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Distribution and quantitative patterns of T lymphocytes in the female reproductive tract and ovary throughout the oestrus cycle of Angora Goats

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Summary

The present study was designed to evaluate the distribution of alpha-naphthyl acetate esterase (ANAE) positive T lymphocytes in the female reproductive tract and ovary throughout the estrous cycle of Angora Goats. Tissue samples were collected on days 5, 10 and 16 of the oestrus cycle and then fixed in formol-sucrose fixative (pH 6.8) and stored for 22 hours at +4°C. The samples were then additionally fixed in Holtz' solution under the same conditions they were in the first fixation. Cryostat sections of 8 µm thickness were stained for alpha naphthly acetate esterase activity at pH 6.4. The ANAE positive T lymphocytes were mainly located in the epithelium, lamina propria and around blood vessels in other region of connective tissues. Density of ANAE positive T lymphocytes in the ovarian and uterine tissues was highest on day 10 and 16 respectively. It was concluded that the estrous cycle may have been responsible for variations in the distribution of ANAE positive T lymphocytes in goat ovarian and reproductive tract tissues.

Key words: reproductive system, lymphocytes, goat

During the estrous cycle in domestic animals the reproductive tract – and the uterine endometrium in particular, undergoes proliferation and differentiation in response to the changes in sex steroid hormone levels. Additionally, the female genital tract is exposed to the environment; like it is the bronchial and intestinal epithelium and so it is part of the mucosal system (26). This is a local immune system that constitutes a defense mechanism against infection, and also has to deal with the fetal placental unit and is cyclically exposed to allogenic sperm (27).

Leucocytes, mainly neutrophils and macrophages, accumulate in the endometrium and cervix, and then some of them migrate across the epithelium into the uterine and cervical lumen (5, 20, 28). Genital tract mucosae plays a special role within the mucosal immune system. Just as in the mammary gland, lymphocyte migration in uterine tissue is greatly influenced by hormonal changes during pregnancy (6, 22).

Hormones play a very important role in regulating host immunity in the genital tract, distinguishing it from the other mucosal tissues in the organism (8). Hormone levels fluctuate during the reproductive cycle, influencing immune surveillance and disease susceptibility. Humoral and cellular adaptive immune effector mechanisms operate in the reproductive tract (8, 11). The normal healthy female genital tract harbours few T cells, but infections with *Chlamydia trachomatis* result in the recruitment of CD4+ and CD8+ T cells (7, 32).

Non-specific esterase is widely distributed in various cell types. Cytochemical esterase activity is commonly used to differentiate leukocyte types and leukaemia cells (10). Alpha-naphthyl acetate esterase is a non-specific esterase. The pattern of esterase activity revealed by this method provides a discriminating marker for mature T-lymphocytes, which show dense, localized, dot-like positive responses (12, 17).

The reproductive immune system of sheep has been widely studied (15, 25, 29). There have, however, been a limited number of studies concerning the reproductive immune systems of goats (18, 27) and very few about possible changes in lymphocyte subpopulations in the reproductive tracts of these animals. The aim of the present study was to establish the pattern of changes in lymphocyte numbers in the reproductive tract and ovary throughout the estrous cycle of Angora Goats.

Material and methods

Animals. Animals were purchased from the Lalahan Livestock Central Research Institute – a government organization located near the capital of Ankara. Adult female goats were randomly divided into three groups of 4 goats each. A fertile male goat was also placed into a separate pen nearby. Estrous behaviour was monitored twice daily using the male goat. The waist region of the buck was covered with a cloth to avoid mating.

Tissue collection. Healthy animals were selected for the study by clinical examination. Reproductive tissues collected from the goats all displayed normal histology. Tissue samples from the cervix, uterus (corpus and horns), fallopian tubes (isthmus and ampulla) and ovaries, including corpus luteum, were collected following laparatomy on days 5, 10 and 16 of the estrous cycle. The day of estrous was accepted as day 0.

Histological procedure. For ANAE demonstration (2), a piece of tissue from each anatomical region was fixed in formol-sucrose solution (pH 6.8) and stored for 22 hours at +4°C. Following the first fixation, the samples were additionally fixed in Holtz' solution under the same conditions they were in the first fixation. Cryostat sections 8 µm thick were cut and stained for alpha naphthly acetate esterase (Sigma, Germany) activity at pH 6.4 for 10 min (24). The incubated preparations were then washed in distilled water and counterstained with 1% methyl green for 30 min. Following dehydration in increasing concentrations of ethanol, the preparations were cleaned in xylene and mounted in DPX. After applying ANAE enzyme stain, the tissues were examined under a light microscope. The regional distribution of ANAE positive lymphocytes was compared in 6 anatomically distinct tissues throughout the estrous cycle and the ANAE positive lymphocyte population in them was estimated subjectively. Each tissue was awarded an arbitrary score of 1 to 4 points according to whether it was considered to have exceptionally large numbers (++++), large numbers (+++), moderate number (++), or few (+) ANAE positive lymphocytes (31). Ten microscopic fields in five sections (8 µm thickness) of each anatomical zone were evaluated using a × 40 objective. Adjacent lumen was not included.

Results and discussion

ANAE positive lymphocytes were observed throughout the reproductive tract and ovaries on days 5, 10 and 16 of the estrus cycle. There was a considerable variation among the different tissues in terms of ANAE positive lymphocyte density. There was also a variation between the location and specific tissues (tab. 1). The majority of ANAE positive lymphocytes displayed in a continuous fashion under the layer of reaction product on their cytoplasm. In a light microscope examination, ANAE positive lymphocytes had 1-3 distinct, red-brownish granules representing the reaction product by which they recognized T-lymphocytes. In some lymphocytes the numbers of granules were noticeably higher. No reaction product was present in B-lymphocytes. The results of ANAE staining are shown in table 1. In all compartments, the macrophages were observed to exhibit diffuse staining at pH 6.4.

Ovaries. ANAE positive lymphocytes in the ovarian cortex and medulla were observed in the cellular stroma, interstitial areas between follicles and corpora lutea regardless of the estrous cycle day. However, the ANAE positive lymphocytes were more concentrated in the ovarian cortical zone than in the medulla region (tab. 1, fig. 1).

Uterus and Cervix. ANAE positive lymphocytes located in the luminal epithelial layer and lamina propria of the endometrium were considered. These layers plus the endometrial glands were also considered in the case of uterus horn and corpus. They were also observed in both organs, around blood vessels between muscle groups in tunica muscularis (fig. 2, 3, 4).

Tab. 1. ANAE positive lymphocytes distributions in Angora Goat female reproductive organs

Estrous Days	Ovary	Uterus		Cervix	Uterine Tubes	
		Corpus	Horn		Isthmus	Ampulla
5	++	++	+	++	+	+
10	+++	++++	+++	++	++	+
16	++	+++	+++	+++	++	+

Explanations: (+) – few; (++) – moderate number; (+++) – large numbers; (++++) – exceptionally large numbers of ANAE positive lymphocytes; n = 4 for each estrous day group

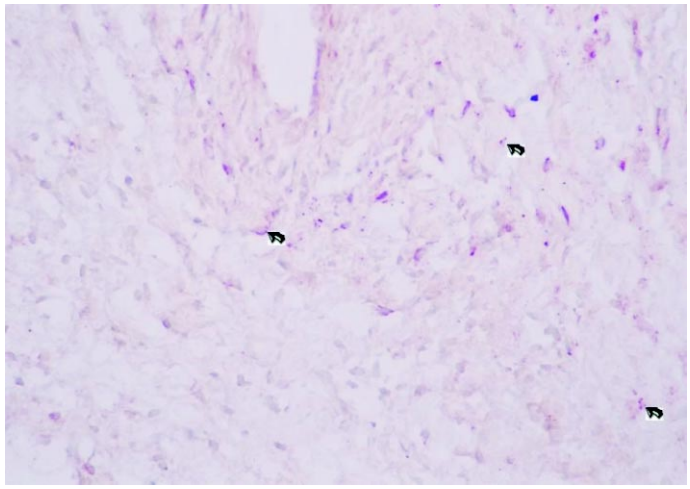

Fig. 1. Alpha-napththyl acetate esterase (ANAE) staining (at 16 days, ovary). Arrows: ANAE-positive T lymphocyte, ANAE, 360 ×

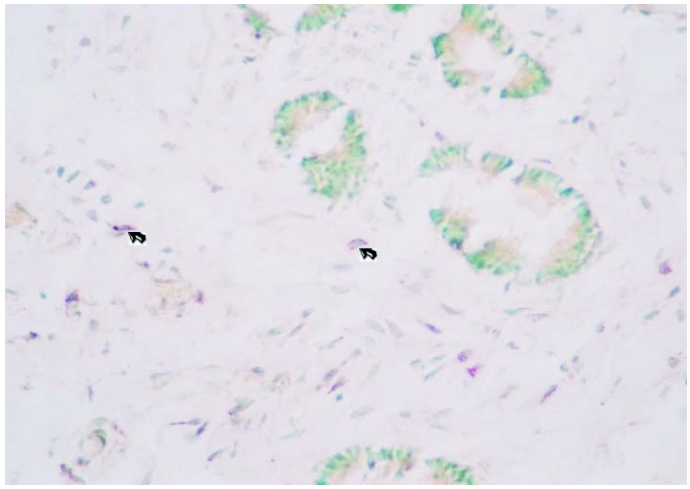

Fig. 2. Alpha-napththyl acetate esterase (ANAE) staining (at 10 days, cornu uteri). Arrows: ANAE-positive T lymphocyte, ANAE, 360 ×

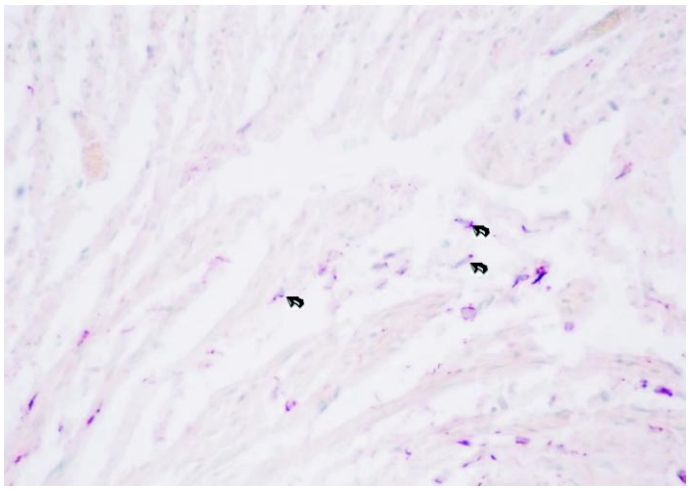

Fig. 3. Alpha-napththyl acetate esterase (ANAE) staining (at 5 days, cervix uteri). Arrows: ANAE-positive T lymphocyte, ANAE, 360 ×

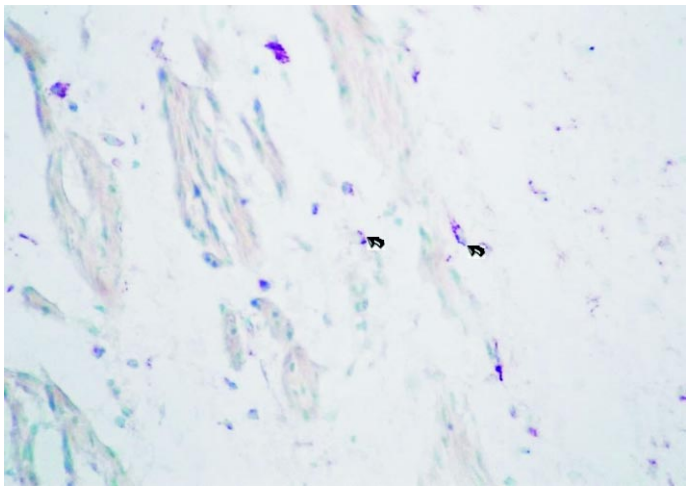

Fig. 4. Alpha-napththyl acetate esterase (ANAE) stainin/g (at 10 days, corpus uteri). Arrows: ANAE-positive T lymphocyte, ANAE, 360 ×

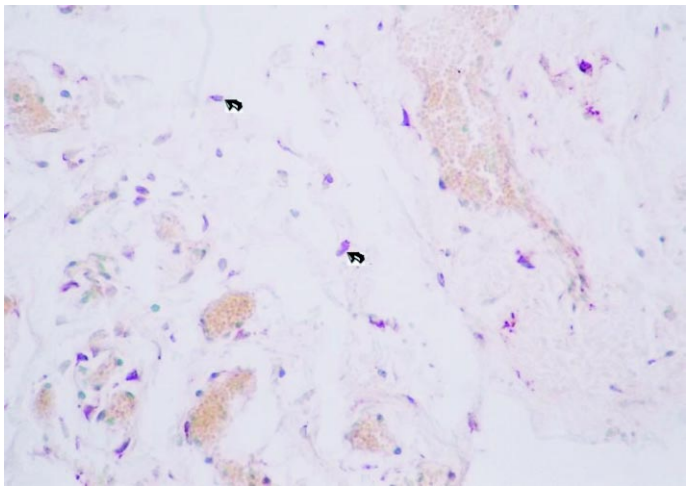

Fig. 5. Alpha-napththyl acetate esterase (ANAE) staining (at 16 days, uteri tube - isthmus). Arrows: ANAE-positive T lymphocyte, ANAE, 360 ×

Uterine Tubes. The lowest number of ANAE positive lymphocytes were observed in the uterine tubes, and had increased by days 10 and 16 in both the isthmus and ampulla (tab. 1). ANAE positive lymphocytes shown in the lamina propria of mucosal membran were considered (fig. 5).

The present study was carried out to determine whether or not the distribution of ANAE positive lymphocytes in the Angora Goat reproductive system changes when it is exposed to the stage-dependent estrous cycle. Certain conditions such as temperature and especially pH may play an important role in detecting alpha-naphthyl acetate esterase activity. Optimal pH for non-specific esterase activity is limited between pH 5 and 8 (3). The present study revealed that most of the ANAE positive lymphocytes were located close to the lamina epithelium and between the uterine endometrial glands (fig. 2). Table 1 shows the distribution of ANAE positive lymphocytes during the increasing days of the estrous cycle and the highest number of the above lymphocytes was observed on days 10 and 16 of the estrus cycle both in the corpus uteri and uterine horns. It seems that in Angora goats, just as in a similar way to other domestic animals and humans (23, 27, 33), lymphocytes are though to be involved in maintaining the sterile environment of the uterine lumen.

In many studies the best reaction for ANAE positivity, measured in peripheral blood, has been reported to be at pH 5.8 in humans (13), pH 5.8 in dogs, sheep and goats (1), and pH 7.2 in chickens (17). The present study demonstrated that the best reaction of the lymphocytes against ANAE staining was obtained at pH 6.4. Staining patterns were similar to those reported for T lymphocytes of lymphoid tissue, and resembled those results described by Aştı et al. in Angora Goat lymphoid tissue (2).

It has been reported that $CD8^+$ lymphocytes are located between the epithelial cells of the uterine mucosa and endometrial glands in goats (18) and the same study, also reported the presence of $CD8^+$ lymphocytes in the stroma, corresponding to the medial region of the lamina propria. $CD4^+$ lymphocytes, however, were abundant in the subepithelial stroma. In contrast, no $CD4^+$ cells were reported in the medial region of the lamina propria (27). The $CD8^+$ T lymphocyte subpopulation was the largest in the endometrial epithelium, glandular epithelium and the stroma. Lee et al. described the distribution pattern of this subpopulation as non-uniform in sheep uterus (14), while Meeusen et al. reported that $CD8^+$ T lymphocytes are located in all intraepithelial regions (19). In the goat uterus, all the lymphocyte subpopulation disappeared from the caruncular area of the placentome during gestation, while the number of $CD4^+$ and $CD8^+$ T lymphocytes decreased drastically in the inter-caruncular area (18). The suppression of all immunocompetent lymphocytes in the caruncular area could be a mechanism preventing immune response against the conceptus, because chorionic binucleated cells have a paternal genetic component. If paternal antigens were expressed, these cells would probably attract maternal immunocompetent cells (18, 30). Although cells displaying $CD4^+$ and $CD8^+$ antigens were found in the both cervix and uterine endometrium, a qualitative observation showed that they were more abundant in the cervix. This fact indicates a massive lymphocyte infiltration

involved in immune cellular response at the entrance door of the reproductive tract (27).

Butterworth et al. have observed that $CD4^+$ lymphocytes are abundant and widely distributed in the normal feline female reproductive tract (4). According to the same study, $CD8^+$ cells were the most prevalent immune cells in the feline female reproductive tract. In addition, $CD8^+$ cells were also reported in the germinal epithelium and stroma of the cats' ovaries (4). It also demonstrated that the submucosal area of the reproductive tract of *Rhesus* contains very few $CD4^+$ T lymphocytes (21). However, a moderate number of $CD4^+$ T lymphocytes were reported in the mucosal and submucosal areas of the feline reproductive tract (4) whereas $CD4^+$ (T) and $CD21^+$ (B) lymphocytes were rarely found within either the cyclic or pregnant luminal epithelium or in the uterine lumen of cows (16). Another study, however, identified intraepithelial $CD8^+$ lymphocytes in sheep (9). Lee et al. reported a relatively high population of lymphocytes in the subepithelial stroma of ovine uterus throughout the estrous cycle, and they also demonstrated that the number of intra-epithelial lymphocytes was only reduced between days 18 and 46 of pregnancy (14, 15).

In the present study, variations in distribution of ANAE positive T lymphocytes were observed in specific regions of the reproductive tract and during cyclical changes in the same anatomical regions. On day 5 the cervix, uterus, isthmus and ovaries contained less ANAE positive T lymphocyte than on the days of 10 and 16 of estrous cycle. In contrast, no distribution of ANAE positive T lymphocytes changes was observed in the ampulla. This may suggests that ANAE positive T lymphocytes are specifically sensitive to hormonal alterations in goats.

In conclusion, the results of light microscopic morphology, the location and quantitative distributions of T lymphocytes in the female goat reproductive tract organs and ovaries seem to be similar to results reported from the other domestic animal species. The study also observed that the distribution of T lymphocytes may vary depending on the physiological changes during the oestrus cycle in the reproductive tract and ovary of Angora Goats.

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