch. However, a syndrome of vaginitis, poor conception, early embryonic death, embryonal and fetal resorption, abortion, stillborn pups, weak newborns and/or neonatal death has been suggested to be caused by these smallest of free-living microorganisms (Feldman and Nelson, 2004). A stage of oestrus cycle may influence the bacterial flora. Presence of bacteria in uterus during proestrus and oestrus was not uncommon; these bacteria are members of the vaginal flora (Olson and Mather, 1978; Watts *et al.*, 1998). Bacteria were rarely present in the normal bitches during other stage of the oestrus cycle (Watts *et al.*, 1996; Schultheiss *et al.*, 1999). Noguchi *et al.* (2003) cultured a large number of microorganisms during oestrus than during metoestrus and anoestrus. In another study, the number and species of bacteria isolated did vary with the stage of the ovarian cycle (Bjurström and Linde-Forsberg, 1992).

A large number of organisms are cultured during oestrus than diestrus or anoestrus and these are retrieved from bitches with reproductive tract disease from normal bitches (Hirsh and Wiger, 1977). It has been also reported that It has been also reported that the type of bacteria isolated do not appear to vary with the different stages of the oestrus cycle but increased number of organisms appear to be present during proestrus and oestrus

(Allen and Dagnall, 1982; Baba *et al.*, 1983). According to some researchers, uterine microflora reflects the bacterial flora of the vagina and cervix (Baba *et al.*, 1983; Watts *et al.*, 1996).

The purpose of this study was to investigate vaginal, cervical and uterine bacterial flora in clinically normal ovariohysterectomized bitches of various breeds during the different stages of the oestrus cycle.

MATERIALS AND METHODS

Animals: Fifty-five clinically healthy mixed-bred bitches, aged 1.5-3 years, weighing between 15 and 30 kg were used in this study. The stages of the sexual cycle were determined based on history, physical examination and vaginal cytology. Bitches were clinically examined at the time of sample collection for swelling of vulva and presence of discharge. All the animals were normal and there were no gross lesions in the reproductive tract.

Ovariohysterectomy operation: The bitches were anaesthetised by an injection of xylazine (2mg kg⁻¹ b.w., Alfazyne-Alfasan, the Netherlands) and ketamin HCl (15 mg kg⁻¹ b.w., Alfamine-Alfasan, the Netherlands). Ovariohysterectomy were performed by medial laparotomy, according to the routine methods (Stone, 1995).

Vaginal cytology: Vaginal cytology samples obtained daily throughout the study period by sterile cotton swabs. Thereafter, the smears were stained using Giemsa stain and observed under light microscopy and percentage of cell types was determined and interpreted as previously described by Olson (1986).

Microbiological culture: Samples for microbiological culture were collected from the vagina, cervix and uterus at all stage of the reproductive cycle. Vaginal swabs were collected before the operation, while uterine and cervical swabs were taken during the operation. Steril plastic swabs were used for sampling. The outer surface of vagina was disinfected using alcohol 90% before sampling. After sampling, each swab was put into a tube containing 3 mL sterile 0.9% NaCl solution and vortexed in order to release whole collected material from the swabs to the aqueous phase. These materials in tubes were centrifuged 3000 g for 10 min.

Sediments were used as inocula. Sediment was inoculated onto 5% ovine blood agar (Oxoid), Mac Conkey (Oxoid) agar plates were incubated in aerobic conditions for 24-48 h at 37°C. In addition, sediment was also inoculated to Hayflick's Medium Broth tubes and

agar plates were incubated in 5% CO₂ for 5-7 days at 37°C for Mycoplasma isolation. After incubation period, a single colony was subcultured and identified by biochemical tests, results were interpreted in accordance with the recommendations of Barrow and Feltham (1995) and Poveda (1998).

RESULTS AND DISCUSSION

Bacteria were found in the vagina during the oestrus cycle. No bacteria were isolated from the cervix and the uterus in all bitches during all stages of sexual cycle. From a total 55 bitches in different stages of reproductive cycle (Anoestrus = 11, Prooestrus = 16, Oestrus = 13, Metoestrus = 9 and Pregnant = 6), 61 bacterial organisms were isolated. Details on the vaginal culture in 55 bitches at the different stages of sexual cycle are shown in Table 1. The commonest isolates were Yeast (45.4%) and *E. coli* (30.9%). These organisms including *Proteus* sp. (20%), *Streptococcus* sp. (12.7%) and *Enterococcus* sp. (1.8%) were isolated from all bitches. The highest rate of isolation of bacteria per dog was recorded among the prooestrus (1.4), followed by oestrus (1.2), pregnant (1.0), metoestrus (0.9) and anoestrus (0.8). It was confirmed that different bacteria could grow as well as the same bacteria at the different stages of oestrus cycle and vaginal flora of mixed character was isolated (Günay *et al.*, 2004; Laurusevičius *et al.*, 2008).

According to some researchers, uterine microflora reflects the bacterial flora of the vagina and cervix (Osbaldiston, 1978; Baba *et al.*, 1983; Watts *et al.*, 1996). Similarly, Zdunczyk *et al.* (2006) and Janowski *et al.* (2008a, b) reported that similar bacterial floras were present in the vagina and uterus of bitches. In contrast, other researchers have found the uterus devoid of microflora (Olson and Mather, 1978; Dhaliwal *et al.*, 1998). In the study, no bacteria were isolates from the uterus. The findings were in agreement with data obtained by Olson and Mather (1978) and Dhaliwal *et al.* (1998).

In the present study, no mycoplasmas were cultured samples from the vagina, cervix and uterus in this study. The findings were similar to those of Watts *et al.* (1996), who found no mycoplasmas in the vagina and uterus of healthy bitches. On the contrary, Bjurström and Linde-Forsberg (1992) found mycoplasma from the vagina of normal bitches. Some researchers (Baba *et al.*, 1983; Wadas *et al.*, 1996) reported that mycoplasmas have also been isolated from both healthy uteri and pyometra infections. However, in some cases no microorganisms at all could be isolated from diseased uteri (Osbaldiston, 1978; Wadas *et al.*, 1996). The reason of these differences could be related to variation in

Table 1: Bacteria isolated from the vagina of bitches at the different stages of the oestrus cycle

Stage of oestrus cycle	Type of bacteria					No. of bacteria	Bacteria per bitch
	<i>Streptococcus</i> sp.	<i>E. coli</i>	<i>Proteus</i> sp.	<i>Enterococcus</i> sp.	<i>Yeast</i> sp.		
Anoestrus (n = 11)	9.09% (1)	27.2% (3)	-	-	45.4% (5)	9	0.8
Proestrus (n = 16)	12.5% (2)	31.25% (5)	37.5% (6)	6.25% (1)	50% (8)	22	1.4
Oestrus (n = 13)	15.4% (2)	30.8% (4)	23% (3)	-	53.9% (7)	16	1.2
Metoestrus (n = 9)	11.1% (1)	22.2% (2)	22.2% (2)	-	33.3% (3)	8	0.9
Pregnant (n = 6)	16.6% (1)	50% (3)	-	-	33.3% (2)	6	1.0
Total (n = 55)	12.7% (7)	30.9% (17)	20% (11)	1.8% (1)	45.4% (25)	61	1.2

composition of canine vaginal flora in different breeds. A larger of bacteria is cultured during oestrus than metoestrus and anoestrus (Noguchi *et al.*, 2003) and the highest bacterial count was obtained in proestrus (Janowski *et al.*, 2008a; Laurusevičius *et al.*, 2008) and oestrus than other stages of sexual cycle (Baba *et al.*, 1983; Allen and Dagnall, 1982).

The lowest isolation was recorded in proestrus and oestrus bitches (Holst, 1986; Mshelia *et al.*, 2001). The highest bacterial count was recorded in the proestrus and oestrus bitches in the study. The results were in agreement with the findings of Noguchi *et al.* (2003), Allen and Dagnall (1982), Baba *et al.* (1983), Janowski *et al.* (2008a) and Laurusevičius *et al.* (2008) but not with the findings of Holst (1986) and Mshelia *et al.* (2001). In the study, lower rates of bacteria were isolated from metoestrus, anoestrus and pregnant animals. These findings were in agreement with results reported by Schultheiss *et al.* (1999). The stage of the cycle did not alter the types of bacteria isolated but increased numbers were present in proestrus and oestrus (Allen and Dagnall, 1982; Baba *et al.*, 1983).

CONCLUSION

The results of this study showed that, an increased number of bacteria were appeared during proestrus and oestrus in vaginal flora. These organisms could be considered as a part of the normal canine vaginal flora. The results of the present study have indicated that no bacteria and mycoplasma were isolated in cervix and uterus in healthy bitches during the different stages of reproductive cycle.

The study showed that uterus was sterile and the uterine microflora did not reflect the vaginal and cervical microflora. It can be said that bacterial isolation in healthy bitches is important because bacteria can become pathogenic and it could have an influence on the future fertility.

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