Induction of proteinases in the human preovulatory follicle of the menstrual cycle by human chorionic gonadotropin

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Objective: To explore the temporal expression in granulosa and theca cells of key members of the MMP and ADAMTS families across the periovulatory period in women to gain insight into their possible roles during ovulation and early luteinization.
Design: Experimental prospective clinical study and laboratory-based investigation.
Setting: University medical center and private IVF center.
Animal and Patient(s): Thirty-eight premenopausal women undergoing surgery for tubal ligation and six premenopausal women undergoing assisted reproductive techniques.
Intervention(s): Administration of hCG and harvesting of follicles by laparoscopy and collection of granulosa-lutein cells at oocyte retrieval.
Main Outcome Measure(s): Expression of mRNA for matrix metalloproteinase (MMPs) and the A disintegrin and metalloproteinase with thrombospondin-like motifs (ADAMTS) in human granulosa cells and theca cells collected across the periovulatory period of the menstrual cycle and in cultured granulosa-lutein cells after hCG. Localization of MMPs and ADAMTSs by immunohistochemistry.
Result(s): Expression of *MMP1* and *MMP19* mRNA increased in both granulosa and theca cells after hCG administration. *ADAMTS1* and *ADAMTS9* mRNA increased in granulosa cells after hCG treatment, however, thecal cell expression for *ADAMTS1* was unchanged, while *ADAMTS9* expression was decreased. Expression of *MMP8* and *MMP13* mRNA was unchanged. Immunohistochemistry confirmed the localization of MMP1, MMP19, ADAMTS1, and ADAMTS9 to the granulosa and thecal cell layers.
Conclusion(s): The collection of the dominant follicle throughout the periovulatory period has allowed the identification of proteolytic remodeling enzymes in the granulosa and theca compartments that may be critically involved in human ovulation. These proteinases may work in concert to regulate breakdown of the follicular wall and release of the oocyte. (Fertil Steril® 2015;103:826–33. ©2015 by American Society for Reproductive Medicine.)
Key Words: Ovulation, granulosa cell, theca cell, matrix metalloproteinase, ADAMTS

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The human ovarian follicle is supported by a complex network of extracellular matrix (ECM) proteins. The ECM composition of the ovarian follicle, with its granulosa, theca, and stromal cell compartments, is dependent on the cell type, and this composition changes throughout the different stages of follicular growth, ovulation, and luteinization (1).

In the human, the granulosa cell compartment is composed of steroidogenic cells supported by the ECM proteins laminin (2), type IV collagen (3), and type VI collagen (3, 4). This granulosa cell layer is separated from the theca interna by a basement membrane, or basal lamina. This basement membrane is composed of a

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lattice-type network of type IV collagen intertwined with a mesh of laminin (5, 6) and is stabilized by the binding of other proteins such as entactin, nidogen, perlecan, collagen type XVIII, and the glycoprotein usherin (5). In the theca cell compartment, collagen type III is present in both the theca interna and the theca externa, while collagen type I is only present in the theca externa of the human follicle (6). In the stroma outside of the theca, collagens I and III are distributed in concentric layers in the capsular stroma, with bundles of collagens connecting these layers to form a lattice (7). Collagen type I is present in larger quantities in the outer layers of the follicle wall, while collagen type III showed the inverse distribution, with higher abundance in the more central parts of the capsular stroma (6).

The abundance of ECM proteins in the follicular wall has led to the hypothesis that their degradation is paramount for follicular rupture to occur (6, 8, 9). This concept has been supported by morphological observations that as ovulation approaches in the human, there is a decrease or fragmentation in the immunostaining intensity of type I, III, and VI collagens in the perifollicular stroma (4, 6). This fragmented or discontinuous immunostaining for type VI collagen was evident predominantly in the apical area rather than in the base of the preovulatory follicle (4, 10). Okamura and colleagues examined the human apical wall by electron microscopy and observed a loss of collagen in the theca externa and tunica albuginea at the follicular apex. After rupture, the theca and tunica albuginea are occupied by "scattered fibrillar substance" with a loss of collagen bundles (11).

These morphological changes at the apex of the human follicle are postulated to occur through the actions of a broad array of proteinases including metallo-, serine, and thiol proteinases (9, 12–17). The expression and activity of these proteinases are set in motion by the midcycle surge of LH in numerous species (9, 12–17). However, little is known about the expression of these proteinases in human ovulation owing to the difficulties of collecting human ovarian tissue across the periovulatory period. The human data on proteinase expression and function in ovulation have mostly come from studies of granulosa-lutein cells from IVF, which represent cells from an artificial hyperstimulated cycle, with no possibility of comparing expression to earlier stages of the follicle. In the present study, we used separated granulosa cells and theca cells of the dominant follicle at timed intervals across the periovulatory period to investigate the expression and localization of members of the matrix metalloproteinase (MMPs) and the A disintegrin and metalloproteinase with thrombospondin-like motifs (ADAMTS) families that are associated with the ovulatory process in the human. The hCG responsiveness of the in vivo cells was compared with in vitro models using granulosa-lutein cells collected at the time of IVF or virally transformed granulosa cells.

MATERIALS AND METHODS

Materials

Unless otherwise noted, all chemicals and reagents were purchased from Sigma-Aldrich Chemical Co. Molecular biological enzymes, culture media, and additives, Trizol, TaqMan primers, and Mastermix, were purchased from Life Technologies, Inc. Immunohistochemistry reagents for the Starr Trek Avidin-AP labeling system were purchased from Biocare.

Human Follicles Collected Across the Periovulatory Period

Human granulosa and theca cells from periovulatory follicles were collected as described elsewhere (18). The study was approved by the regional human ethics committee of Gothenburg, and informed written consent was obtained from all patients. To obtain high-quality patient material, only women with proven fertility, with regular menstrual cycles, and without hormone medication for at least 3 months were included. The women were monitored with repeated transvaginal ultrasound (TVU) for an average of two cycles to enable planning of follicle collection at one of four time points: pre-, early, late, or postovulatory.

For samples collected at the preovulatory phase, surgery was performed before the LH surge when the dominant follicle was $\geq$14 mm and $\leq$17.5 mm. The remaining patients received an injection of hCG (sc, 250 μg recombinant human chorionic gonadotropin [rhCG], Ovitrelle, Merck Serono) to mimic the endogenous LH surge and underwent surgery after varying lengths of time after rhCG injection: early ovulatory phase (12 to $\leq$18 hours), late ovulatory phase (>18 to $\leq$34 hours), and postovulatory phase (>44 to $\leq$70 hours). Frequent TVU examinations after rhCG administration have determined that rupture occurs approximately 36–38 hours after rhCG administration (19, 20). Samples for the measurement of serum levels of P and E_2 were taken immediately before surgery to confirm the patient's ovulatory phase category (7). The entire intact dominant follicle was excised from the ovary using scissors and without use of diathermy and placed inside a laparoscopic sac to be retrieved through a suprapubic trocar incision and processed intact for immunohistochemistry or bisected for the collection of granulosa and theca cells.

The intact follicles for immunohistochemistry were fixed in 4% paraformaldehyde overnight, embedded in paraffin, and sectioned at 7 μm. For cell isolation, granulosa cells were collected by dissecting the follicle to release the loosely attached cells. The mural granulosa cells were then gently scraped from the follicle wall and pooled with the loosely attached granulosa cells. Theca cells of the interna layer were harvested mechanically from the remnant of the follicle by separating the theca interna layer from the theca externa layer. This theca interna cell layer also contains vascular cells, leukocytes, and fibroblasts (6). The cells were frozen in liquid nitrogen for subsequent processing and analysis of mRNA expression for key MMPs and ADAMTS associated with ovulation (*MMP1*, *MMP8*, *MMP13*, *MMP19*, *ADAMTS1*, and *ADAMTS9*) by real-time reverse transcriptase–polymerase chain reaction (RT-PCR). Theca cells were obtained from all four periovulatory phases, but granulosa cells could not be collected from the postovulatory group since large quantities had been lost at follicular rupture.