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The Effects of Oestrogen and Progesterone on Lymphocyte and Plasma Cell Population in the Oviduct and Uterine Mucosae during Follicular and Luteal Phases in Ewes

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ABSTRACT

Hormonal changes during the oestrous cycle influence the immune cells pattern in ewe's reproductive tract, particularly in the uterus and oviduct. This study was conducted to quantify the number of lymphocytes and plasma cells of the uterus and oviduct under the influences of oestrogen or progesterone. Results showed that the number of lymphocytes in different parts of the uterus was significantly ($p<0.05$) higher during the follicular phase as compared to the luteal phase. Nevertheless, in the follicular phase group, the number of lymphocytes was not significantly different between the middle and anterior horn, while in the luteal phase group, the number of lymphocytes was not significantly different between the posterior and middle horns. Similarly, the number of plasma cells was significantly ($p<0.05$) higher in the follicular phase compared to the luteal phase for the different parts of the reproductive tract. In the luteal phase group, on the contrary, the number of plasma cells was not significantly different between the posterior and middle horns and between the anterior horn and oviduct. Thus, the results emphasize that the ewes are much more protected when they are in follicular phase since the number of lymphocytes and plasma cells are higher.

Keywords: Oestrogen, ewe, lymphocytes, plasma cells, progesterone, oviduct, uterine mucosae

INTRODUCTION

During oestrous cycle, the uterine endometrium undergoes proliferation and differentiation in response to the changes in the levels of sex hormones. The uterine endometrium contains cellular elements of the immune system, including lymphocytes, macrophages, plasma cells, and polymorphonuclear leukocytes (Gogolin-Ewens *et al*., 1989; Segerson *et al*., 1991; Gottshall and Hansen, 1992). In certain species, including the human's endometrium, lymphoid aggregates and scattered interstitial lymphocytes are constant features which are thought to be involved in maintaining the sterile environment of the uterine lumen (Cobb and Watson, 1995). However, the female reproductive system is exposed to infectious agents during mating, following copulation, and during parturition (Gogolin-Ewens *et al*., 1989).

Many previous studies have shown that in the normal uterus, leukocyte infiltration occurs at a certain stage of the oestrous cycle of various

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species of animals, including sow, cattle, mice, ewes and human (Zamri-Saad, 1987; Lander-Chachin, 1990; Bischof *et al*., 1994; Kaeoket, 2001; Engelhardt *et al*., 2002; Trundley and Moffett, 2004). The qualitative estimation of T lymphocytes subsets in ovine endometrium during follicular and luteal phases has been reported (Lee *et al.*, 1988). However, the sample collection was done in the abattoir, where careful clinical examination of the animals was not conducted before slaughter. Hence, the ovarian hormones were not analysed and determination of luteal and follicular phases were solely based on the appearance of ovaries. Thus, the quantitative determination of lymphocytes subsets in ovine endometrium at peak levels of oestrogen and progesterone has never been done in both cyclic and pregnant ewes.

MATERIALS AND METHODS

Animals

Fourteen adult, cycling ewes were used in this study. They were randomly divided into two different groups, namely follicular phase and luteal phase groups, with 7 ewes allocated for each. The ewes were kept in raised slatted floor house with an open environment. They were fed with commercial pellets and water was provided *ad libitum*. The ewes were kept in the same house throughout the experimental period with the temperature ranging from 26.8ºC to 32.8ºC and 84.90% to 98.40% humidity.

The ewes were synchronized into oestrus by using the progesterone sponge (Chronogest®) containing 40 mg flugestone acetate intravaginally, for 12 days. At sponge removal, the ewes were given an intramuscular injection of 350-450 IU of pregnant mare serum gonadotropin (PMSG) (Folligon®). Blood samples were collected every alternate day in order to determine the hormonal profiles by using RIA technique. The ewes of luteal phase and follicular phase were slaughtered at the peak level of progesterone and estradiol, respectively.

Sample Collection and Processing

Immediately after slaughtering, both sides of the middle portion of the Fallopian tube (ampulla region), anterior, middle and posterior parts of the uterine horns samples were fixed, processed accordingly and stained with Haematoxylin and Eosin.

Morphometry Evaluation

Morphometry evaluation was done according to the modified method proposed by Segerson *et al*. (1991). Six arbitrarily chosen microscopic fields were analysed in each of the two sections obtained from different tissues of each ewe. The morphometry evaluation was done using a light microscope, with objective × 40 and eyepieces × 10 with 1mm^2 ocular micrometers. Cell counting was performed by using an ocular reticule (ocular micrometer, 10mm × 10 mm, with 100 squares) (Leitz Wetzlar, Germany) placed onto the left side of the eyepiece of the light microscope. Only the optimal sections (free of artefacts) and correct orientation were accepted for counting. For each section, the cell counts were performed at x400 magnifications by movement of the ocular micrometer across the entire epithelium area in a non-overlapping manner.

The number of lymphocytes and plasma cells of the mucosal area (within the surface and glandular epithelium of the endometrial glands), expressed as cells per mm^2 field, were counted and recorded. Similarly, the number of lymphocytes and plasma cells in the surface epithelium of the oviduct (ampulla) and within the stromal area were also recorded. The mean cell counts and the morphometric parameters were calculated.

Statistical Analysis

The cells counts were analyzed using 2-way ANOVA (SPSS version 17.0) to compare the differences between the groups and at different anatomical parts of the reproductive tract. This

was followed by the Duncan multiple comparison test in the event of significant ANOVA findings. All the statistical tests were conducted at 95% confidence level and the differences of $P<0.05$ were considered significant.

RESULTS AND DISCUSSION

Population of Lymphocytes in the Uterus and Oviduct

The lymphocytes were localized in the luminal epithelium, as well as in the glandular epithelium and in some areas of the stroma immediately beneath these epithelia in all parts of reproductive tract during both follicular and luteal phases (*Fig. 1A-1B*). In the oviduct, the lymphocytes were preferentially localized near the border between the propria-submucosa and the epithelium regardless the phases of the oestrous cycle (*Fig. 1C-1D*).

The numbers of lymphocytes were significantly higher ($P<0.05$) in all parts of the reproductive tract during the follicular phase as compared to the luteal phase (Table 1). The highest lymphocyte count was recorded in the posterior horn for both groups, while the least count was recorded in the oviduct of both groups. The increased neutrophils and lymphocyte counts during the follicular phase might be due to their active involvement in the phagocytosis of sperms following mating (Bischof *et al.*, 1994). Moderate to high densities of leukocyte infiltrations were observed in the uterus of the non-pregnant mice, and reached the minimum

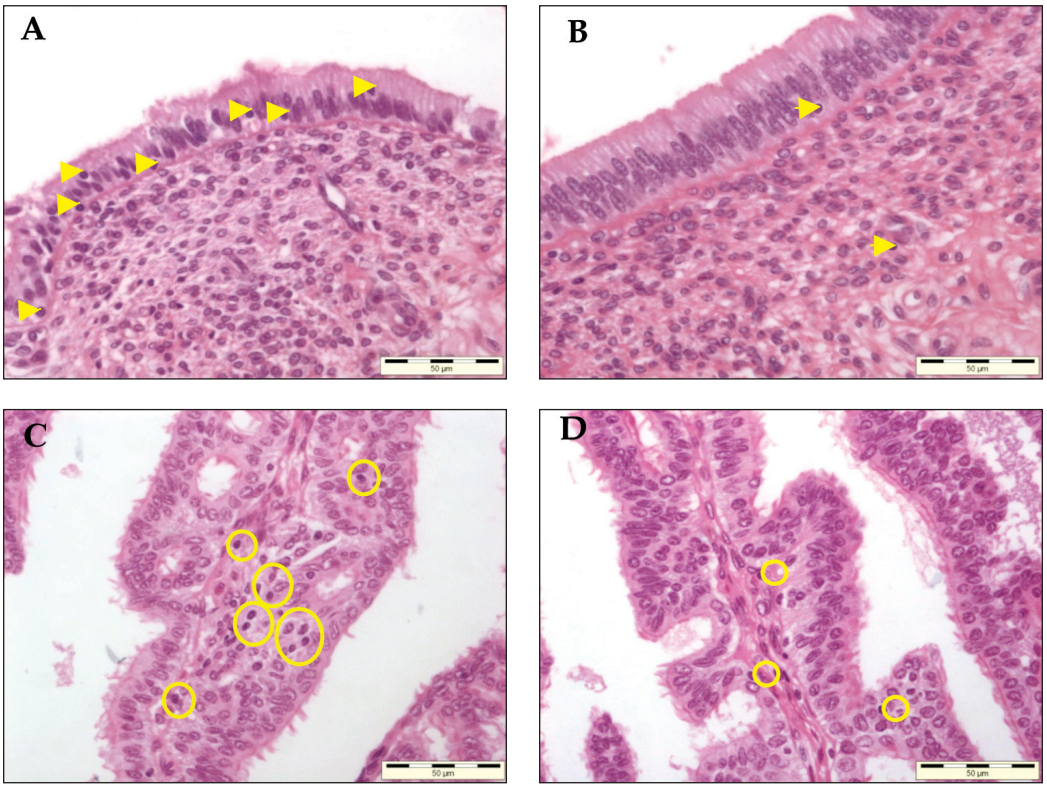

Fig. 1: Photomicrographs of the posterior horn of the uterus during follicular phase (A) luteal phase (B), and in ampulla of follicular (C) and luteal phase (D) showing the distribution of lymphocytes near the border between the propria-submucosa and the epithelium (H&E, X400). Note the remarkable difference in the lymphocyte population (H&E, X400)

TABLE 1
Total count of lymphocytes (number of cells per mm^2) at different parts of the reproductive tract during follicular and luteal phases of ewes

Different parts of the reproductive tract	Follicular phase group (mean ± S.E.)	Luteal phase group (mean ± S.E.)
Posterior horn	114.80 ± 20.00*	49.60 ± 2.44[b]
Middle horn	65.37 ± 2.62[a]	43.25 ± 3.32[b]
Anterior horn	59.00 ± 5.20[a]	24.25 ± 2.39*
Oviduct	31.10 ± 3.37*	22.50 ± 2.98*

[ab]Means with similar superscripts within the columns did not differ significantly at p=0.05
*Means with asterisks within columns differed significantly at p=0.05. (n=14)

during pregnancy. However, the infiltrations were abundant during post-parturient as it is important in the removal of the placental debris and bacterial contamination (Zamri-Saad, 1987).

The Population of Plasma Cells in the Uterus and Oviduct

Similar to the lymphocytes, the plasma cells were mostly distributed within the luminal epithelium (*Fig. 2A*). However, they were sparsely distributed and rarely found in the glandular epithelium. The plasma cells were localized mostly in the areas of the stroma, preferentially near to the glands beneath these epithelia (*Fig. 2B*). In contrast, the numbers of the plasma cell counts were much lower than lymphocytes, especially in the oviduct (ampulla region), as presented in Table 2.

There were significant differences (P<0.05) between the two phases in each part of the reproductive tracts. The number of the plasma cells was found to be highest in the posterior horn in both groups, followed by the middle, anterior horn and the least in the oviduct (P<0.05). Meanwhile, the mean number of the plasma cells was significantly different in all the parts of the reproductive tract during the follicular phase (P<0.05). During the luteal phase, however, the number was not significantly different between the posterior and middle horns, as well as between the anterior horns and the oviduct (P>0.05). The number of plasma cells was found to decrease from the posterior horns (highest) to the oviduct (lowest) for both the phases.

A gradual decrease in the population of these cells, from the posterior horn progressing

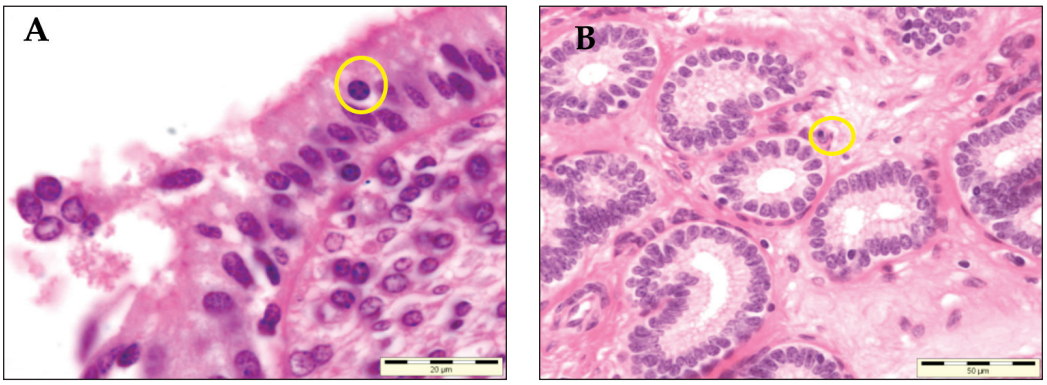

Fig. 2: Photomicrographs show the plasma cell at high magnification located in the luminal epithelium (A) (H&E X1000) and near the gland within the endometrium (H&E, X400)

TABLE 2
The average number of plasma cells (number of cells per mm^2) at the different parts of the reproductive tract during the follicular and luteal phases of ewes

Different parts of the reproductive tract	Follicular phase group (mean ± S.E.)	Luteal phase group (mean ± S.E.)
Posterior horn	5.70 ± 0.47*	2.00 ± 0.33[a]
Middle horn	3.00 ± 0.36*	1.70 ± 0.26[a]
Anterior horn	1.70 ± 1.11*	0.80 ± 0.24[b]
Oviduct	0.50 ± 0.12*	0.30 ± 0.15[b]

[ab]Means within the columns with same superscripts did not differ significantly at $p<0.05$
*Means with asterisks within columns differed significantly at $p=0.05$. (n=14)

towards the anterior horn, would probably signify the need for pathogen surveillance. Thus, such a phenomenon would have existed for two main reasons. First, infection usually stems from the exterior, i.e. via the vagina into deeper parts of the tract. Here, lies the need for a much more vigil inflammatory response and surveillance. However, the lesser number of the cells in the oviduct reflects the least contaminated area and prevention of unnecessary phagocytic activity especially during implantation. Alternatively, environmental modifications of leucocytes also have major pregnancy-associated functions that include facilitation of implantation, modulation of maternal uterine vasculature, supply of growth factors to the placenta, promotion of trophoblast differentiation and facilitation of parturition (Hunt *et al*., 2000). Furthermore, the fewer plasma cells in the Fallopian tube and endometrium than lower down the tract might be due to the lack of antigenic stimulation in the sterile environment of the upper tract (Brandtzaeg, 1997; Vaerman and Ferin, 1974).

Similar findings in the different parts of the sow oviduct were observed, whereby it was found to be significantly lower from the lower part to the upper portion. This finding indicates different immune functions within various parts of the oviduct (Jiwakanon *et al.*, 2005). Hussain *et al*. (1983) revealed that there is a cyclic variation in the distribution of plasma cells in the reproductive tract of the sow and possibly ewes, whereby oestrus is accompanied by a general increase in the number of plasma cells, whereas during dioestrus the cell counts were usually low. The reduction in the number of plasma cells during the luteal phase may reflect the amount of antibody production. When the production of antibody is lesser, the immune system will be impaired and the ability of the animals to combat infections will be lower.

Progesterone is responsible for slowing infiltration of leukocytes into the uterus at the luteal phase. This suggests that the difference in bactericidal activities between the follicular and luteal phases is closely related to the difference in the infiltrating rate of leukocytes into the uterus (Matsuda *et al*., 1985). Progesterone has been shown to affect activities of immune cell directly or indirectly. Directly, lymphoid tissue has receptors for progesterone and indirectly, progesterone induces the synthesis of uterine proteins that eventually inhibit the proliferation of lymphocytes (Staples *et al*., 1983; Low and Hansen, 1988) and neutrophil activity (Seals *et al*., 2003). Furthermore, progesterone induces the presence of γδ T cells which actually suppresses the lymphocytes (Majewski *et al*., 2001).

In contrast, oestrogen was found to increase uterine blood flow (Dickson *et al*., 1969), and this might contribute to the increasing numbers of these cells infiltrated on the uterine and adjacent tissues. In the endometrium, a higher level of oestrogen during the follicular phase would increase the permeability of blood capillaries (Keys, 1988) and this might contribute to the higher population of immune cells infiltrated to the adjacent tissues.

CONCLUSIONS

Hormonal changes during the oestrous cycle influence the immune cells pattern in the ewe's reproductive tract, particularly in the uterus and oviduct. In more specific, progesterone inhibits lymphocyte proliferation that resulted in the lesser number of lymphocytes population in the uterus during the luteal phase. These findings corresponded with the previous studies on other various species, such as porcine (Kaeoket *et al*., 2001; Jiwakanon *et al*., 2005; Hussain *et al*., 1983), bovine (Cobb and Watson, 1995), caprine (Perez-Martinez *et al*., 2002) and ovine (Segerson *et al*., 1991). During the oestrous cycle, the period of the luteal phase is longer than the follicular phase. This study has shown that the ewes were much more protected when they were in the follicular phase since the number of lymphocytes and plasma cells were found to be higher. Thus, the ewes would be more susceptible to infection during the luteal phase and this might have to be considered when diagnosing pathological cases.

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