Levels of the epidermal growth factor-like peptide amphiregulin in follicular fluid reflect the mode of triggering ovulation: a comparison between gonadotrophin-releasing hormone agonist and urinary human chorionic gonadotrophin

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Objective: To detect differences in follicular fluid (FF) levels of amphiregulin (AR), depending on mode of triggering final oocyte maturation.
Design: Prospective randomized trial.
Setting: Three IVF units.
Patient(s): Ninety-six patients undergoing IVF–intracytoplasmic sperm injection.
Intervention(s): Ovulation triggered with either urinary hCG or GnRH agonist (GnRH-a). Controls: 15 FF samples from small antral follicles (3–9 mm) and 12 FF samples from natural cycle.
Main Outcome Measure(s): Follicular fluid concentration of AR, P_4, E_2, vascular endothelial growth factor, and inhibin B.
Result(s): Significantly lower levels of AR were found in FF from the GnRH-a group versus the hCG group, 51 $\pm$ 3.5 versus 71 $\pm$ 6.0 ng/mL. In FF from natural cycles, levels of AR were significantly higher than those of GnRH-a triggering but significantly lower than those of urinary hCG triggering. In small antral follicles only 5 out of 15 follicles contained measurable amounts of AR. When urinary hCG and GnRH-a triggering were compared, FF P_4 was significantly higher after urinary hCG triggering, whereas no difference was seen regarding E_2, vascular endothelial growth factor, and inhibin B. A total of 14% more metaphase II oocytes and 11% more transferable embryos were obtained after GnRH-a triggering.
Conclusion(s): This study suggests that oocyte competence is linked to granulosa cell AR secretion. (Fertil Steril® 2011;95:2034–8. ©2011 by American Society for Reproductive Medicine.)

Key Words: Amphiregulin, VEGF, GnRH agonist, GnRH antagonist, in vitro fertilization, triggering of ovulation

The pivotal role of the midcycle surge of gonadotrophins for inducing ovulation and resumption of meiosis is well recognized. However, the physiologic processes by which FSH and LH secure the release of a fertilizable oocyte at ovulation involve a complicated network of regulatory substances. Animal studies have shown that members of the epidermal growth factor (EGF)-like family act as mediators of LH action on the preovulatory follicle (1, 2). On stimulation with LH-like activity mural granulosa cells of preovulatory follicles respond with a rapid and massive synthesis of EGF-like peptides, that is, amphiregulin (AR), epiregulin, and betacellulin (1–5). Especially AR and epiregulin have been shown to be potent mediators of oocyte maturation by stimulating EGF receptors in the cumulus cells (3, 6). Recently, isolated murine cumulus enclosed oocytes have been shown dose-dependently to respond to AR by resuming meiosis (7). In humans, AR becomes expressed abundantly in preovulatory follicles on exposure to the midcycle surge of gonadotrophins or hCG, and the concentration of AR has been shown to correlate inversely with the fertilization rate (8).

During ovarian hyperstimulation, the use of GnRH antagonist protocols for the prevention of a premature LH surge allows ovulation to be induced with a bolus of GnRH agonist (GnRH-a) as an alternative to hCG (9–11). In the pituitary GnRH-a displaces the GnRH antagonist, which activates the GnRH receptor, resulting in a surge of gonadotrophins (flare-up), similar to the natural midcycle surge of gonadotrophins. The surge of FSH elicited simultaneously with LH potentially could have a positive impact on oocyte maturation, LH receptor formation in granulosa cells, and cumulus expansion (12–16). The aim of the present study was to explore possible differences in follicular fluid (FF) hormones after triggering of final oocyte maturation with either GnRH-a or hCG, focusing on AR.

MATERIALS AND METHODS

Patients and Hormonal Treatment

Follicular fluid was obtained from 96 patients with normal gonadotrophin levels undergoing IVF–intracytoplasmic sperm injection (ICSI) and

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TABLE 1

Follicle-stimulating hormone consumption, GnRH antagonist consumption, and E_2 levels during follicular phase in GnRH-a versus urinary hCG groups.

	Total	GnRH-a	Urinary hCG
Started cycles, total	96	48	48
FSH stimulation days, median (range)	8 (6–12)	8.5 (6–10)	8 (6–12)
FSH consumption, IU (mean ± SEM)	1,475 ± 39	1,501 ± 54	1,450 ± 58
First antagonist day, SD median (range)	6 (5–10)	6 (5–9)	7 (5–10)
Antagonist consumption, mg (mean ± SEM)	0.98 ± 0.03	1.00 ± 0.04	0.95 ± 0.04
Se-E_2 (pmol/L), S2	230 ± 13	239 ± 25	221 ± 9
Se-E_2 (pmol/L), S7	2,465 ± 194	2,504 ± 250	2,424 ± 302
Se-E_2 (pmol/L), hCG day	4,071 ± 294	3,999 ± 313	4,142 ± 498
Se-E_2 (pmol/L), OPU day	2,443 ± 144	2,389 ± 183	2,494 ± 222

Note: OPU = oocyte pickup; Se = serum; S2 = stimulation day 2; S7 = stimulation day 7.

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participating in a prospective randomized, three-center study. Details on patient characteristics and hormonal treatment given have been published previously (17). In brief, patients received ovarian stimulation followed by cotreatment with a standard GnRH antagonist protocol; when at least two follicles had reached a size of 17 mm, patients were assigned randomly into two groups: the GnRH-a group or the hCG group. The GnRH-a group received final oocyte maturation with a single bolus of 0.5 mg buserelin SC (Suprefact; Hoechst, Hørsholm, Denmark). The hCG group received 10,000 IU of hCG SC for final oocyte maturation. Oocyte pickup was performed 34 hours later in both groups. The oocyte maturity was assessed in patients undergoing ICSI. The study was approved by the Ethics Committee of Viborg County, project number 20060036MCH, ClinicalTrials.gov NCT00627406.

Follicular Fluid Sampling

Two follicular samples without contamination of flushing medium, from the first follicle aspirated on each side, were collected. To eliminate granulosa cells the fluid was centrifuged at 500 × *g*. A total of 146 FF samples were included; of these 73 FF samples were obtained from the GnRH-a group and 73 from the hCG group. Fifteen FF samples from small antral follicles (3–9 mm) and 12 preovulatory FF samples from natural cycles, aspirated after the midcycle surge but before ovulation, served as controls. Samples were stored at −20°C. All measurements were performed on individual follicles, and the results are given as the mean of measurements of individual follicles.

Hormone Measurements

Amphiregulin was measured with use of a commercially available sandwich ELISA kit (DY262; R&D Systems Europe, Abingdon, United Kingdom). According to the manufacturer the assay does not show any cross-reactivity toward EGF, EGF receptor, heparin-binding–EGF, or transforming growth factor α at a concentration of 50 ng/mL. The assay standard curve covered a concentration range from 1.5 to 1,000 pg/mL, and FF samples were diluted 1:500 with use of the reagent diluent to be covered by the standard curve. Samples were diluted parallel to the standard curve, and the interassay variation of a sample containing 59.8 ng/mL was 6.9% (N = 10).

Vascular endothelial growth factor (VEGF) was measured with use of a commercially available sandwich ELISA kit (DY293b; R&D Systems Europe). According to the manufacturer the assay does not show any cross-reactivity toward VEGF-C and VEGF-D at a concentration of 50 ng/mL. The assay standard curve covered a concentration range from 2 to 2,000 pg/mL, and FF samples were diluted 1:10 to be covered by the standard curve. The interassay variation of a sample containing 1,536 pg/mL was 14% (N = 28).

Inhibin B was measured with use of a specific ELISA kit according to the manufacturer's instruction (Oxford Bio-innovation kit; Biotech-IgG, Copenhagen, Denmark). Before measurement, irrespective of whether samples derived from small antral or preovulatory follicles, all FF samples were diluted 1:100 or 1:500 in serum obtained from a pool of five postmenopausal women (with no inhibin B activity). The FF samples were pretreated with sodium dodecyl sulfate, heated, and exposed to hydrogen peroxide before they were applied to the wells of the plate and incubated overnight at room temperature. Subsequently, the plates were washed and incubated with detection antibody for 3 hours at room temperature. Substrate solution was applied and incubated for 1 hour. The amplifier solution was added, and the plates were read with an ELISA reader at 490 nm with its reference at 620 nm (coefficient of variation <7%).

Estradiol and P were measured, with use of commercially available RIA kits (DSL-43100 and DSL-4300; Diagnostics System Laboratories, Webster, TX). Samples for both assays were diluted to 1:1,000 in steroid-free serum before measurement.

Statistical Methods

Comparison of outcomes between the two groups was performed by Student's *t*-test for parametric data and by Fisher's exact test for nonparametric data. The program used for analysis was SPSS 15 (SPSS, Inc., Chicago, IL). A P value $< .05$ was considered to be statistically significant.

RESULTS

Patient Characteristics and Stimulation

No significant differences regarding demographic data were found. The total exogenous FSH consumption, total dose of GnRH antagonist, duration of stimulation, and E_2 levels during the follicular phase did not differ between the GnRH-a and hCG groups (Table 1). The distribution of IVF and ICSI cycles performed did not differ between the two groups.

Follicular Fluid Contents of Hormones After Gonadotrophin-releasing Hormone–Agonist and Human Chorionic Gonadotrophin Triggering and in the Natural Cycle

Significantly lower levels of AR were found in FF from the GnRH-a group as compared with the hCG group, 51 ± 3.5 versus 71 ± 6.0 ng/mL (mean ± SD) (P=.003). In preovulatory FF from natural menstrual cycles, levels of AR were 68 ± 25 ng/mL, a level significantly higher than that of GnRH-a triggering but significantly lower than that of hCG triggering (P<.001) (Table 2). No difference was seen regarding VEGF, 1,199 ± 83 pg/mL versus 1,192 ± 91 pg/mL, between the GnRH-a and hCG groups, respectively. However, the VEGF level of the natural cycle preovulatory FF, 2,248