

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 follicle.

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.

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HUMAN OVULATION

Studies on collagens, gelatinases and tissue inhibitors of metalloproteinases

Akademisk avhandling

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Anna Karin Lind

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Avhandlingen baseras på följande delarbeten:

- I 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 Obstetrica et Gynecologica Scandinavica 2006;00:1-9.
- II Gelatinases and their tissue inhibitors during human ovulation: increased expression of tissue inhibitor of matrix metalloproteinase-1. A-K. Lind, B. Weijdegård, P. Dahm-Kähler, K. Sundfeldt and M. Brännström
Mol Hum Reprod. 2006;Oct 27. Epub ahead of print.
- III 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.
- IV 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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