



UNIVERSITI PUTRA MALAYSIA

***MORPHOLOGY AND MUCOSAL IMMUNITY OF THE OVIDUCT AND
UTERUS DURING FOLLICULAR AND LUTEAL PHASES IN EWES***

INTAN SHAMEHA BINTI ABDUL RAZAK

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By

INTAN SHAMEHA BINTI ABDUL RAZAK

**Thesis submitted to the School of Graduate Studies, Universiti Putra
Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of
Philosophy**

October 2009

*I wish to dedicate this thesis to my inspired late Father,
Abdul Razak bin Abdul Motalib
Who always wanted me to, and now your wishes come true.
And to my precious children,
Aidiel Ikmal, Ariff Iskandar, Annuralisa Izmira &
Azzalea Irdina
May this masterpiece be your inspiration for your future
endeavors...*

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
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Chairman: Md Zuki Abu Bakar @ Zakaria, PhD

Faculty: Veterinary Medicine

Reproductive diseases result in major production losses in the sheep industry. Although extensive studies on mucosal surfaces of the female reproductive tract had been conducted, very little information particularly the mechanism is known. Furthermore, defining the mucosal immunity is complicated by the critical interface between the endocrine and immune systems. Thus, an approach to gain sight into the pathogenesis of the reproductive diseases by investigating the local uterine cellular immune response under the precise hormonal influences throughout the estrous cycle was undertaken.

Fourteen ewes were synchronized into estrus and the plasma samples were collected every alternate day for hormonal profiles using radioimmunoassay (RIA) techniques. The ewes were divided into two groups: follicular and luteal phases groups (n=7). All the ewes were observed for three consecutive cycles before they were slaughtered at the peak of the follicular and luteal phases. The ewes started to exhibit signs of estrus between 24 to 36 hours following the intravaginal sponges' removal. The average estradiol level during the peak of the follicular phase was 5.44 pg/ml for the first cycle, 4.85 pg/ml for the second and 4.25 pg/ml for the third cycle. The ewes started the luteal phase which peaked at day 9 with the average progesterone value of 4.21 ng/ml and 4.65 ng/ml respectively. The estrus period occurred for 30.6 ± 0.65 hours, luteal phase period ranged between 12 to 14 days, making the complete cycle of 15 days. Average vaginal mucous resistances in ewes were recorded for three consecutive cycles using Draminski® estrous detector. The daily plasma concentrations of estradiol and progesterone were significantly correlated ($p < 0.01$) with the estrus detector reading at ($r = -0.924$) and ($r = 0.705$), respectively.

At slaughter, samples from the anterior, middle and posterior horns and the oviducts were taken and processed accordingly for light and electron microscopy. Under light microscopy, the number of lymphocytes in

different parts of the uterus was significantly ($p < 0.05$) higher during the follicular phase compared to the luteal phase. However, 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 horn. Similarly, the quantity of plasma cell revealed that the number was significantly ($p < 0.05$) higher in the follicular phase compared to the luteal phase for the different parts of the reproductive tract. However, in the luteal phase group, the number of plasma cells was not significantly different between the posterior and middle horn and between the anterior horn and oviduct.

Ampullae were taken and processed accordingly for scanning (SEM) and transmission electron microscopy (TEM). During the follicular phase, the population of secretory cells was less than the luteal phase, while the number of ciliated cells was higher than the luteal phase. The secretory cells were rounded, turgid with intact microvilli in the follicular phase, but in the luteal phase the surfaces were broken and some secretions were oozing out.

The TEM examination revealed that during the follicular phase, the secretory cells had blunt processes at the apex with intact microvilli, but during the luteal phase, the cytoplasmic protrusion on the secretory cells

exhibited an increase in volume. Numerous secretory granules with different sizes and electron density were found in both phases. During the follicular phase, the secretory cells were at the preparatory stage while they were actively secreting during luteal phase. The present study revealed marked cyclic changes and differences of the secretory cells during these two phases of the estrous cycle. Results of current study also suggested that the secretory granules were released by exocytosis and the apocrine would be the mode of secretion at estrus while the merocrine is the mode of secretion at luteal phase.

In vitro experiments were conducted on the adhesion and colonization of *E.coli* to the uterus explants of both the follicular and luteal phases ewes. Scanning electron microscopy showed there were significant ($p<0.05$) differences between the groups at all different post-inoculation times except for the negative control group and between 180 and 360 minutes post-inoculation for both groups. Hence, the adhesion of *E. coli* to uterus during follicular phase was significantly ($p<0.05$) lower than the luteal phase. It seems that the intensity of adhesions increases with time.

This present study contributes to the new knowledge on the endocrinology of Malin crossbred ewes where complete estradiol and progesterone

hormonal profiles were obtained for the first time. In addition, the vaginal mucous resistance data recorded using the Draminski® estrous detector might be useful as the guideline in determining the exact timing of the estrus phase during the estrous cycle.

Present results demonstrated that the mucosal immunity is lower during peak luteal phase than follicular phase in the ewes since the number of lymphocytes and plasma cells were higher during peak follicular phase. The morphological evaluations confirmed that during follicular phase, the uterus and the oviduct are at the preparatory stage for the fertilization process while during luteal phase, the cellular immune response is modified to minimize rejection in the case if the ewe conceived. The *in vitro* challenged of the uterine explants confirmed the severity of the bacterial colonization during luteal phase than the follicular phase.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**MORFOLOGI DAN KEIMUNAN MUKOSA OVIDUKTUS DAN
UTERUS SEMASA FASA FOLIKEL DAN LUTEUM PADA BIRI-BIRI**

Oleh

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Penyakit berkaitan reproduksi telah mengakibatkan kerugian besar kepada industri ternakan. Walaupun kajian terhadap permukaan mukosa tragus reproduktif betina telah dijalankan secara meluas, masih sedikit maklumat berkaitan mekanismenya diperolehi. Tambahan lagi, penakrifan keimunan mukosa semakin rumit oleh hubungkait kritikal antara sistem endokrin dan imun. Oleh itu, pendekatan terhadap patogenesis penyakit reproduksi ini melalui pengkajian tindakbalas keimunan sel pada rahim di bawah pengaruh hormon yang tepat di sepanjang kitaran estrus telah dijalankan.

Empat belas bebiri kacukan Malin telah diselaraskan masa estrusnya supaya berlaku secara serentak, dan sampel plasma telah diambil selang sehari

untuk mendapatkan profil hormon menggunakan teknik radioimmunoassai (RIA). Bebiri ini dibahagikan kepada dua kumpulan: kumpulan fasa folikel dan luteum (n=7). Kesemua bebiri ini diperhatikan untuk tiga kitaran estrus berturut-turut sebelum mereka disembelih samada pada waktu puncak fasa folikel atau luteum. Bebiri ini mula menunjukkan ciri-ciri biang di antara 24 hingga 36 jam selepas span intravagina dicabut. Purata paras estradiol semasa waktu puncak fasa folikel ialah 5.44 pg/ml untuk kitaran pertama, 4.85 pg/ml untuk kitaran kedua dan 4.25 pg/ml bagi kitaran ketiga. Bebiri ini mula berada pada fasa luteum yang memuncak pada hari ke-9 dengan purata paras progesteron 4.21 ng/ml, 4.65ng/ml dan 4.2ng/ml untuk kitaran pertama, kedua dan ketiga. Tempoh masa estrus berlaku selama 30.6 ± 0.65 jam, dan jangkamasa fasa luteum ialah antara 12 hingga 14 hari, menjadikan satu kitaran estrus lengkap ialah selama 15 hari. Purata bacaan rintangan mukus pada vagina telah direkodkan bagi tiga kitaran berturut-turut menggunakan pengesan estrus Draminski®. Kandungan estradiol dan progesteron dalam plasma harian di dapati berkorelasi secara signifikan ($r=-0.924$) dan ($r=0.705$) pada ($P=0.01$) dengan bacaan pengesan estrus Draminski®.

Selepas disembelih, sampel dari bahagian anterior, tengah dan posterior tanduk rahim serta oviduktus diambil dan diproses sewajarnya (mengikut

prosedur) untuk mikroskop cahaya dan elektron. Di bawah mikroskop cahaya, bilangan limfosit pada bahagian uterus berbeza semasa fasa folikel adalah lebih tinggi secara signifikan ($p < 0.05$) berbanding semasa fasa luteum. Walaubagaimanapun, di dalam kumpulan fasa folikel, bilangan limfosit didapati tidak berbeza secara signifikan ($p > 0.05$) di antara tanduk tengah dan anterior, dan di dalam kumpulan luteal, bilangan limfosit didapati tidak berbeza secara signifikan di antara tanduk posterior dan tengah. Begitu juga dengan kuantiti sel plasma, di mana didapati bilangannya adalah tinggi secara signifikan ($p < 0.05$) semasa fasa folikel berbanding dengan fasa luteum pada bahagian-bahagian yang berbeza di dalam saluran pembiakan. Walaubagaimanapun, di dalam kumpulan fasa luteum, bilangan sel plasma tidak berbeza dengan signifikan di antara tanduk posterior dan tanduk tengah serta antara tanduk anterior dan oviduktus.

Ampula telah diambil dan diproses sewajarnya untuk mikroskop elektron imbasan dan transmisi. Semasa fasa folikel, populasi sel rembesan adalah berkurangan berbanding dengan semasa fasa luteum, sementara bilangan sel silia didapati lebih banyak semasa fasa luteum. Semasa fasa folikel, sel rembesan didapati berbentuk bulat, membengkak dan mempunyai mikrovili pada permukaannya, tetapi pada fasa luteum, permukaan sel ini

telah pecah dan terdapat rembesan yang mula keluar. Pemeriksaan mikroskop elektron transmisi (TEM) mendapati semasa fasa folikel, sel rembesan mempunyai lanjutan berbentuk tumpul dan bermikrovili pada bahagian apeksnya, manakala, semasa fasa luteum, pertambahan unjuran sitoplasma sel rembesan terjadi. Banyak granul rembesan yang berbilang saiz dan densiti elektron pada kedua-dua fasa. Semasa fasa folikel, sel rembesan di dalam keadaan bersedia dan menjadi aktif semasa fasa luteum. Kajian ini mendapati terdapat perubahan dan perbezaan berkisar yang ketara dan pada sel rembesan semasa kedua fasa kitaran estrus. Hasil penemuan kajian ini juga mencadangkan granul rembesan dibebaskan melalui proses eksositosis; dan modnya ialah secara apokrin semasa tempoh biang manakala merokrin adalah mod rembesan semasa fasa luteum.

Eksperimen *in vitro* pelekatan dan pengkolonian *E.coli* terhadap eksplan mukosa uterus fasa folikel dan luteum telah dijalankan. Pemeriksaan mikroskop elektron imbasan menunjukkan terdapat perbezaan yang signifikan ($p < 0.05$) di antara semua kumpulan dan tempoh pascaeraman kecuali pada kawalan negatif, dan antara 180 dan 360 minit pascaeraman bagi kedua kumpulan. Pelekatan *E. coli* juga didapati lebih rendah secara signifikan ($p < 0.05$) semasa fasa folikel berbanding fasa luteum. Ini menunjukkan keamatan perlekatan bertambah dengan masa.

Hasil penemuan ini telah menyumbang kepada maklumat baru dalam bidang endokrinologi bebiri baka kacukan Malin di mana profil hormon estradiol dan progesteron yang lengkap telah dapat direkodkan buat pertama kalinya. Maklumat ini dilengkapi dengan data bacaan rintangan mukus vagina yang direkod menggunakan alat pengesan estrus Draminski® di mana ianya dapat dimanfaatkan sebagai panduan semasa menentukan fasa estrus semasa kitaran estrus.

Hasil kajian ini juga telah membuktikan tahap keimunan mukosa semasa waktu puncak fasa luteum adalah lebih rendah berbanding fasa folikel memandangkan bilangan limfosit dan sel plasma yang lebih tinggi semasa waktu puncak fasa folikel. Penilaian morfologi telah dapat mengesahkan semasa fasa folikel, rahim dan oviduktus berada dalam keadaan bersedia untuk proses persenyawaan tetapi semasa fasa luteum, tindakbalas keimunan sel diubahsuai bagi meminimumkan penolakan sekiranya kehamilan berlaku. Cabaran *in vitro* telah membuktikan pengkolonian bakteria yang lebih teruk semasa fasa luteum berbanding fasa folikel.

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APPROVAL SHEET

I certify that an Examination Committee has met on **14th October 2009** to conduct the final examination of **Intan Shameha Abdul Razak** on her Doctor of Philosophy thesis entitled “**Morphology and Mucosal Immunity of the Oviduct and Uterus during Follicular and Luteal Phases in Ewes**” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Doctor of Philosophy.

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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.

INTAN SHAMEHA ABDUL RAZAK

Date: 25 November 2009

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