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Histopathological investigation of endometrium in repeat breeder cows

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ABSTRACT

This study consisted of comparative histopathological examination of endometrium in repeat breeder as well as fertile cows. In fertile and repeat breeder cows, hyperaemia, edema, and focal haemorrhages with the desquamated epithelial cells in gland lumens were observed. Subclinical endometritis was determined in 8 repeat breeder cows (53.33%). Inflammatory cell infiltration, degeneration and desquamation in the epithelial lamina, glandular dilatation and periglandular fibrosis were determined in 8 (53.33%), 5 (33.33%), 3 (20.0%) and 2 (13.33%) of cows having endometritis respectively.

It was concluded that subclinical endometritis was one of the reasons of repeat breeder syndrome, and histopathological investigation on biopsies from uterine horns and body would be a useful diagnostic tool.

Key words: Cow, Endometrium, Histopathology, Oestrus cycle, Repeat breeder cows

Repeat breeder syndrome is considered as one of the most important causes of infertility in dairy cattle. Cows younger than 10 years, calved at least once, having regular sexual cycle without any abnormal discharge and clinical disorder in their genital organs, and which did not conceive even 3 matings with a fertile bull or 3 artificial inseminations can be described as repeat breeder cows (Zemjanis 1980, Ayolan 1984, Lafi and Kaneene 1988, Alacam 1994).

Subclinical endometritis (I. grade) is seen in repeat breeder cows (Singh *et al.* 1983, De Bois and Manspeaker 1986, Apaydin 1987, Apaydin *et al.* 1991a, b). The incidence has been reported as 16.3% (Sagartz and Hardenbrook 1971), 37.7% (Seitaridis *et al.* 1977), 50.0% (Rao and Kotayya 1980), 81.0% (Apaydin 1987), 55.0% (Biolatti *et al.* 1991), 86.2% (Apaydin *et al.* 1991a) and 52.8% (Kakar *et al.* 1997) in repeat breeder cows, and 64.0% (Rao and Kotayya 1980) in buffaloes.

Diagnosis of subclinical endometritis is only possible by examining the biopsy samples histopathologically (Seitaridis *et al.* 1977, Shirai *et al.* 1979, Singh *et al.* 1983, Apaydin 1987). In repeat breeder cows, Messier *et al.* (1984) found periglandular fibrosis, diffuse mast cell infiltration and ulcerative lesions in superficial epithelia. Whereas, some lesions like neutrophil, lymphocyte and plasma cell infiltration, and cystic dilatation and periglandular fibrosis in glands were reported in repeat breeder buffaloes (Singh *et al.* 1983). Gustafsson *et al.* (1985) reported that focal lymphocyte and erythrocyte accumulation, and cystic glands in endometrium were observed less in heifers never been inseminated than in repeat breeder heifers. In uterine body, atrophy in glands, inflammatory cell infiltration and edema in connective tissue; and in uterine horn, edema, neutrophil and lymphocyte infiltration, and dilatation and necrosis in uterine glands were reported by Shahzada *et al.* (1997) investigating the endometrial biopsy samples of repeat breeder buffaloes. Apaydin *et al.* (1991a, b) reported intensive polymorphonuclear leukocyte, lymphocyte and plasma cell infiltration in lamina propria, partial destruction in epithelial layer, reduction in the size of gland epithelia and in some samples more severe connective tissue increase around gland in repeat breeder cows.

This study was carried out to examine endometrium histopathologically in oestrus and on the eighth day of oestrus cycle (dioestrus) in repeat breeder cows.

MATERIALS AND METHODS

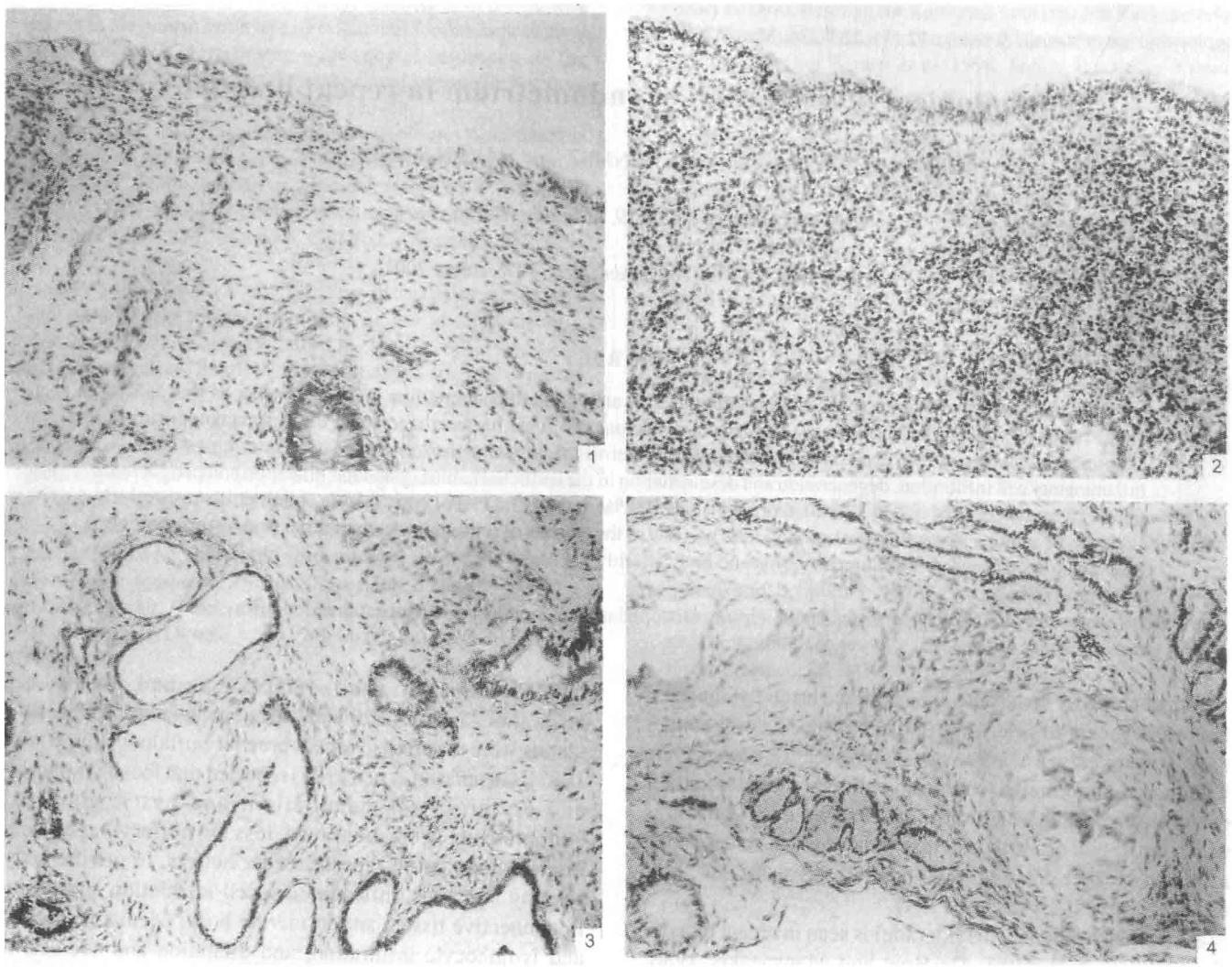
Cows (5 fertile and 15 repeat breeder) between 3 and 6 years of age, having no clinical or metabolic disease after the parturition and raised in the same management conditions at Karacabey Stud Farm in Bursa, Turkey, were used.

Fertile cows consisted of the ones that diagnosed as pregnant after the first or second insemination following the biopsy. Repeat breeder cows were chosen among non-

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Figs 1-4. **1.** Desquamation of epithelium of the left uterine horn of a repeat breeder cow. H & E ×200. **2.** Diffuse inflammatory cell infiltration in the left uterine horn of a repeat breeder cow. H & E × 200. **3.** Dilatation of endometrial glands in the right uterine horn of a repeat breeder cow. H & E ×200. **4.** Periglandular fibrosis in the right uterine horn of a repeat breeder cow. H & E ×200.

Table 1. Histological findings in the endometriums of fertile and repeat breeder cows in oestrus and dioestrus

	Hyperemia	Edema	Haemorrhage	Secretion and epithelal cells in glandular lumen	Degeneration and desquamation in epithelial lamina	Cell infiltration	Dilatation of glands	Periglandular fibrosis
Oestrus								
Fertile n=5	5/5	5/5	3/5	3/5	0/5	0/5	0/5	0/5
RB n=15	7/15	7/15	10/15	13/15	4/15	3/15	2/15	0/15
Dioestrus								
Fertile n=5	3/5	2/5	5/5	5/5	0/5	0/5	0/5	0/5
RB n=15	12/15	12/15	15/15	15/15	2/15	8/15	2/15	2/15

RB, Repeat breeder; n, number of cows. No significant difference between fertile and RB groups at oestrus and dioestrus stages (P>0.05).

Table 2. Localization of histological findings in the endometriums of fertile and repeat breeder cows in oestrus and dioestrus

	Hyperaemia			Edema		Haemorrhage			Secretion and epithelial cells in glandular lumen			Degeneration and desquamation in epithelial lamina			Cell infiltration			Dilatation in glands			Periglandular fibrosis		
	Left uterine horn	Right uterine horn	Uterine body	Left uterine horn	Right uterine horn	Left uterine horn	Right uterine horn	Uterine body	Left uterine horn	Right uterine horn	Uterine body	Left uterine horn	Right uterine horn	Uterine body	Left uterine horn	Right uterine horn	Uterine body	Left uterine horn	Right uterine horn	Uterine body	Left uterine horn	Right uterine horn	Uterine body
Oestrus fertile																							
n= 5	4/5[a]	5/5[a]	4/5[a]	4/5[A]	5/5[a]	4/5[a]	3/5[a]	1/5[a]	2/5[a]	3/5[a]	3/5[a]	0/5[a]	0/5[a]	0/5	0/5[a]	0/5[a]	0/5[a]	0/5[a]	0/5	0/5[a]	0/5	0/5	0/5
RB																							
n= 15	4/15[a]	3/15[b]	4/15[a]	3/15[B]	3/15[b]	4/15[a]	3/15[a]	7/15[a]	9/15[a]	11/15[a]	8/15[a]	3/15[a]	3/15[a]	0/15	3/15[a]	4/15[a]	1/15[a]	1/15[a]	0/15	1/15[a]	0/15	0/15	0/15
Dioestrus fertile																							
n= 5	2/5[a]	2/5[a]	1/5[a]	1/5[a]	1/5[a]	1/5[a]	5/5[a]	5/5[a]	4/5[a]	5/5[a]	4/5[a]	0/5[a]	0/5	0/5	0/5[a]	0/5[a]	0/5[a]	0/5[a]	0/5[a]	0/5	0/5[a]	0/5[a]	0/5
RB																							
n= 15	8/15[a]	8/15[a]	8/15[a]	7/15[a]	7/15[a]	6/15[a]	9/15[a]	12/15[a]	13/15[a]	13/15[a]	12/15[a]	2/15[a]	0/15	0/15	6/15[a]	5/15[a]	5/15[a]	1/15[a]	2/15[a]	0/15	1/15[a]	2/15[a]	0/15

RB: Repeat breeder, n: number of cows. Different superscripts in the same column differ significantly; a, b; P<0.01; A, B; P<0.05.

pregnant cows which calved at least once previously, had normal oestrus discharge and cycle, and even inseminated at least three-times (Zemjanis 1980, Ayalon 1984, Lafi and Kaneene 1988, Alacam 1994).

In fertile and repeat breeder cows, 4mm × 11 mm biopsy samples from both uterine horns and uterine body were obtained by biopsy forceps in oestrus and on the eighth day (dioestrus) of the same oestrus cycle. Samples were fixed in 10% neutral formalin and serial sections of 5 μ thickness were obtained by routine paraffin embedding technique. Haematoxylin and eosin staining (Luna 1968) was carried out and stained sections were evaluated under light microscope. Fisher's exact test was applied to analyze the results statistically (Instat 1990-1993).

RESULTS AND DISCUSSION

Histological findings in the endometriums of fertile and repeat breeder cows are summarized in Tables 1 and 2.

In this study, 8 out of 15 repeat breeder cows (53.53%) were diagnosed histopathologically as subclinical endometritis (I. grade). Biopsy samples obtained from fertile cows failed to reveal any significant pathological lesions. Our results varied from the observations made by other workers (Sagartz and Hardenbrook 1971, Seitaridis *et al.* 1977, Rao and Kotayya 1980, Apaydin 1987, Apaydin *et al.* 1991 a, Biolatti *et al.* 1991, Kakar *et al.* 1997). This may be due to variation in the clinical selection of repeat breeder cows.

Hyperaemia, edema, haemorrhage, secretion and desquamation of epithelial cells in glands observed in biopsy samples obtained in oestrus and dioestrus of both fertile and repeat breeder cows indicated that these are normal histological changes during oestrus cycle which do not affect the fertility. Similar conclusions have been made by Priedkalns (1981), Ohtani *et al.* (1993), Ohtani and Okuda (1995).

Epithelial degeneration and desquamation was observed only in the epithelial lamina of 5 cows with subclinical endometritis (Fig. 1). Similar reports were made by Messier *et al.* (1984), Apaydin *et al.* (1991 a, b), Sar *et al.* (1996).

Cellular infiltration was not seen in endometriums of fertile cows. In 8 of repeat breeder cows (53.53%), focal or diffuse inflammatory cell infiltration consisting of lymphocyte and plasma cell with a few polymorphonuclear leukocyte in lamina propria was determined (Fig. 2). Infiltrative changes are considered an important indicator of endometritis in repeat breeder cows (Messier *et al.* 1984, Apaydin *et al.* 1991 a, b, Sar *et al.* 1996), heifers (Gustafsson *et al.* 1985) and also in buffaloes (Singh *et al.* 1983, Shahzada *et al.* 1997).

Findings on endometrial glandular dilatation (Fig.3) corroborate with observations made by Messier *et al.* (1984), Apaydin *et al.* (1991 a, b) in cows, Gustafsson *et al.* (1985) in heifers and by Singh *et al.* (1983), Shahzada *et al.* (1997) in buffaloes.

In this study, periglandular fibrosis was only detected in 2 cows of repeat breeder group in dioestrus (Fig. 4). Similar

findings were also made in repeat breeder cows (Messier *et al.* 1984, Apaydin *et al.* 1991 a, b, Sar *et al.* 1996) and buffaloes (Singh *et al.* 1983, Shahzada *et al.* 1997).

It can be concluded that subclinical endometritis is a major contributor to the repeat breeder syndrome in cows and biopsy studies of uterine body and its horns can be a good diagnostic tool.

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