UNIVERSITI PUTRA MALAYSIA

IMMUNOLOGICAL STATUS OF THE REPRODUCTIVE TRACT OF EWES DURING FOLLICULAR AND LUTEAL PHASES

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MASTER OF VETERINARY SCIENCE
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2009

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By

MORTETA H. AL-MEDHTIY

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Veterinary Science

IMMUNOLOGICAL STATUS OF THE REPRODUCTIVE TRACT OF EWES DURING FOLLICULAR AND LUTEAL PHASES

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August 2009

Chairperson: **Assoc. Prof. Dr. Md Zuki Abu Bakar @ Zakaria, PhD**

Faculty: **Veterinary Medicine**

The levels of uterine Secretory-IgA (S-IgA) and numbers of plasma cells were measured to observe the differences between two stages of estrous cycle (follicular and luteal phases) in the healthy cycling non pregnant ewes. Twelve local crossbreed (Malin) ewes were used in this study and they were divided into two groups of 6 animals each according to the stages of estrous cycle. All ewes were subjected to estrous synchronization and were allowed to undergo one natural estrous cycle after the removal of the sponge. All animals were slaughtered at the end of the experiment. The uterine mucus was harvested by flushing with a mixture of protease inhibitor cocktail in distilled water. For both stages, the level of uterine S-IgA was quantified by using ELISA and Methyl Green Pyronine staining was used to quantify the plasma cell in the mucosa of the uterine horn and oviduct of ewe's reproductive tract. H&E stain was used to evaluate the histological changes of endometrium during follicular and luteal phases of ewe.

The protocol of the study was approved by the Faculty's animal care and use committee (animal utilization protocol number: 08R26/Jun 08-May 09). The S-IgA level and the number of plasma cells in the reproductive tract of ewe were significantly higher ($P<0.01$) in the follicular phase as compared to those values in the luteal phase.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

STATUS KEIMUNAN TRAKUS REPRODUKTIF SEMASA FASA FOLIKEL DAN LUTEUM PADA BIRI-BIRI

Oleh

MORTETA H. AL-MEDHTIY

Ogos 2009

Pengerusi: **Prof. Madya Dr. Md Zuki Abu Bakar @ Zakaria, PhD**

Fakulti: **Perubatan Veterinar**

Tahap Secretory-IgA (S-IgA) dalam uterus dan bilangan sel plasma telah dihitung untuk memerhatikan perbezaan antara dua fasa kitaran estrous (fasa folikel dan luteum) bagi bebiri betina yang sihat dan tidak bunting. Dua belas ekor bebiri betina baka kacukan Malin telah digunakan dalam kajian ini dan bebiri tersebut telah dibahagikan kepada dua kumpulan yang setiap satunya mempunyai enam ekor bebiri berdasarkan peringkat kitar estrous. Semua bebiri tadi telah diselaraskan masa estrusnya dan dibiarkan mengalami satu kitaran estrus secara semulajadi selepas span dikeluarkan. Semua haiwan dalam kajian ini telah disembelih pada akhir eksperimen. Mukus dari uterus dipungut melalui pengepaman dengan campuran larutan penghalang protease danair suling. Untuk kedua-dua peringkat, tahap Secretory-IgA (S-IgA) telah dikuantifikasi dengan menggunakan ELISA dan kaedah pewarnaan Methyl Green Pyronine. Kaedah pewarnaan ini telah digunakan untuk memerhatikan sel-sel plasma dalam tisu tanduk uterus dan oviduktus pada trakus genitalia bebiri. Protokol untuk kajian ini telah diluluskan oleh Faculty's Animal Care and Use Committee (nombor protokol penggunaan haiwan: 08R26/Jun 08-May 09). Tahap S-

IgA dan bilangan sel-sel plasma dalam saluran reproduksi bebiri betina ialah tinggi secara signifikan ($P<0.01$) pada fasa folikel berbanding kepada fasa luteum.

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I certify that a Thesis Examination Committee has met on **20 August 2009** to conduct the final examination of Morteta H AL-Medhtiy on his thesis entitled **"IMMUNOLOGICAL STATUS OF THE REPRODUCTIVE TRACT OF EWES DURING FOLLICULAR AND LUTEAL PHASES"** in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the degree of Master of Veterinary Science.

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

MORTETA H. AL-MEDHTIY

Date:

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LIST OF ABBREVIATIONS

S-IgA	Secretory-Immunoglobulin A
%	Percentage
˚C	Degree Celsius
µg	Microgram
µl	Micro liter
ABTS	2, 2', azino-bis (3-ethyl benzathiazoline-6-sulphonic acid) /H2O2
APCs	Antigen-Presenting Cells
ASC	Antigen Secreting Cell
BSA	Bovine Serum Albumin
CD	Cluster of Differentiation
CI	Confidence Interval
CL	Corpus Luteum
E. coli	*Escherichia coli*
ELISA	Enzyme-linked Immunosorbent Assay
FSH	Follicle Stimulating Hormone
g	Force of gravity
h	Hour
H&E	Haematoxylin and Eosin
IFN-γ	Interferon-Gamma
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-12	Interleukin-12

IQR	Inter-Quartile Range
ISCs	Immunoglobulin-Secreting Cells
IU	International Unit
kg	Kilogram
LH	Luteinizing Hormone
M	Molarity
mg	Milligram
MGP	Methyl Green Pyronin
min	Minute
ml	Mililiter
mm	Millimeter
n	Number
nm	Nanometer
p	Probability
P4	Progesterone hormone
PBS	Phosphate Buffer Saline
PGF2α	Prostaglandin F2α
pIgA	Polymeric Immunoglobulin A
pIgR	Polymeric Immunoglobulin receptor
PKC	Palm Kernel Cake
PMSG	Pregnant mare serum gonadotropin
s/c	Subcutaneous
SC	Secretory Component
SEM	Standard Error Mean

CHAPTER 1

INTRODUCTION

The animal's resistance to disease is related to the proper function of the immune system. Therefore, any factor that could alter the female immune system status such as diseases, stressors, hormonal changes etc., would affect the animal's health status. Sheep and cattle are known to have a suppressed immune function during the luteal phase (Gottshall and Hansen, 1992; Ramadan *et al.*, 1997). This represents significant risk to diseases when the animal is exposed to disease agents during the luteal phase.

To our knowledge, there is no previous reports regards to the level of S-IgA and the number of plasma cells in the reproductive tract of healthy non-pregnant cycling ewes. Quantifications of both S-IgA in the uterine secretions and plasma cells throughout mucosal of uterine horn endometrium and oviduct ampulla during normal estrous cycle of healthy cycling non pregnant ewes have never been carried out previously.

Purpose of the study to prepare a perfect immunological base by measuring immune parts those are affected by estrous cycle such as immunoglobulins, immunoglobulin-producing cells. The results can be used as a benchmark, which will help to derive the right vaccines suitable with the animal's stages of estrous cycle.

There is insufficient information from previous researches which support this important aspect of the immunological segments inside the ewes' reproductive tract which is necessary to ensure the safety of this complicated system (McNeilly, 2008).

The sheep have proved to be a useful model for studying the actions of progesterone on uterine immune function because of the ease of surgical manipulation on the reproductive tract as well as the large quantities of uterine secretions and lymphocytes that can be obtained (Hansen, 1998).

Sheep as a suitable model for human diseases, that bacterial infection in sheep may be used for the benefit of both human and veterinary medicine (Papp and Shewen, 1997).

The female reproductive system consists of the internal reproductive organs which consist of ovaries, oviducts, the uterus and the vagina and the external genitalia consist of clitoris, the labia majora and the labia minora. The reproductive organs are incompletely developed and remained in a state of rest until gonadotropic hormones are secreted by the pituitary gland. Accordingly, these events are considered as a signal to launching or start of the puberty. In sheep, puberty occurs at about 6 to 9 months and the age at first ovulation (Jainudeen *et al.*, 2000). Thereafter, many changes take place in the entire reproductive system, including further differentiation of the reproductive organs that eventually crowned by the first estrus. The estrous cycle includes many hormonal, histological and physiological changes. A growth and development occurred for the ovum during follicular phase until it reaches a mature ovum known as Graafian follicle. During this stage, the estrogen hormones are

released by the follicles. Estrogen has many actions on endometrium and myometrium causing proliferation and hypertrophy, and development of female sexual characteristics. Highest level of estrogen concentration in the blood, leads to production of luteinizing hormone (LH) by the pituitary gland. The LH plays an essential role in the ovulation process. After the ovum has been released from the Graafian follicle, the remaining follicular cells and the surrounding theca interna will form corpus luteum (CL). Corpus luteum will secrete the hormone progesterone (P4). P4 has many effects on the endometrium, and plays an essential role to maintain the pregnancy, if fertilization occurs. Otherwise, P4 prepares the endometrium to receive another estrous cycle (Leslie and James, 2001; Eurell and Frappier, 2006). These hormones (progesterone and estrogen) play a very important role in regulating host immunity in the reproductive tract, distinguishing this from other mucosal sites (Entrican and Wheelhouse, 2006). In sheep, ovarian sex hormones have obtained influent on uterine immune functions (Ramadan *et al.*, 1997). The hypothesis of this study was that S-IgA (secretory-immunoglobulin A) and plasma cells levels increased during follicular phase and decreased in the luteal phase in the normal, cycling and non-pregnant ewes.

Objectives

The present study was carried out with the following objectives:

1. To quantify the immunoglobulin A (IgA) level in the uterine flushing during follicular and luteal phases.
2. To quantify the plasma cells in the oviductal (ampulla) and uterine horn mucosa during follicular and luteal phases.

CHAPTER 2

LITERATURE REVIEW

2.1 Anatomy of the Reproductive Tract of the Ewe

The female genital tract includes the following organs: 2 ovaries, 2 oviducts, uterus and vagina, which are considered the internal genital organs or internal reproductive organs (Figure 2.1).

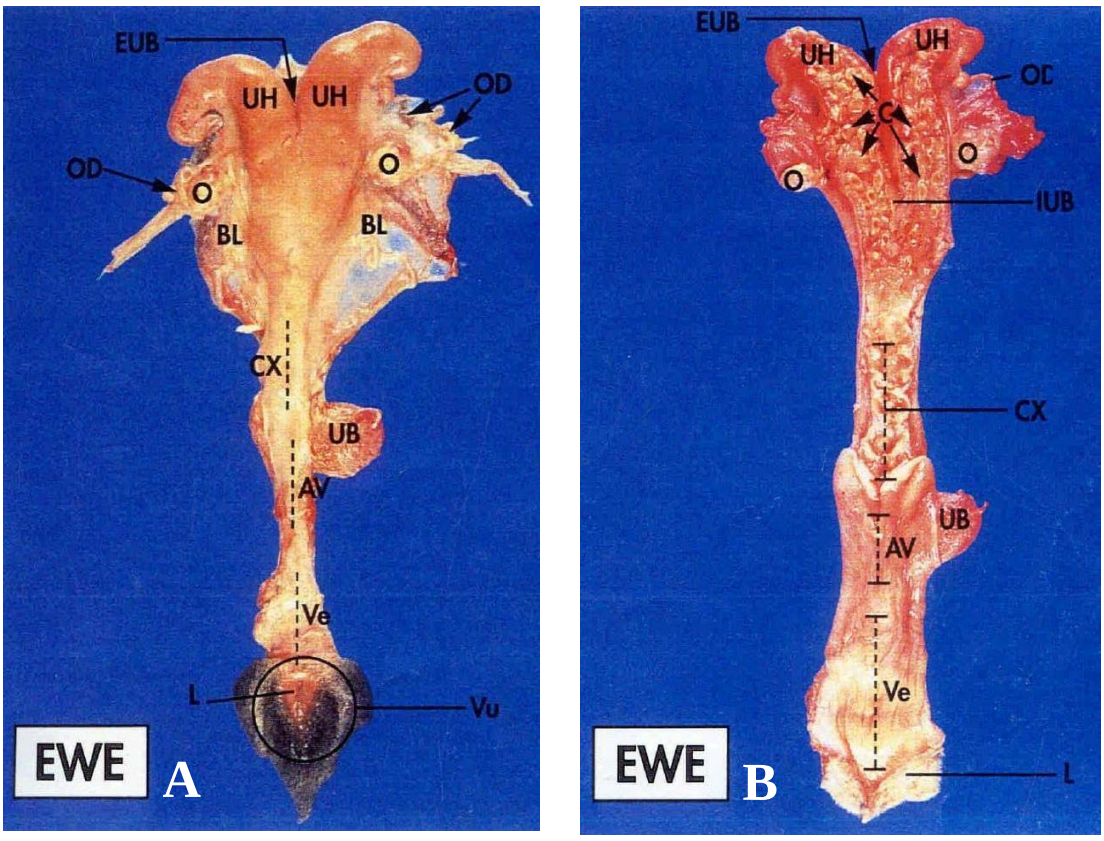

Figure 2.1: (A) Unopened reproductive tract (B) opened reproductive tract**.** (A, B) = dorsal view of the reproductive tract of ewe. **AV**: Anterior vagina, **BL**: Broad ligament, **C**: Caruncle, **CX**: Cervix, **EUB**: External uterine bifurcation, **IUB**: Internal uterine bifurcation, **L**: Labia, **O**: Ovary, **OD**: Oviduct, **Ve**: Vestibule, **Vu**:

The external genital organs or external genitalia are the clitoris, the labia majora and the labia minora.

There is a ligament supporting the internal genital organs, which is known as the broad ligament. This ligament consists of the mesovarium, which supports the ovary; the mesosalpinx, which supports the oviduct; and the mesometrium, which supports the uterus. In sheep, the broad ligament attached to the dorsolateral in the region of the ileum. Thus, like horns of ram, the uterus is arranged with dorsal convexity while the ovaries are located near the pelvis (Sisson, 1975; Jainudeen *et al.,* 2000).

Ovaries are the reproductive glands, which produce the female germ cells, the ova and are homologous to the testes of the male (Nickel *et al.,* 1979). Both exocrine (egg release) and endocrine (steroidogenesis) functions are performed by the ovaries (Jainudeen *et al.,* 2000). The ovaries are located in the abdominal cavity. They are morphologically almond-shaped but slightly flattened, weigh about 1-2 g each, 1.5 cm long and 1-1.8 cm wide in sheep and goat. These data are just averages because the ovaries vary in shape, weight and size with the stage of the estrous cycle (Nickel *et al.,* 1979). The mesovarium is the cranial part of the broad ligament, which supports the ovary (Jainudeen *et al.,* 2000). Within close proximity in the level of the fifth lumbar vertebra, the mesovarium holds the ovaries in position lateral to the coiled uterine horns. The blood vessels, lymphatics and nerves are observed in this ligament and they enter the ovary at the hilus. The proper ligament of the ovary is muscular and extends from the uterine end of the ovary to the mesometrium. A suspensory ligament has been demonstrated in sheep and goat (Nickel *et al*., 1979).

Oviduct or fallopian tube or uterine tube is a muscular tube, which acts as a channel to convey oocyte after released from the ovary to the uterus, and fertilization occurs here. The oviduct in the small ruminants is about 15-16 cm in length. The oviduct consists of four anatomical regions initiated from the open ends, the infundibulum that is located at the ovarian end of the uterine tube that has characteristic of fringed projections known as fimbriae. The latter, help to capture the oocyte after it is launched from ovary in to the abdominal cavity towards the uterus. Following the infundibulum, is the ampulla, which is a wide portion where fertilization usually occurs. After the ampulla portion is the isthmus, which is, seems narrowed, much convoluted portion between the ampulla and the uterus. Finally, the oviduct opens into the uterine horn through the uterine ostium and marks the site of the uterotubal junction. This junction in ruminant seems gradual (Nickel *et al.,* 1979; Gartner and Hiatt, 2001; Konig and Liebich, 2007). In farm mammals, the ovary lies in an open ovarian bursa. The bursa in sheep and cattle is wide and open. This bursa is a pouch consists of a thin peritoneal fold of mesosalpinx. The cranial portion of the broad ligament is the mesosalpinx that is attached to a suspended loop at the upper part of the uterine tube (Jainudeen *et al.,* 2000). Blood vessels and nerves reach the organ within the mesosalpinx, which is received from the broad ligament that is the common suspension of the female genital tract (Konig and Liebich, 2007).

Uterus is a muscular tube, located in the abdominal cavity in most of the domestic mammals. The anatomy of this organ varies in shape and internal organization with the species, age and physiological activity, which do hormones control. The uterus facilitates in receiving the fertilized ovum, implantation, nourishment of the fetus until birth and expels the embryo through the birth canal at the end of gestation by

muscular contraction. In domestic mammals, the uterus belongs to the intermediate form (uterus bicornis). In ruminants, there is a septum limited to the cranial part of the uterine body, and extend almost as far as the cervix (Nickel *et al.,* 1979; Konig and Liebich, 2007). The uterus of the small ruminants is similar to that of the cow (Nickel *et al.,* 1979). The uterus comprises uterine horns, body and cervix. The cranial end of uterus is stretched to diverge to two uterine horns as an extension of the uterine body. They extended to the ovaries and connecting with the oviducts. In ruminants, each horn is been coiled ventrally on itself with the first convexity facing dorsocranially (Konig and Liebich, 2007). The uterine horns are slightly longer and united on tips of the horns by a common peritoneal covering. There is only one intercornual ligament at the bifurcation of the horns. The body of the uterus is almost short in distance, which is about 3 cm in length. The broad ligaments are muscular and extensive. They arise cranially from the area ventral to the tuber coxae and the lateral wall of the pelvic cavity, and end along the mesometrial border of the uterine horns. In point of the lateral surface of the broad ligament, round ligament of the uterus is origin. The broad ligaments contain large amounts of smooth muscle fibers that raised the uterus towards the rectum or ventrally decrease in the more relaxed state. Cervix is a sphincter structure, which rises into the caudal vagina. The cervix is a fibrous organ, its wall is composed of external longitudinal muscle layer, a vascular layer and an internal circular muscular layer, which contains connective tissue characterized by toughness, high density and a pale mucus membrane (Nickel *et al.,* 1979). The cervix lumen is characterized by its constriction and thickness of its wall. The cervical canal projects are varying among farm mammals. In ruminants, the cervix has transverse-like interlocking prominences known as annular ring or plicae circulares. These will grow up to dissimilar degrees in different species. Usually, in