HUMAN OVULATION

Studies on collagens, gelatinases and tissue inhibitors of metalloproteinases

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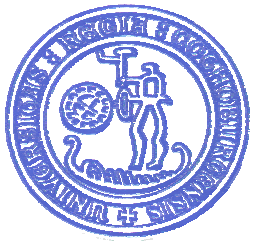

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Sweden 2006

ABSTRACT

Lind, Anna Karin. 2006. **HUMAN OVULATION – Studies on collagens, gelatinases and tissue inhibitors of metalloproteinases.** Department of Obstetrics and Gynecology, The Sahlgrenska Academy at Göteborg University, Sahlgrenska University Hospital, S-413 45 Göteborg, Sweden.

Ovulation is a highly regulated process, which involves the degradation and rupture of healthy tissue of the ovarian follicle and the extrusion of a fertilizable oocyte. This unique biological process is initiated by the LH-surge, which induces major vascular changes and remodelling of the extracellular matrix (ECM) in and around the follicle. The collagens of the ECM make up the tensile strength of the follicle wall and breakdown of these proteins seems to be a prerequisite for follicular rupture to occur. There is now robust evidence that matrix metalloproteinases (MMPs) and their endogenous tissue inhibitors (TIMPs) are crucial in this process. Earlier studies in this field have been mostly animal studies.

The general aims of this thesis were to investigate the distribution of three types of collagens in the human ovary and to explore the expression patterns of the gelatinases, MMP-2 and MMP-9, together with their endogenous inhibitors, TIMP-2 and TIMP-1, during ovulation.

The study was approved by the human Ethics Committee of Sahlgrenska Academy, Göteborg University. Informed written consent was obtained from all women participating in the study.

Women, planned for laparoscopic sterilization participated in the study. They were closely monitored by transvaginal ultrasound. Surgery was performed during either of four distinct ovulatory phases and the dominant follicle and its adjacent stroma was excised. Granulosa and theca cells were harvested. Whole ovarian sections from premenopausal women undergoing oophorectomy at surgery for cervical cancer or due to familial predisposition to ovarian cancer were also obtained.

The distribution of collagen types I, III and IV in biopsies of the perifollicular stroma from four distinct ovulatory phases as well as in whole ovarian sections was investigated by immunohistochemistry (paper I). Collagen types I and III were abundant in the perifollicular stroma around the periovulatory human follicle. These types of collagen were also present in a typical concentric layered pattern in the stromal capsule of the ovary making up a scaffold for the ovarian tissue. Collagen type IV was present in the basal lamina that separates the granulosa from the theca cells. The staining intensity of collagens type I and III in the perifollicular stroma decreased from preovulatory stage throughout ovulation indicating a degradation of the follicle wall.

In paper II-IV, MMP-2, MMP-9 and TIMP-2 and TIMP-1 were found to be present in the stroma-, theca and granulosa cells compartment of the periovulatory follicle. The protein levels of these enzymes and inhibitors were examined by Western Blot in paper II. In paper II-IV mRNA expression was demonstrated by real time PCR and the protein distribution by immunohistochemistry. An increase of TIMP-1 was seen in the perifollicular stroma both on the protein- and mRNA- level. In the granulosa- and theca cells compartments a large increase in TIMP-1 and MMP-9 mRNA was demonstrated. Immunostaining for MMP-2, MMP-9, TIMP-2 and TIMP-1 was visualized in the different compartments of the periovulatory folllcle.

In summary, the abundance of collagens in the human follicular wall and their specific localization suggest that major site-directed degradation of collagens is necessary for follicular rupture to occur. This is the first study that has demonstrated an ovulation-associated expression of the ECM remodelling enzyme, MMP-9, together with its endogenous inhibitor, TIMP-1, in and around the human follicle at ovulation. Increasing knowledge concerning these aspects of human ovulation could contribute to the development of clinical treatments of anovulation and also to the discovery of new contraceptive strategies that could inhibit ovulation on the ovarian level without the side effects of the oral contraceptives used today.

Key words: ovulation, ovary, human, follicle, collagen, MMP and TIMP.

ISBN-10: 91-628-7014-9
ISBN-13: 978-91-628-7014-0

Energy rightly applied can accomplish anything
Nellie Bly
(1864-1922)

To Olivia & Jonathan
and my parents
Vera & Tage Skoglund

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A-K. Lind, B. Weijdegård, P. Dahm-Kähler, K. Sundfeldt and M. Brännström
Mol Hum Reprod. 2006;Oct 27. Epub ahead of print.

II **Collagens in the human ovary and their changes in the perifollicular stroma during ovulation**
A-K. Lind, B. Weijdegård, P. Dahm-Kähler, J. Mölne, K. Sundfeldt and M. Brännström
Acta obstetrician et Gynecologica 2006;00:1-9.

III **Increased expression of MMP-9 and its endogenous inhibitor TIMP-1 in theca cells during human ovulation and luteinisation**
A-K. Lind, B. Weijdegård, P. Dahm-Kähler, K. Sundfeldt and M. Brännström
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IV **Temporal differences in expression of matrix metalloproteinases – 2 and 9 (MMP-2, MMP-9) and of tissue inhibitors of metalloproteinase – 1 and 2 (TIMP-1, TIMP-2) in granulosa cells during human ovulation**
A-K. Lind, P. Dahm-Kähler, B. Weijdegård, K. Sundfeldt and M. Brännström.
Manuscript.

ABBREVIATIONS

ACE	angiotensin converting enzyme
ADAM	family proteins with a disintegrin-like and metalloprotease domain
ADAMTS	family ADAM with thrombospondin type I repeatsmembrane-type MMPs MT-MMPs
Ang I	angiotensin I
Ang II	angiotensin II
BL	basal lamina
CA	corpus albicans
cDNA	complementary DNA
CL	corpus luteum
COX	cyclooxygenase
CSFs	colony stimulating factors
C_T	threshold cycle
DNA	deoxyribonucleic acid
E_2	oestradiol
ECM	extracellular matrix
EDN2	endothelin-2
EMMPRIN	extracellular matrix metalloproteinase inducer
ET	embryo transfer
FACITs	fibril-associated collagens with interrupted triple helices
FACITs	fibril-associated collagens with interrupted triple helices
FSH	follicle stimulating hormone
FSHR	FSH-rececptor
GIFT	gamete intrafallopian transfer
GnRH	gonadotropin-releasing hormone
hCG	human chorionic gonadotropin
IF	interferons
IL	interleukins
IVF	in vitro fertilization
KO	knockout
LH	luteinising hormone
LH	luteinizing hormone
LHR	lutropin/choriogonadotropin receptor
MCP-1	monocyte chemotactic protein-1

MMPs	matrix metalloproteinases
mRNA	messenger RNA
NO	nitric oxide
NOS	nitric oxide synthase
NSAID	non-steroid-anti-inflammatory drug
OD	optical density
OSE	ovarian surface epithelium
P_4	progesterone
PA	plasminogen activator
PAI-1, PAI-2	PA inhibitor-1 and -2
PCOS	polycystic ovarian syndrome
PCR	polymerase chain reaction
PG	prostaglandins
PKA	protein kinase A
PMSG	pregnant mare's gonadotropin
PR	P_4 receptor
proMMP	precursor form of MMP
PVDF	polyvinylidene fluoride
RAS	renin-angiotensin system
RNA	ribonucleic acid
RT	reverse transcription
s.c.	subcutaneous
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel electrophoresis
TA	tunica albuginea
TE	theca externa
TGF-β	transforming growth factor-β
TI	theca interna
TIMPs	tissue inhibitor of metalloproteinases
TNF	tumor necrosis factor
tPA	tissue-type PA
TVU	transvaginal ultrasound
uPA	urokinase-type PA
VEGF	vascular endothelial growth factor