UNIVERSITI PUTRA MALAYSIA

MORPHOLOGICAL AND MOLECULAR CHANGES IN EMBRYOMATERNAL INTERACTIONS IN A RAT MODEL AT OPTIMAL AND ELEVATED ENVIRONMENTAL TEMPERATURES

HUDA YAHIA HAMID

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By

HUDA YAHIA HAMID

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

March 2012

Dedication

To

My late mother, Alawia Khogali

and

My late father, Yahia Hamid,

who always live in my heart.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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March 2012

Chairman: Professor Md Zuki Bin Abu Bakar, PhD

Faculty: Veterinary Medicine

Early embryonic development, successful implantation, optimal fetal and placental development, and maintenance of a pregnancy are critically dependent on intact embryo-maternal interactions. Today, assisted reproductive technology (ART) is widely used to overcome some causes of infertility; however, the success rate remains low as a result of failures in communication between the transferred embryo and the endometrium. Failure in embryo-maternal communication is responsible for reproductive wastage, resulting in enormous economic loss. The mechanism that regulates embryo-maternal interaction has remained elusive and is not well understood. A better understanding of early embryo-maternal communication and the identification of the external factors that could interfere with embryo-maternal crosstalk will improve reproductive success in both humans and animals. The present study focuses on the maternal signals, which are responsible for two-thirds of pregnancy losses. Thus, the main objectives of this study were to define the

morphological and molecular changes that occur in the maternal tissue in response to the presence of the embryo and to determine the effects of elevated ambient temperatures on the morphological and molecular responses of maternal tissue to embryonic signals.

In the present study, implantation time was determined using blue dye injection (1% Chicago Sky Blue 6B). A radioimmunoassay (RIA) was used to estimate the plasma estradiol and progesterone levels during peri-implantation. Morphological changes in the rat oviduct and uterus during early pregnancy were examined under light and electron microscopes. Localization of transforming growth factor β1 (TGFβ1), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and their receptors TGFβR1, VEGFR2 (Flk-1), FGFR1 (Flg) in the oviduct and uterus was determined by immunohistochemistry. TGF β1, VEGF, and bFGF gene expression levels in the rat uterus were examined by real-time quantitative RT-PCR.

The effects of elevated ambient temperatures on implantation time, number of implantation sites, plasma estradiol and progesterone concentrations, morphological changes, and the expression levels of TGFβ1, bFGF, and VEGF in the uterus during peri-implantation were also examined. Further, the effects of exposure to elevated temperatures at different stages of pregnancy on gestation length, litter size, neonatal deaths, sex ratio, birth weight, and offspring growth were also determined.

In the present study, implantation was initiated on day 5 and established by day 7 of pregnancy in the rats kept under optimal temperatures (23±1°C), while the rats exposed to elevated temperatures (33±2°C) exhibited delayed implantation and a

reduced number of implantation sites. Moreover, the exposure to elevated temperatures resulted in changes in the patterns of plasma progesterone and estradiol levels during peri-implantation.

Exposure to elevated ambient temperatures during early pregnancy resulted in prolonged gestation, reduced litter size, increased neonatal death, and a sex ratio biased toward males. Moreover, elevated temperatures adversely affected the birth weight and growth of offspring. Exposure to elevated ambient temperatures during the pre- and peri-implantation periods had stronger adverse effects on reproductive outcomes and offspring growth than post-implantation exposure.

Rats maintained under optimal conditions exhibited many morphological changes in the oviduct and uterus during early pregnancy. In the oviduct, the secretory cells were predominant during the first 4 days of pregnancy. On days 5 through 8 of pregnancy, however, the ciliated cells were predominant. In the uterus, extensive infiltration of leukocytes was observed in the endometrium during pre-implantation, whereas during implantation, the number of leukocytes decreased. Additionally, considerable changes occurred in the apical plasma membrane of the uterine epithelial cells. Such changes included changes in the length and density of the microvilli, flattening of the uterine epithelial cells, loss of the small secretory droplets seen near the epithelial cells, and the appearance of cytoplasmic protrusions (pinopods). The uterine luminal epithelial cells gradually lost their microvilli and became very flat. Pinopods were observed at the apical surface as early as day 2 of pregnancy. The incidence of pinopods increased gradually up to day 5 of pregnancy, when the pinopods became abundant and appeared in doughnut shaped.

In rats exposed to elevated ambient temperatures, there was a reduced incidence of pinopods on day 5 compared to the rats maintained under optimal temperatures. On day 6 of pregnancy, the rats exposed to elevated temperatures did not show flattening of the apical membrane.

Immunohistochemistry showed that TGFβ1, TGFβR1, VEGF, VEGFR2 (Flk-1), bFGF, and FGFR1 (Flg) were expressed in both the oviduct and uterus throughout the first 8 days of pregnancy; however, the distribution and intensity of immunostaining for each protein varied depending on the day of pregnancy. In the oviduct, high immunoreactivities of these growth factors and their receptors were observed while the embryo was present in the oviduct. In the uterus, TGFβ1, TGFβR1, VEGF, Flk-1, bFGF, and Flg were detected in the endometrium, i.e., in the epithelial and stromal cells.

Both immunohistochemical analysis and real time RT-qPCR revealed that TGFβ1, VEGF, and bFGF were found in the uterus at relatively lower levels during pre-implantation but that their expression levels increased dramatically during peri-implantation. Expression of TGFβ1 increased significantly on day 5 (8.72 ± 1.20 - fold increase versus day 0, $P < 0.001$) and day 5.5 (13.22 ± 1.80 - fold increase versus day 0, $P < 0.001$). A dramatic increase in TGFβ1 expression was detected on day 6 of pregnancy (46.38 ± 8.57 - fold increase versus day 0, $P < 0.0001$). Elevated expression of bFGF was observed immediately before implantation on day 5 (13.04 ± 1.79 - fold increase versus day 0, $P < 0.0001$), whereas VEGF showed elevated expression on day 5.5 (52.40 ± 6.50 - fold increase versus day 0, $P < 0.0001$).

The TGFβ1 mRNA levels in the uteri of rats exposed to elevated temperatures on days 5, 5.5, and 6 of pregnancy were significantly ($P < 0.01$) lower than the expression levels in the uteri of rats kept under optimal temperatures at the same stages of pregnancy. VEGF and bFGF expression in the uteri of rats exposed to elevated temperatures on days 5 and 5.5 were significantly ($P < 0.01$) lower than the expression levels in uteri of rats maintained under optimal temperatures at the same stages of pregnancy, while high expression levels were observed on day 6 of pregnancy.

In conclusion, implantation in rats was initiated on day 5 and established by day 7 of pregnancy. Elevated ambient temperatures can delay implantation and reduce the number of implantation sites. Elevated temperatures disturb the plasma estradiol and progesterone levels during peri-implantation. Elevated temperatures can also lead to prolonged gestation time, decreased litter size, neonatal death, and a sex ratio biased toward males. Additionally, exposure to elevated temperatures during early pregnancy has adverse effects on offspring growth. The exposure of pregnant rats to elevated temperatures during the pre- and peri-implantation stages has stronger adverse effects on reproductive performance than post-implantation exposure. Morphological alterations in the apical plasma membrane indicate endometrial receptivity. The expression patterns of TGFβ1, VEGF and bFGF and their receptors in the oviduct indicate that these growth factors contribute to early embryonic development. High expression levels of TGFβ1, VEGF, and bFGF during peri-implantation suggest that these growth factors play roles in implantation in rats. Exposure of pregnant rats to elevated temperatures affects the responses of uterine tissue (i.e., both morphological and molecular responses) to embryonic signals,

leading to delay or failure of implantation. Overall, this study reveals that morphological alterations and growth factors (TGFβ1, VEGF, and bFGF) are involved in embryo-maternal interactions and that elevated environmental temperatures interfere with the embryo-maternal crosstalk during peri-implantation, leading to implantation delay/failure. These findings can help in the diagnosis and treatment of non-receptive endometria and abnormal embryo-maternal interactions.

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

PERUBAHAN MORPHOLOGI DAN MOLEKUL DALAM INTERAKSI EMBRIO-IBU DALAM MODEL TIKUS PADA SUHU PERSEKITARAN YANG OPTIMUM DAN YANG TINGGI

Oleh

HUDA YAHIA HAMID

Mac 2012

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Pembangunan awal embrio, kejayaan implantasi, pembangunan optimum janin dan plasenta, dan penyelenggaraan bagi kehamilan amat bergantung kepada interaksi embrio-ibu yang utuh. Kegagalan dalam komunikasi embrio-ibu bertanggungjawab bagi pembaziran pembiakan, mengakibatkan kerugian ekonomi yang besar. Hari ini, teknologi pembiakan bantuan (ART) digunakan secara meluas untuk mengatasi beberapa penyebab kemandulan; bagaimanapun, kadar kejayaan masih rendah akibat kegagalan dalam komunikasi antara embrio yang dipindahkan dan endometrium. Mekanisme yang mengawal interaksi embrio-ibu masih sukar difahami dan tidak difahami dengan baik. Pemahaman yang lebih baik dalam komunikasi awal embrio-ibu dan mengenal pasti faktor-faktor luaran yang boleh mengganggu interaksi embrio-ibu akan meningkatkan kejayaan pembiakan dalam kedua-dua manusia dan haiwan. Kajian ini memberi tumpuan kepada isyarat ibu, yang bertanggungjawab bagi dua pertiga daripada kerugian kehamilan. Oleh itu, objektif utama kajian ini

adalah untuk mentakrifkan perubahan morfologi dan molekul yang berlaku dalam tisu ibu sebagai tindak balas kepada kehadiran embrio dan juga untuk menentukan kesan suhu tinggi persekitaran ke atas tindakbalas morfologi dan molekul tisu ibu kepada isyarat embrio.

Dalam kajian ini, masa implantasi telah ditentukan dengan menggunakan suntikan pewarna biru (1% Chicago Blue Sky 6B). Radioimunocerakinan (RIA) telah digunakan untuk menganggarkan paras plasma estradiol dan progesteron semasa peri-implantasi. Perubahan morfologi dalam oviduct dan rahim tikus semasa awal kehamilan telah diperiksa di bawah mikroskop cahaya dan elektron. Penyetempatan faktor pertumbuhan pengubah β1 (TGFβ1), faktor pertumbuhan vaskular endotelium (VEGF) yang, faktor pertumbuhan fibroblast asas (bFGF), dan reseptor mereka TGFβR1, VEGFR2 (Flk-1), FGFR1 (Flg) dalam oviduct dan uterus telah ditentukan oleh Immunohistokimia. TGF β1, VEGF dan bFGF ungkapan tahap gen dalam rahim tikus telah diperiksa oleh masa sebenar kuantitatif RT-PCR.

Kesan suhu tinggi persekitaran ke atas masa implantasi, bilangan tapak implantasi, kepekatan estradiol dan progesteron plasma, perubahan morfologi, dan tahap ungkapan TGFβ1, bFGF, dan VEGF dalam rahim semasa peri-implantasi turut diperiksa. Di samping itu, kesan pendedahan kepada suhu tinggi di pelbagai peringkat kehamilan pada panjang kandungan, saiz kelahiran, kematian neonatal, nisbah jantina, berat lahir, dan pertumbuhan anak-anak juga ditentukan.

Dalam kajian ini, implantasi bermula pada hari ke 5 dan lengkap pada hari ke 7 kehamilan di dalam tikus yang disimpan di bawah suhu optimum (23 ± 1°C),

manakala tikus yang terdedah kepada suhu tinggi (33 ± 2°C) menunjukkan kelewatan implantasi dan bilangan yang berkurangan bagi tapak implantasi. Tambahan pula, pendedahan kepada suhu tinggi menyebabkan perubahan dalam corak progesteron plasma dan paras estradiol semasa peri-implantasi.

Pendedahan kepada suhu tinggi ambien pada awal kehamilan menyebabkan kehamilan yang panjang, mengurangkan saiz kelahiran, meningkatkan kematian neonatal dan nisbah jantina yang condong kepada lelaki. Selain itu, suhu tinggi menjejaskan berat kelahiran dan pertumbuhan anak-anak. Pendedahan kepada suhu tinggi ambien pada masa pra- dan peri-implantasi mempunyai kesan yang lebih teruk ke atas hasil-hasil pembiakan dan pertumbuhan anak daripada pendedahan selepas implantasi.

Tikus yang dikekalkan di bawah keadaan optimum menunjukkan banyak perubahan morfologi dalam oviduct dan uterus semasa awal kehamilan. Dalam oviduct, sel-sel pengeluaran adalah dominan dalam tempoh masa 4 hari pertama kehamilan. Pada hari ke 5 hingga 8 kehamilan, bagaimanapun, sel-sel bersilia adalah dominan. Dalam rahim, infiltrasi sel leukosit banyak diperhatikan dalam endometrium semasa pra-implantasi, manakala semasa implantasi, bilangan leukosit menurun. Selain itu, perubahan yang agak besar berlaku dalam membran plasma apeks sel-sel epitelium rahim. Perubahan tersebut termasuklah perubahan dalam panjang dan ketumpatan mikrovilus, kesamarataan sel-sel epitelium rahim, kehilangan pengeluar titisan-titisan kecil yang dilihat berhampiran sel-sel epitelium, dan keadaan unjuran cytoplasmic (pinopods). Sel-sel epitelium lumen rahim kehilangan mikrovilus secara beransur-ansur dan menjadi sangat rata. Pinopods diperhatikan di permukaan apikal seawal

hari ke 2 kehamilan. Kehadiran pinopods meningkat secara beransur-ansur sehingga hari ke 5 kehamilan, apabila pinopods menjadi banyak dan kelihatan dalam bentuk donat.

Dalam tikus yang terdedah kepada suhu ambien yang tinggi, pinopods berkurangan pada hari ke 5 berbanding dengan tikus yang disimpan di bawah suhu optimum. Pada hari ke 6 kehamilan, tikus-tikus yang terdedah kepada suhu tinggi tidak menunjukkan kesamarataan membran apikal.

Immunohistokimia menunjukkan TGFβ1, TGFβR1, VEGF, VEGFR2 (Flk-1), bFGF dan FGFR1 (Flg) terdapat dalam kedua-dua oviduct dan rahim sepanjang 8 hari pertama kehamilan, namun, pengedaran dan intensiti immunostaining bagi setiap protein berbeza bergantung kepada hari kehamilan. Dalam oviduct, immunoreactivities yang tinggi bagi faktor-faktor pertumbuhan dan reseptor diperhatikan semasa embrio hadir dalam oviduct itu. Dalam rahim, TGFβ1, TGFβR1, VEGF, Flk-1, bFGF dan Flg dikesan dalam endometrium, iaitu, dalam sel-sel epitelium dan stroma.

Kedua-dua analisis immunohistochemical dan masa nyata RT-qPCR mendedahkan bahawa TGFβ1, VEGF dan bFGF ditemui dalam rahim pada paras yang agak rendah semasa pra-implantasi tetapi tahap ungkapan mereka meningkat secara mendadak semasa peri-implantasi. Ekspresi TGFβ1 meningkat dengan ketara pada hari ke 5 (8.72 ± 1.2 kali ganda berbanding hari 0, $P < 0.001$) dan hari ke 5.5 (13.22 ± 1.8 - kali ganda berbanding hari 0, $P < 0.001$). Peningkatan dramatik dalam TGFβ1 ungkapan dikesan pada hari ke 6 kehamilan (46.38 ± .8.57 - kali ganda berbanding

hari 0, $P < 0.0001$). Ungkapan tinggi bFGF diperhatikan sebaik sebelum penanaman pada hari ke 5 (13.04 ± 1.79 - kali ganda berbanding hari 0, $P < 0.0001$), manakala VEGF menunjukkan ungkapan tinggi pada hari ke 5.5 (52.4 ± 6.5 kali ganda berbanding hari 0, $P < 0.0001$).

Tahap TGFβ1 mRNA dalam rahim tikus yang terdedah kepada suhu tinggi pada hari ke 5, 5.5 dan 6 kehamilan adalah lebih rendah ($P < 0.01$) daripada tahap ungkapan dalam rahim tikus yang disimpan di bawah suhu optimum di peringkat kehamilan yang sama. Ungkapan VEGF dan bFGF dalam rahim tikus yang terdedah kepada suhu tinggi pada hari ke 5 dan 5.5 adalah lebih rendah ($P < 0.01$) daripada tahap ungkapan dalam rahim tikus yang diselenggarakan di bawah suhu optimum di peringkat kehamilan yang sama, manakala tahap ungkapan tinggi diperhatikan pada hari ke 6 kehamilan.

Kesimpulannya, implantasi dalam tikus telah dimulakan pada hari ke 5 dan dilengkapkan pada hari ke 7 kehamilan. Suhu persekitaran yang tinggi boleh melambatkan implantasi dan mengurangkan bilangan tapak implantasi. Suhu tinggi mengganggu paras estradiol dan progesteron plasma semasa peri-implantasi. Suhu bertingkat juga boleh membawa kepada masa matang yang panjang, penurunan saiz kelahiran, kematian neonatal, dan nisbah jantina yang condong kepada lelaki. Selain itu, pendedahan kepada suhu tinggi semasa awal kehamilan mempunyai kesan buruk ke atas pertumbuhan anak-anak. Pendedahan tikus yang mengandung kepada suhu tinggi semasa peringkat pra-dan peri-implantasi mempunyai kesan buruk yang kuat terhadap prestasi pembiakan daripada pendedahan selepas implantasi. Perubahan morfologi dalam membran apikal plasma menunjukkan kesediaan menerima oleh

endometrium. Corak ungkapan TGFβ1, VEGF, dan bFGF dan reseptor mereka dalam oviduct menunjukkan bahawa faktor-faktor pertumbuhan menyumbang kepada pembangunan embrio awal. Tahap ungkapan yang tinggi TGFβ1, VEGF, dan bFGF semasa peri-implantasi mencadangkan bahawa faktor-faktor pertumbuhan memainkan peranan dalam implantasi dalam tikus. Pendedahan tikus yang mengandung kepada suhu tinggi memberi kesan kepada tindak balas tisu rahim (iaitu, jawapan kedua-dua morfologi dan molekul) isyarat embrio, yang membawa kepada kelewatan atau kegagalan penanaman. Oleh itu, perubahan morfologi dan faktor pertumbuhan (TGFβ1, VEGF, dan bFGF) terbabit dalam komunikasi awal embrio-ibu. Penemuan ini boleh membantu dalam diagnosis dan rawatan kes-kes seperti non-receptive endometria dan ketidaknormalan interaksi embrio-ibu.

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I certify that a Thesis Examination Committee has met on 6 March 2012 to conduct the final examination of Huda Yahia Hamid Fadlalla on her thesis entitled "Morphological and Molecular Changes in Embryo-Maternal Interactions in a Rat Model at Optimal and Elevated Environmental Temperatures" in accordance with the Universities and University College 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 Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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Date:

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.

HUDA YAHIA HAMID

Date: 6 March 2012

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LIST OF ABBREVIATIONS

µg	microgram
µM	micromolar
µl	microlitre
µm	micron
bFGF	basic fibroblast growth factor
C	celcius
cm^3	cubic centimeter
C_T	cycle threshold
DNA	deoxyribonucleic acid
FGFR1/Flg	fibroblast growth factor receptor 1
Flk1	fetal liver kinase-1
g	gram
h	hour
H&E	haematoxylin and eosin
IS	implantation sites
M	molar
min	minutes
mg	milligram
ml	milliliter
mm^3	cubic millimeter
mRNA	messenger ribonucleic acid
ng	nanogram
nm	nanomolar

OD	optical density
PBS	phosphate buffer saline
PCR	polymerase chain reaction
pg	picogram
RER	rough endoplasmic reticulum
RNA	ribonucleic acid
RT-qPCR	real time quantitative polymerase chain reaction
SEM	scanning electron microscope
TEM	transmission electron microscope
TGFβ	transforming growth factor beta
TGFβR1	transforming growth factor beta receptor 1
VEGF	vascular endothelial growth factor

CHAPTER ONE

INTRODUCTION

Early embryonic development, successful implantation, optimal fetal and placental development, and maintenance of a pregnancy are critically dependent on intact embryo-maternal interactions. The embryo-maternal dialogue starts immediately after fertilization and is exerted through both local and systemic signaling (Barnea, 2001). The presence of a biochemical network of embryo-maternal communication implies that various secreted molecules constitute a signal-response mechanism. This mechanism is crucial for the process of embryo implantation in mammals (Cocchiara *et al.,* 1996).

Communication between the embryo and mother and their reciprocal effects on each other are not fully understood, and thus constitute an exciting and unsolved problem in reproductive medicine (Simon *et al.*, 2001). Problems in the signaling mechanisms play a crucial role in early embryonic mortality since a high rate of embryonic mortality occurs during early pregnancy (Goff, 2002).

In farm animals, one of the most important problems in reproduction and breeding is early embryonic death, which has a substantial economic impact (Wolf *et al*., 2003). In cattle, for instance, 40% of total embryonic losses occur during early pregnancy; i.e. from day 8 to 17 of pregnancy (Humbolt, 2001; Thatcher *et al*., 2001). Early embryonic loss is considered a principal obstacle to the reproductive success in all

domestic animals (Johnson *et al*., 2003), as 25% of successful fertilizations do not result in viable fetuses (Roberts *et al*., 1984).

In humans, early loss of pregnancy is a significant clinical problem. Although the human population is growing rapidly and is projected to reach nine billion by 2050, 15% of married couples worldwide have no children and 25% of them experience unexplained infertility (Cahill and Wardle, 2002; Wang and Dey, 2006). Currently, assisted reproductive technology (ART), such as *in vitro* fertilization and embryo transfer (IVF-ET), is widely used to overcome some causes of infertility, such as tubal scaring and male factors. Nevertheless, the rate of successful pregnancies is only 30% (Wang and Dey, 2006), and subsequent delivery rates are only 10-30% (Adamson *et al*., 2006). The low success rate of ART is due to implantation failure, which occurs when the embryo is transferred to a non-receptive uterus; such failures occur despite excellent blastocyst quality, indicating the pivotal role of the endometrium in implantation.

Non-receptive endometria are responsible for about two-thirds of implantation failures, while the embryos are responsible for only one-third of failures (Simon *et al*., 1998; Ledee-Bataille *et al.,* 2002). Uterine receptivity may be affected by internal as well as external factors.

The maternal environments during pre- and peri-implantation are thought to play a major role in the success of pregnancy, offspring viability and offspring health; most inherited diseases manifest at these early stages of pregnancy.

The definite mechanism that regulates the embryo-maternal dialogue until now is elusive and is not well understood. A better understanding of early embryo-maternal communication, and the external factors that could interfere with it, will help to increase the pregnancy rate by improving embryo transfer process, avoiding embryonic losses, and assisting in the diagnosis and treatment of non-receptive endometrium. Knowledge of the cells and molecules that are involved in embryo-maternal crosstalk, particularly during peri-implantation period, could help to solve two contrasting global problems by alleviating female infertility as well as by helping to develop novel contraceptives.

Identifying reliable markers of uterine receptivity will lessen the need to transfer multiple embryos to increase the success rate in *in vitro* fertilization and embryo transfer programs and therefore avoid the complications of multiple pregnancies. Not only is early maternal-embryonic interaction important for successful pregnancy, but it could also exert a significant influence on adult life in the future (e.g. body weight, diseases and likely behavior).

The current study focused on the maternal signals, which is responsible for two-thirds of the pregnancy failures. Particular attention was paid to the morphology of the oviduct and uterus during the presence of the embryo in these organs, and to the expression of transforming growth factor (TGFβ1), vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (bFGF) in the maternal tissue during pre and peri-implantation period.

Perrier d'Hauterive *et al*. (2005) reported that TGFβ1 enhances the production of leukaemia inhibitory factor (a pro-implantation cytokine) from the endometrium. Moreover, over expression of Lefty (a TGFβ1 antagonist) in the mouse uterus reduces the number of implantation sites (Tang *et al*., 2005). VEGF and basic FGF are implicated in angiogenesis, i.e., an essential process in the reproductive cycle (Friesel *et al*., 1989; Bates, 2010). Thus, these growth factors seem to have pivotal roles during pregnancy. It would be significant to determine the expression patterns of these growth factors during pre-, peri- and post- implantation. Knowledge of these expression patterns will help in the diagnosis and treatment of such cases of infertility and also in the prevention and treatment of early pregnancy disorders.

Heat stress has a major influence on fertility and significantly reduces the reproductive and productive efficiency of farm animals (Jordan, 2003; Rensis and Scaramuzzi, 2003; Bloemhof *et al.,* 2008; Kornmatitsuk *et al*., 2008; Udompraset and Williamson 1987). Several experimental studies in mice have shown that heat exposure for 12 h soon after mating compromises embryonic development, due to the effects of heat on the oviduct rather than on the embryo (Ozawa *et al.,* 2002; Ozawa *et al.,* 2003; Matsuzuka *et al.,* 2005; Sakamoto *et al.,* 2008). As implantation is the most sensitive process in pregnancy, the effects of exposure to elevated ambient temperatures on the maternal responses to implantation should be investigated.

This study aimed to generate more meaningful information about the maternal signals during pre-, peri- and post-implantation periods. The secondary focus is the effect of elevated ambient temperatures (an external factor) on implantation,

maternal signals during peri-implantation, reproductive outcomes and offspring growth.

Thus, the hypothesis of this study was that the morphology of the oviduct and endometrium and the expression of local growth factors change in response to embryonic signals and, additionally, the environmental conditions affect embryo-maternal crosstalk.

The main objective of this study was to define the morphological and molecular changes that occur in the maternal tissue in response to the presence of the embryo.

The specific objectives were:

i. to determine the time of implantation in rats and the concentrations of plasma progesterone and estradiol during peri-implantation,

ii. to define the morphological changes in the oviduct and uterus during early pregnancy,

iii. to determine the distribution and expression pattern of the growth factors and their receptors in the oviduct and uterus during early pregnancy,

iv. to determine the effects of elevated ambient temperatures on implantation, concentrations of plasma progesterone and estradiol, morphological changes and growth factor expressions during peri-implantation, as well as on reproductive outcomes and offspring growth.

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