



UNIVERSITI PUTRA MALAYSIA

***MICROSCOPIC CHANGES IN OVARIES IN RELATION TO INFLAMMATORY
MEDIATORS OF BLOOD PLASMA IN NORMAL AND SUPEROVULATED RATS***

BANULATA GOPALSAMY

FPV 2013 15



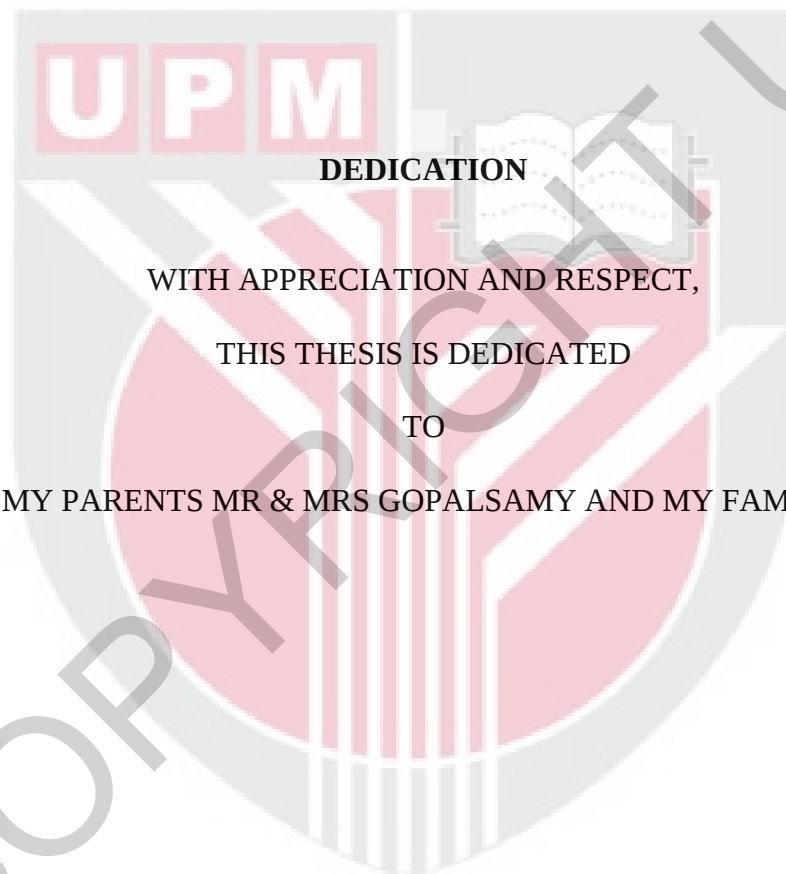
**MICROSCOPIC CHANGES IN OVARIES IN RELATION TO
INFLAMMATORY MEDIATORS OF BLOOD PLASMA IN
NORMAL AND SUPEROVULATED RATS**

By

BANULATA GOPALSAMY

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

August 2013



DEDICATION

WITH APPRECIATION AND RESPECT,

THIS THESIS IS DEDICATED

TO

MY PARENTS MR & MRS GOPALSAMY AND MY FAMILY

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

**MICROSCOPIC CHANGES IN OVARIES IN RELATION TO
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Chairman: Associate Professor Shanthi Ganabadi, PhD

Faculty: Veterinary Medicine

Superovulation is an important treatment widely used in transgenic animals and in breeding industry. However, many problems on ovulation, fertilization, embryo recovery and viability rates were encountered when superovulation treatment was carried out. The study was carried out to evaluate the difference in the follicular development and inflammatory mediators of rats in the different phases of the oestrous cycle and to compare the changes that occur in superovulated rats and control rats. Six rats (n=6) from each phase of the oestrus cycle (dioestrus, proestrus, oestrus and metoestrus) were euthanised to observe the inflammatory changes that takes place throughout the cycle. In another experiment, rats were administered exogenous gonadotropin to superovulate and the rats were sacrificed 8 hour post hCG (n=6), 18 hours post hCG (n=6) and control rats (n=6) were euthanised at the oestrus phase of the cycle. Serial histological sections of ovaries were made to

observe the follicle development that occurs within the ovaries and Enzyme linked immunosorbent assay (ELISA) was carried out to analyse the inflammatory mediators in the blood plasma. Data were subjected to statistical analysis using SPSS software version 16.0.

In the experiment to study the normal oestrous cycle, the ovarian weight was highest during the proestrus stage as many large follicles were present at this stage. The number of healthy and unhealthy follicles were relatively unaltered throughout the cycle but the diameters of large follicles increased significantly ($P<0.05$) from dioestrus to proestrus. Plasma Interleukin 8 (IL-8) and Nerve Growth Factor (NGF) was significantly ($P<0.05$) increase during proestrus but IL-8 level reduced in the next phases whereas NGF was maintained at a high level until the end of the cycle. Prostaglandin E₂ (PGE₂) concentration was however consistent throughout the cycle.

In another experiment to study the inflammatory process in superovulated rats, the highest ovarian weight was recorded in 8 hours post hCG group as most of the large follicles were present in those ovaries. The number of healthy large follicles were significantly increased ($P<0.05$) in superovulated rats (both 8 hours post hCG and 18 hours post hCG groups) compared to control rats but the diameter of the follicles were not significantly different ($P>0.05$) in superovulated and control rats. The level of IL 8 was significantly increased ($P<0.05$) in 8 hours post hCG rats but PGE₂ and NGF levels were not significantly different ($P>0.05$) than control rats.

The outcome of this study showed that IL-8 had significantly ($P<0.05$) higher levels of production during proestrus and in 8 hours post hCG rats compared to control rats.

The other mediator remains the same throughout the cycle and is not different in superovulated rats from control rats. Since only IL-8 was increases in superovulated rats it does not provide enough evidence to conclude if the inflammation level were increased when rats were superovulated. Therefore, further research on other inflammatory markers should be carried out to study the inflammation process that occurs as a result of the superovulatory treatment.



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

**PERUBAHAN MIKROSKOPIK OVARI DAN PERKAITANNYA DENGAN
PENGANTARA KERADANGAN DALAM PLASMA DARAH DALAM
TIKUS NORMAL DAN DISUPEROVULASI**

Oleh

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Pengerusi: Professor Madya Shanthi Ganabadi, PhD

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Superovulasi merupakan satu rawatan yang digunakan secara luas untuk haiwan transgenik dan dalam industri pembiakan. Walaubagaimanapun, banyak masalah dalam pengovulan, persenyawaan, dapat semula embrio dan kadar terus hidup telah dihadapi semasa rawatan superovulasi dilakukan. Kajian ini telah dijalankan bagi menilai perbezaan dalam perkembangan folikel dan perantara keradangan pada tikus dalam setiap fasa dalam kitar ovulasi dan membandingkan perubahan yang berlaku pada tikus yang disuperovulasi dan tikus kawalan.

Enam tikus ($n=6$) daripada setiap fasa dalam kitar estrus (dioestrus, proestrus, oestrus dan metoestrus) telah dieuthanasia untuk memerhati perubahan keradangan yang berlaku sepanjang kitaran estrus. Dalam eksperimen yang lain, tikus telah diberi gonadotropin eksogenus untuk superovulasi dan tikus tersebut telah dieuthanasia 8

jam selepas hCG (n=6), 18 jam selepas hCG (n=6) dan tikus kawalan (n=6) dibunuh pada fasa estrus dalam kitaran estrus. Potongan histologi bersiri pada ovari telah dilakukan untuk memerhati pembentukan folikel yang berlaku dalam ovari dan 'Enzyme linked immunosorbent assay (ELISA) telah dijalankan bagi menganalisis pengantara keradangan dalam plasma darah. Data telah di analisis secara statistik dengan menggunakan perisian SPSS versi 16.0.

Dalam eksperimen untuk mempelajari proses keradangan dalam tikus berkitaran estrus normal, berat ovari adalah tertinggi pada fasa proestrus kerana banyak folikel besar hadir pada fasa tersebut. Bilangan folikel sihat dan tidak sihat tidak menunjukkan perubahan sepanjang kitaran estrus tetapi diameter folikel besar meningkat dengan perubahan ketara ($P < 0.05$) daripada dioestrus kepada proestrus. Plasma Intereleukin 8 (IL-8) dan Faktor Pertumbuhan saraf (NGF) telah meningkat secara ketara ($P < 0.05$) semasa proestrus tetapi paras IL-8 berkurang pada fasa seterusnya sementara NGF dan kekal pada tahap yang tinggi sehingga ke akhir kitar ovulasi. Kepekatan Prostaglandin E_2 (PGE_2) pula konsisten sepanjang kitar oestrus.

Dalam eksperimen untuk mempelajari proses keradangan pada tikus yang disuperovulasi, berat ovari yang tertinggi telah direkodkan dalam kumpulan 8 jam selepas hCG kerana kebanyakan folikel besar hadir pada ovari-ovari tersebut. Bilangan folikel besar yang sihat telah meningkat dengan perubahan ketara ($P < 0.05$) dalam tikus yang telah disuperovulasi (kedua kumpulan 8 jam selepas hCG dan 18 jam selepas hCG) dibandingkan dengan kumpulan kawalan tetapi diameter folikel-folikel tersebut tidak mempunyai perubahan ketara ($P > 0.05$) dalam tikus yang disuperovulasi dan tikus kawalan. Paras IL-8 telah meningkat secara ketara ($P < 0.05$)

dalam tikus 8 jam selepas hCG tetapi paras PGE_2 dan NGF tidak berubah secara ketara ($P>0.05$) daripada tikus kawalan.

Hasil kajian ini menunjukkan bahawa IL-8 mempunyai penghasilan yang ketara ($P<0.05$) pada proestrus dan dalam tikus 8 jam selepas hCG berbanding tikus kawalan. Perantara-perantara yang lain kekal sepanjang kitar ovulasi dan tidak berbeza dalam tikus yang telah disuperovulasi berbanding tikus kawalan. Oleh kerana hanya IL-8 telah meningkat dalam tikus yang telah disuperovulasi, ia tidak memberi bukti yang mencukupi bagi menyimpulkan samaada paras keradangan telah meningkat bila tikus telah disuperovulasi. Oleh itu, kajian selanjutnya pada penunjuk keradangan yang lain perlu dijalankan bagi mempelajari proses keradangan yang berlaku akibat rawatan superovulasi.

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I certify that a Thesis Examination Committee has met on 26 August 2013 to conduct the final examination of Banulata a/p Gopalsamy on her thesis entitled "Microscopic Changes in Ovaries in Relation to Inflammatory Mediators of Blood Plasma in Normal and Superovulated Rats" 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 Master of Science.

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Declaration by graduate student

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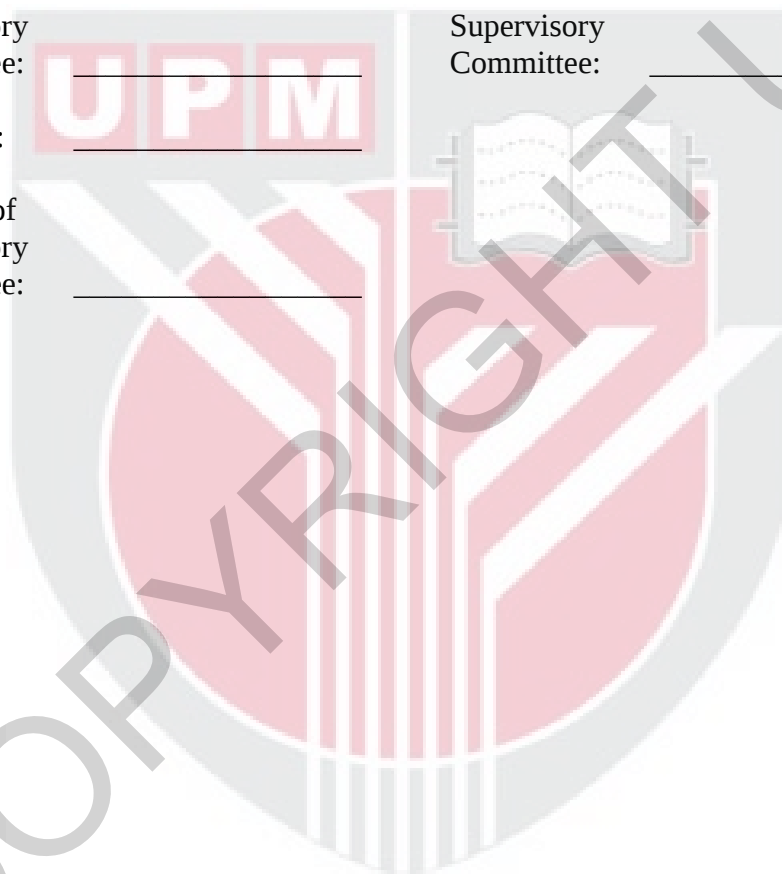


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