

OVULATION: INTRA-OVARIAN MECHANISMS

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademien, Göteborgs universitet kommer att offentligen försvaras i stammen, blå stråket 6, Sahlgrenska Universitetssjukhuset, Göteborg, den 19 december 2019, klockan 09.00 av Farnosh Zakerkish Sørensen.

Fakultetsopponent: Peter Humaidan, Professor of Obstetrics and Gynecology, Faculty of Health, Aarhus University, Aarhus, Denmark.

Avhandlingen baseras på följande delarbeten

- I. Rosewell KL, Al-Alem L, Zakerkish F, McCord L, Akin JW, Chaffin CL, Brännström M, Curry TE Jr. Induction of proteinases in the human preovulatory follicle of the menstrual cycle by human chorionic gonadotropin. *Fertil Steril* 2015;103:826-833
- II. Puttabytappa M, Al-Alem LF, Zakerkish F, Rosewell KL, Brännström M, Curry TE Jr. Induction of tissue factor pathway inhibitor 2 by hCG regulates periovulatory gene expression and plasmin activity. *Endocrinology* 2017;158:109-120
- III. Zakerkish F, Brännström M, Carlsohn E, Sihlbom C, Van der Post S, Thoroddsen A. Proteomic analysis of follicular fluid during human ovulation. Revision 1, submitted to *Acta Obstet Gynecol Scand*
- IV. Zakerkish F, Thoroddsen A, Dahm-Kähler P, Olofsson J, Brännström M. Expression patterns of osteoprotegerin (OPG) and receptor activator nuclear factor kappa B ligand (RANKL) in human follicles during ovulation. In manuscript
- V. Zakerkish F, Soriano MJ, Novella-Maestre E, Brännström M, Diaz-Garcia C. Differential effects of the immunosuppressive calcineurin inhibitors cyclosporine-A and tacrolimus on ovulation in a murine model. Revision 1, submitted to *Hum Reprod Open*

SAHLGRENKA AKADEMIN
INSTITUTIONEN FÖR KLINISKA VETENSKAPER



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Abstract

Background Ovulation is the central biological process involved in the menstrual cycle of women. Specifically, ovulation involves the tissue remodelling of the preovulatory follicle to achieve the rupture of the exterior follicle wall with the extrusion of the oocyte. The ovulatory process involves many mediators that cooperatively and redundantly carry out changes that are necessary for ovulation, normal progression and natural conception. Increased knowledge of mammalian ovulation is important regarding many aspects of female fertility, such as the treatment of anovulation, ovarian stimulation in assisted reproduction, and the prevention of ovarian hyperstimulation. Another aspect is that it may lead to the development of new strategies for contraception.

Aims: The general aim of this study was to increase knowledge regarding the intra-ovarian regulation of ovulation, which was achieved via studies on protease expression, the expression and regulation of the protease inhibitor, the proteome profile in follicular fluid, the expression of osteoprotegerin (OPG), the receptor activator of the nuclear factor kappa B ligand (RANKL), and the effects of calcineurin inhibitors on ovulation.

Methods: Granulosa cells, theca cells, follicular fluid, and whole follicles were obtained from women at four different stages of the ovulatory process. Expression, proteome profile, and immunohistochemistry were performed. Granulosa lutein cells were used for the cell culture from women undergoing in vitro fertilisation (IVF). Immature Sprague-Dawley rats were primed with pregnant mare's serum gonadotropin to induce maturation and subsequent ovulation, that was triggered 48 hours later with human chorionic gonadotropin (hCG). In vivo experiments in this animal model as well as in vitro experiments on its cells and tissues were conducted.

Expression patterns were studied via a quantitative, real-time polymerase chain reaction (RT-PCR) and a microarray. Proteins were quantified and identified by mass spectrometry isobaric tags for relative and absolute quantification (iTRAQ), and localization was performed with immunohistochemistry. Assays were also used for the assessment of plasmin activity, leukocyte distribution, steroid levels, and levels of mediators/pharmacological agents in the blood.

Results: Paper I indicate that an ovulatory trigger induces expression in the human granulosa and theca cells of certain proteases from the matrix metalloproteinase (MMP) as well as a disintegrin and metalloproteinase with thrombospondin-like motifs (ADAMTs) family. Paper II presents data on the increased expression of the protease inhibitor tissue factor pathway inhibitor 2 (TFPI2) in the ovulating follicle of the human and rat. Moreover, the down-stream signalling pathways and effects on a large number of mediators were also characterized. Paper III use a modern proteomic technique to identify more than 500 proteins in the follicular fluid during ovulation, with 25 showing level changes during human ovulation. Paper IV identifies OPG and RANKL as potential mediators in the intra-ovarian events of ovulation. Paper V demonstrates that cyclosporine-A, but not tacrolimus, negatively influences ovulation in the rat.

Conclusion: The results of the thesis provide information on the roles and functions of several new mediators in ovulation.

Key words: *A disintegrin and metalloproteinase with thrombospondin-like motifs, animal model, calcineurin, cyclosporine-A, follicle, follicular fluid, granulosa cell, human, human chorionic gonadotropin, immunohistochemistry, matrix metalloproteinase, menstrual cycle, osteoprotegerin, ovary, ovulation, plasmin, protease, proteomic, rat, receptor activator of nuclear factor kappa B ligand, tacrolimus, theca cell, tissue factor pathway inhibitor 2*