The Ovulatory Process
Studies in the human and the rabbit

Akademisk avhandling
Som med vederbörligt tillstånd av Medicinska Fakulteten, Sahlgrenska Akademin vid Göteborgs Universitet för avläggande av doktorsexamen i medicinsk vetenskap kommer att offentligen att försvaras i föreläsningssalen, kvinnokliniken, Sahlgrenska Universitetssjukhus, Sahlgrenska, Göteborg
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av

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Avhandlingen baseras på följande arbeten:

I Dahm-Kähler P, Löfman C, Fujii R, Axelsson M, Janson P.O, Brännström M. An intravital microscopy method permitting continuous long-term observations of ovulation *in vivo* in the rabbit. Human Reproduction, 21; 624-631, 2006.

II Dahm-Kähler P, Runesson E, Lind A-K, Brännström M. Monocyte chemotactic protein-1 (MCP-1) in the follicle of the menstrual- and the IVF-cycle. Molecular Human Reproduction. *Access online, Jan.2006. In press.*

III Dahm-Kähler P, Ghahremani M, Lind A-K, Sundfeldt K, Brännström M. Monocyte chemotactic protein-1 (MCP-1) and macrophages in the perifollicular stroma during the human ovulatory process. *In manuscript*

IV Dahm-Kähler P, Höjer S, Brännström M. The pressure inside the follicle of the human ovary: novel methodology and measurement. *Submitted.*

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ABSTRACT:
Dahm Kähler, Pernilla. 2006. **THE OVULATORY PROCESS - Studies in the human and in the rabbit.** Department of Obstetrics and Gynecology. Sahlgrenska Academy at Göteborg University, Sahlgrenska University Hospital, S-413 45 Göteborg, Sweden.

Background and general summary: Ovulation is the cascade of events ending with follicular rupture and oocyte extrusion. This ovulatory process is triggered by the LH surge, which induces biochemical and biophysical alterations within the preovulatory follicle. The ovulatory process is an inflammation-like process and also involves alterations in hemodynamics and pressure within the follicle. A model for investigations of the events of ovulation *in vivo* in the rabbit was developed. The expression and regulation of two macrophage specific chemokines in the human ovary were also explored. To enable studies of the intrafollicular pressure (IFP) in the human ovary a new method was developed. The aims of these studies were to develop methodologies for studies of ovulation and to more specifically study one component of the inflammation-like response at ovulation.
Methods and results: An intravital microscopy method, permitting long-term observation of the rabbit ovary *in vivo*, was developed. During anaesthesia the ovary of an eCG/hCG primed rabbit was submerged into a specially designed organ chamber with a microscopy lens close to the ovary. Video recordings documented ovulations, which occurred around 12 h after hCG. The sequence of typical features of ovulation were; vascular shut down in the follicular apex, petechiae in the follicular wall, formation of a cone-shaped structure over the rupture site, bulky bleeding at the site of follicular rupture and a steady extrusion velocity of granulosa cells and the oocyte (Paper I).
The presence and regulation of two macrophage specific chemokines, monocyte chemoattractant protein-1 (MCP-1) and monocyte inflammatory protein-1α (MIP-1α), were investigated in the human ovary during menstrual and IVF cycles. The levels of MCP-1 were markedly higher in follicular fluid as compared to blood plasma in both menstrual- and IVF-cycles. The follicular fluid to plasma difference in MCP-1 levels in menstrual cycles increased from the follicular phase to the late ovulatory phase. Theca cells from follicles of menstrual cycles secreted both MCP-1 and MIP-1α during basal conditions and the secretion increased by addition of IL-1. Granulosa-lutein cells secreted MCP-1 under basal condition and also MIP-1α after IL-1 (Paper II).
MCP-1 expression and macrophage density were evaluated in the perifollicular stroma during precise phases of the ovulatory process in the human. Women, planned for laparoscopy, were monitored closely by transvaginal ultrasound and when the dominant follicle was 15-17mm in diameter 21 out of the 28 women received rhCG. Surgery was performed at four distinct ovulatory phases and the dominant follicle and its adjacent stroma was collected. The mRNA levels of MCP-1 in the perifollicular stroma increased from the preovulatory to late ovulatory phase and declined during post ovulatory phase. Immunoblot confirmed presence of macrophages and MCP-1 receptor CCR2 in the stroma. There was a tendency to higher macrophage density during the two earlier ovulatory phases (Paper III)**.**
A methodology for *in vivo* measurements of the IFP in the human ovary was developed. A pressure sensor was inserted into the follicular antrum, in ovaries of women undergoing laparotomy. The ovarian arteries and veins were dissected free to enable manipulation during pressure measurement. The baseline IFP was positive and stable. When the ovarian veins were blocked the IFP increased and then declined after clamping of the ovarian artery. The pressure inside ovarian non-follicular cysts was considerably lower and no alterations were seen during vascular manipulation (Paper IV).
Taken together, the present studies present two new methodologies that enable *in vivo* examinations of the ovulatory process and do also show that the chemokine MCP-1 is expressed and under cytokine regulation during the ovulatory process of the human.

Keywords: ovulation, ovary, rabbit, human, follicle, MCP-1, intrafollicular pressure.
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