Comparison of gene expression profiles in granulosa and cumulus cells after ovulation induction with either human chorionic gonadotropin or a gonadotropin-releasing hormone agonist trigger

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Objective: To explore differences in follicle transcriptomes in patients having oocyte maturation with either a bolus of hCG or GnRHa.
Design: Cumulus cells (CC) and mural granulosa cells (MGC) were isolated from preovulatory follicles in patients undergoing controlled ovarian stimulation, prospectively randomized to GnRHa or hCG triggering.
Setting: University-based facilities for clinical services and research.
Patient(s): Twenty women with indication for IVF or intracytoplasmic sperm injection treatment were randomly allocated to hCG or GnRH agonist (GnRHa) trigger.
Intervention(s): MGC and CC were collected from individual follicles in connection with oocyte retrieval.
Main Outcome Measure(s): RNA was extracted, labeled, amplified, and hybridized on HumanGene1.0ST GeneChip Affymetrix array. Expression data were robust multichip average normalized and compared using Partek and Ingenuity software. Array data were confirmed with reverse transcription–polymerase chain reaction analysis.
Result(s): Comparing the transcriptomes between the groups, 391 and 252 genes were differentially expressed (fold change >1.5) in CC and MGC, respectively. The enriched bionetworks showed that CC genes highly represented "lipid metabolism and small molecule biochemistry" (network score, 41), while in MGC, the top network was "cardiovascular development and function and cellular movement" (network score, 50). For both CC and MGC, the regulator analysis suggested LH as the upstream regulator for the difference observed. In CC, the LH receptor was more highly expressed after GnRHa trigger, while in MGC, genes involved in angiogenesis such as angiopoietin 1 and semaphorin 3A were down- and up-regulated, respectively, in GnRHa- as compared with hCG-triggered patients.
Conclusion(s): The comparisons between somatic cell transcriptomes from GnRHa- and hCG-triggered follicles showed significant functional differences in both CC (steroidogenesis) and MGC (angiogenesis) compartments. (Fertil Steril® 2013;100:994–1001. ©2013 by American Society for Reproductive Medicine.)
Key Words: Cumulus cells, granulosa cells, gene expression profile, GnRHa trigger, hCG trigger

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Triggering of final oocyte maturation after controlled ovarian stimulation (COS) can be performed with either a bolus of hCG or a bolus of GnRH agonist (GnRHa) in patients cotreated with a GnRH antagonist for IVF/intracytoplasmic sperm injection (ICSI). Randomized

controlled trials in IVF/ICSI comparing the two protocols for ovulation induction have reported the retrieval of a higher number (1–3) and percentage (2, 3) of metaphase II (MII) oocytes and transferable cleavage-stage embryos after GnRHa trigger as compared with the standard hCG triggering and, additionally, comparable rates of positive pregnancy tests after ET (1, 2, 4). However, the initial proof-of-concept studies in IVF/ICSI patients showed a significantly reduced clinical pregnancy rate in the group of patients triggered with GnRHa (2, 5) owing to an insufficient luteal phase (6–9). Subsequent studies have shown that modified luteal support in the GnRHa trigger group overcomes the luteal phase insufficiency, resulting in delivery rates comparable to hCG trigger (8, 10). Moreover, the risk of ovarian hyperstimulation syndrome (OHSS) is significantly reduced or even eliminated after GnRHa trigger as compared with after hCG trigger (11–13).

The detailed comparisons between the two triggering protocols show differences in the periovulatory and luteal endocrine as well as growth factor profile (2, 14–16). Differences in the biology of the follicle were confirmed when analyzing follicular fluid isolated on the day of oocyte retrieval. Thus, pronounced differences were found in gonadotropin levels as well as in progesterone levels, which were significantly higher after hCG trigger as compared with after GnRHa trigger (6, 17). In addition, the follicular fluid composition of amphiregulin (AREG) and vascular endothelial growth factor (VEGF), which may relate to oocyte maturation and competence and reduction in the risk of OHSS, respectively, differed significantly between the two trigger protocols (14, 15).

To further understand the observed effect during the periovulatory and early luteal phase, the present study investigated whether the mode of triggering was reflected in the transcriptome of the somatic cells of the follicle. Thus, the aim of the present study was to explore possible differences in cumulus cell (CC) and mural granulosa cell (MGC) gene expression in patients who had final oocyte maturation with either hCG or GnRHa.

MATERIALS AND METHODS

Patient and Treatment Cycle

Human granulosa cells were collected in the fertility clinic at Skive Regional Hospital, Denmark. Patients with regular cycles referred for IVF or ICSI owing to male factor and/or tubal disease, unexplained infertility, and mild endometriosis participated in the project after written consent. Twenty women (Supplemental Table 1 presents patient demographics and baseline characteristic) underwent COS with recombinant FSH (Puregon, MSD) from cycle day 2 or 3 until the day of ovulation induction. Individually fixed doses of recombinant FSH were used for the first 6 days of stimulation according to the woman's age, body mass index (BMI), basal FSH level, and antral follicle count. After 6 days, the dose was adjusted according to ovarian response. Once the leading follicle reached the size of 13 mm, cotreatment with the GnRH antagonist ganirelix 0.25 mg (Orgalutran, MSD) was initiated and continued until ovulation induction. When at least two follicles reached the size of 17 mm, ovulation was induced. Patients were randomized (sealed envelopes) to either hCG 5,000 IU (Pregnyl, MSD) or GnRHa 0.5 mg. (Suprefact, Sanofi-Aventis) for ovulation induction. Oocyte retrieval was performed 34 hours after hCG or GnRHa administration.

The study was approved by the Danish Scientific Ethical Committee (VN2004/61).

Purification Method

Immediately after oocyte retrieval, CC and MCG were isolated from two separate follicles for each woman. The cells from one of the follicles were used for microarray, and cells from the other follicle were used for polymerase chain reaction (PCR) validation.

MGC aggregates were separated from the follicular fluid with a pipette and transferred to a 15-mL polystyrene test tube (BD Falcon), avoiding aggregates with blood vessels. Contaminating erythrocytes were removed by erythrocyte lysis buffer (Qiagen; 1:4, placed in an ice bath at 4°C for 10 minutes), whereupon the MGC aggregates were washed in Mg^{2+}- and Ca^{2+}-free phosphate-buffered saline (PBS; Sigma). The MGC were transferred with 20 μL RNase inhibitor (Protector RNase Inhibitor, 5 U/L; Roche Diagnostic) to 0.2-mL tubes (MicroAmp, Applied Biosystems), flash frozen in liquid nitrogen (within 30 minutes after oocyte retrieval), and stored at −80°C until RNA extraction.

A piece of the cumulus surrounding the oocyte from the same follicles was mechanically removed (18 G needles), washed in PBS buffer, and snap frozen in a 0.2-mL cryo tube (MicroAmp, Applied Biosystems) in 5 μL of RNase inhibitor and stored at −80°C until RNA extraction.

RNA Purification

RNA from individual samples was purified using Arcturus PicoPure RNA Isolation kit (Applied Biosystems, Life Technologies) according to the manufacturer's instructions but with small modifications. In short, 100 μL of extraction buffer was used for initial lysis of each sample, except for samples exceeding a volume of 70 μL. For these samples, twice the sample volume of extraction buffer was added. After lysis and centrifugation, 80% of the supernatant was transferred to a tube, where it was mixed 1:1 with 70% ethanol. For MGC samples with large volume, several initial transfers and spins were needed to collect all the RNA in the spin tubes. In the end, the RNA was extracted using 12 μL of nuclease-free water, giving a ~10 μL end volume. The quantity and the integrity of the extracted total RNA were determined by Nanodrop (Nanodrop Technologies) and the Bioanalyzer LabChips (Agilent Technologies), respectively. Sufficient high-quality RNA for array analysis was achieved for 10 (GnRHa) and 9 (hCG) samples in MGC and for 10 (GnRHa) and 6 (hCG) samples in CC.

Microarray Analysis

RNA was amplified and labeled using a pico amplification kit according to the manufacturer's instructions. In short, approximately half of the total RNA from each sample (5 μL