Follicular fluid and serum levels of Inhibin A and pregnancy-associated plasma protein A in patients undergoing IVF

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Objective: To elucidate transport of intrafollicular proteins Inhibin A and pregnancy-associated plasma protein A (PAPP-A) across the follicular fluid (FF)/blood barrier.
Design: A retrospective study.
Setting: IVF lab at a university hospital, academic, and industrial research labs.
Patient(s): Fifty-five women undertook the IVF program.
Intervention(s): Follicular fluid aspirations and analysis, blood sample drawing, and serum analysis.
Main Outcome Measure(s): Concentrations of Inhibin A, PAPP-A, and major serum proteins in FF and serum, total amount of PAPP-A, and Inhibin A in FF.
Result(s): The FF/blood barrier permeability was calibrated using major serum proteins. The FF/serum ratio decreased with the molecular mass of proteins, and their FF and serum concentrations were well correlated. In contrast, concentrations of Inhibin A in paired serum and FF samples showed a weak correlation ($r = 0.563$), whereas serum and FF concentrations of PAPP-A were independent of each other. The total amount of Inhibin A in FF correlated well with concentrations of Inhibin A in paired serum samples ($r = 0.858$), whereas the correlation between the total amount of FF PAPP-A and PAPP-A serum concentrations remains poor ($r = 0.215$).
Conclusion(s): These observations suggest that at the day of oocyte retrieval, FF is a major source of serum Inhibin A but not of serum PAPP-A. (Fertil Steril® 2009;91:1739–44. ©2009 by American Society for Reproductive Medicine.)

Key Words: Follicular fluid/blood barrier, protein transport, PAPP-A, Inhibin A, IVF

Ovaries are tissues in which endocrine organs, ovarian follicles, and corpora lutea periodically grow and regress. The developing ovarian follicles are composed of a follicular fluid (FF)-filled antrum containing granulosa cells and oocytes, and a highly vascularized thecal compartment. At ovulation the granulosa cells undergo a transition into luteal parenchymal cells. To control that process, ovarian follicles have to communicate with the environment via FF and blood plasma. In this communication, properties of the FF /blood barrier play an important role, as ovarian proteins facilitate both intraovarian paracrine effects, as well as endocrine feedback effects (1).

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Semipermeable barriers between the blood and various body fluids, separating the environments of both fluids, are well-described phenomena. The best-known examples are blood/brain barrier and a selective ultrafiltration of blood plasma proteins into urine. A similar barrier has been reported for the transport of blood proteins into the ovarian FF with permeability inversely proportional to the protein molecular mass (2) or into a combination of protein pI and molecular mass (3). Only proteins with molecular mass <500 kDa were found to be ultrafiltrated from the blood into FF.

To test the FF–blood barrier permeability for the transport of ovarian proteins into the blood, we have selected two proteins secreted by granulosa cells into the FF at the time of oocyte retrieval: Inhibin A (32 kDa) and pregnancy-associated plasma protein A (PAPP-A) (500 kDa).

Inhibin secretion by granulosa cells and the evidence that ovarian Inhibin A and Inhibin B suppress FSH production has been reported by Ericsson and Hsueh (4). Inhibins are heterodimeric glycoprotein hormones composed of one α (18 kDa) and one β (14 kDa) chain linked by disulphide bonds. Inhibin A consists of $\alpha-\beta_\alpha$ subunits and Inhibin B consists of $\alpha-\beta_\beta$ subunits (5). Meunier et al. (6) reported the expression of inhibin subunits in various tissues; the inhibin α subunit, however, is predominantly expressed in the gonads.

Inhibin A serum concentrations were measured in samples taken at various time points from the start of ovarian stimulation (7, 8). The presence of a measurable concentration of Inhibin A and Inhibin B in serum of stimulated women suggests that the blood/follicle barrier is permeable for them, although the FF concentration of both Inhibin A and Inhibin B is considerably higher (9).

Pregnancy-associated plasma protein A (PAPP-A) was first isolated from human pregnancy serum >30 years ago (10). Only recently it has been shown that proteolytic activity against Insulin-like growth factor binding protein 4 (IGFBP-4) in human FF is identical to PAPP-A (11).

Proteolysis of IGFBP-4 by PAPP-A is an important mechanism regulating FSH-induced ovarian follicle maturation and steroidogenesis in human granulosa cells (12). Pregnancy-associated plasma protein A is a metalloproteinase composed of two 200-kDa disulfide-bound subunits. The most common form of PAPP-A in circulation is the 500-kDa disulfide-bound 2:2 heterotetrameric complex with the proform of eosinophil major basic protein (proMBP), PAPP-A/proMBP (13). Ovarian follicles and generally reproductive tissues are important but not an exclusive [for review, see (14)] source of PAPP-A in nonpregnant women. The reported blood serum levels of PAPP-A are much lower than the levels found in FF, so despite the various sources of the PAPP-A, we believe that PAPP-A is still a good candidate to our study. Hourvitz et al. (15) reported expression of PAPP-A gene in the ovarian follicles. Sjöberg et al. (16) reported PAPP-A presence in human preovulatory FF, luteinized cells of unruptured follicles, and corpus luteum. Sinosich et al. (17) published a concentration range of PAPP-A in FF between 0.317 and 1.595 IU/L. Conover et al. (11) demonstrated measurable concentrations of PAPP-A (1.604 ± 0.315 IU/L) in FF obtained from dominant ovarian follicles in a standard IVF procedure. There are, however, only a few reports showing a relation between blood and FF PAPP-A concentration and transport of PAPP-A from ovarian follicle across the FF/blood barrier (18, 19).

In the present study, FF and serum concentrations of one large (PAPP-A, 500 kDa) and one small (Inhibin A, 32 kDa) protein of ovarian follicle origin was analyzed in patients undergoing regular IVF treatment. Our hypothesis was that although Inhibin A passes the FF/blood barrier rather freely and its serum and FF concentrations are highly correlated, PAPP-A is, because of its high molecular mass, retained within the ovarian follicles and only acts locally. To evaluate the hypothesis, concentrations of Inhibin A and PAPP-A were analyzed in FF and in paired serum samples collected at the time of the oocyte retrieval.

MATERIALS AND METHODS

Female Patients

Patients undergoing regular stimulation for IVF were recruited for the study at the Center of Assisted Reproduction, Department of Obstetric and Gynecology, General Teaching Hospital in Prague. A total of 55 women were recruited for that study. Because of serum volume requirements, patients were separated into two groups. Samples of 32 patients were used for the serum protein study and samples of 23 patients were used for the ovarian protein experiment. Two patients of the latter group suffered with ovarian hyperstimulation syndrome and their samples were excluded from the study. The number of growing follicles ranged from 6 to 27 follicles. Folicular fluid and serum samples were obtained with the patient's permission. All the patients participating in this study signed an institutional review board-approved informed consent form.

All subjects underwent standard treatment protocol: FSH ovarian hyperstimulation using GnRH long agonists or GnRH short antagonist's protocol with hCG induction of the follicular/egg maturation 36 hours before egg collection.

Follicular Fluid Aspiration and Blood Processing

Follicular fluid was obtained from the puncture of dominant ovarian follicles (14 to 22 mm in diameter) or from all follicles as indicated. After oocytes were removed, FF was cleared by centrifugation, and the resulting supernatant was transferred into sterile tubes, frozen at -20°C and stored at -70°C for further analysis. Blood-contaminated FF was excluded from the study. In parallel, samples of blood (5 mL) were taken on the day of oocyte retrieval, allowed to clot, cleared by centrifugation, and the resulting sera were frozen at -20°C and kept at -70°C until assayed.

In the serum protein experiment, only the FF from large dominant follicles was collected and analyzed. In the PAPP-A and Inhibin A experiments, FF from large dominant follicles, as well as FF from all follicles, was collected and analyzed. Fluid from large dominant follicles was collected and analyzed separately. After that, all FF was pooled and analyzed for volume and total concentration of PAPP-A and Inhibin A. The volume and total FF concentration were used to calculate the total amount of FF PAPP-A and FF Inhibin A.

Serum and FF concentrations of Inhibin A were analyzed using enzyme-linked immunosorbent assay kits (Diagnostic System Laboratories, Webster, TX). Concentrations of PAPP-A in serum (ultrasensitive PAPP-A) were analyzed using enzyme-linked immunosorbent assay kits supplied by DRG instruments GmbH (Marburg, Germany). Follicular fluid PAPP-A was assayed by a PAPP-A IRMA kit (Immunotech, Prague, Czech Republic). All the immunodiagnostic kits were processed according to the manufacturer's instructions. For the analysis of FF Inhibin A, the FF samples were diluted 200-fold with calf plasma (Scantibodies Laboratory, Inc., Santee, CA). The linearity of the dilution was verified (recovery percentages obtained in range 84.6% to 109%).

Ten major serum proteins were analyzed using the protein analysis system Immage (Beckman Coulter Inc., Fullerton,