ORIGINAL ARTICLE: ASSISTED REPRODUCTION

Standard human chorionic gonadotropin versus double trigger for final oocyte maturation results in different granulosa cells gene expressions: a pilot study

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Objective: To investigate the messenger RNA (mRNA) expression of reproduction-related genes in granulosa cells (GCs) of patients triggered with hCG compared with patients triggered with GnRH agonist and hCG (double trigger) for final oocyte maturation.
Design: Granulosa cells were obtained at the time of oocyte retrieval, and gene expression was analyzed using quantitative real-time polymerase chain reaction.
Setting: Referral center.
Patient(s): Fifteen women undergoing controlled ovarian hyperstimulation for IVF who received hCG for final follicular maturation and in a subsequent IVF cycle received double trigger.
Intervention(s): Granulosa cells collection.
Main Outcome Measure(s): The expression of genes related to ovarian hyperstimulation syndrome, gap junction, and epidermal-like growth factor in GCs.
Result(s): The mRNA expressions of amphiregulin (2.1 vs. 1, arbitrary unit) and epiregulin (2.5 vs. 1, arbitrary unit) were significantly higher in the double trigger group compared with the hCG group. We found no difference in luteinizing hormone receptor and follicle stimulating hormone receptor mRNA expressions between the two groups. Moreover, although the mRNA expression of pigment epithelium-derived factor (1.5 vs. 1, arbitrary unit) was significantly higher in the double trigger group, no between-group differences were observed in the expression of vascular endothelial growth factor and GnRH receptor. The mRNA expression of conexin43 in cumulus cells (0.7 vs. 1, arbitrary unit) was significantly lower in the double trigger group compared with the hCG group.
Conclusion(s): Our findings suggest that the decreased expression of conexin43 and the increased expression of epiregulin and amphiregulin in the GCs from patients receiving the double trigger may explain the suggested improved oocyte and embryo quality related to the double triggering group. (Fertil Steril® 2016;■:■–■. ©2016 by American Society for Reproductive Medicine.)
Key Words: Double trigger, GnRH agonist, final follicular maturation

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Controlled ovarian hyperstimulation (COH), which combines GnRH antagonist cotreatment and GnRH agonist trigger, has become a common tool aiming to eliminate severe early ovarian hyperstimulation syndrome (OHSS) and to support the concept of an OHSS-free clinic (1–5). However, despite many years of clinical use, the mechanism is still not fully understood. It was suggested that the shorter half-life of the endogenous LH surge induced by GnRH agonist, compared with the continuous high levels of the longer-acting hCG, both of which act by binding to the LH receptor, induces a shorter and milder secretion of vasoactive substances such as vascular endothelial growth factor (VEGF), suggested together with other proinflammatory cytokines to play a fundamental role in the pathophysiology of OHSS (6, 7).

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Moreover, although previous studies have observed higher mature oocytes yield while using GnRH agonist for ovulation triggering (8), lower pregnancy and higher early pregnancy loss rates were also reported (8–10). This poor outcome was explained by the luteal phase insufficiency, caused by GnRH agonist–induced luteolysis and the consequent lower luteal P levels observed in the group of women triggered with GnRH agonist.

Among the possible advantages of GnRH agonist for triggering of ovulation is the endogenous surge of FSH, which has been reported to have several important roles in ovulation and oocyte maturation (11–13), and adding FSH at the time of the hCG trigger was shown by Lamb et al. (14) to enhance oocyte recovery and to improve fertilization rate.

Recently a new treatment modality has been clinically implemented for patients with a high proportion of immature oocytes per number of oocytes retrieved or patients with poor oocytes yield (15, 16). The treatment consists of the coadministration of GnRH agonist and hCG for final oocyte maturation—40 and 34 hours before oocyte pick-up (OPU), respectively. It was assumed that by prolonging the time between ovulation triggering and OPU and the GnRH agonist trigger with the consequent simultaneous induction of an FSH surge, the "double trigger" could overcome any existing impairments in granulosa cell (GC) function, oocyte meiotic maturation, or cumulus expansion, resulting in successful aspiration of mature oocytes, pregnancy, and delivery (17).

In contrast to a sole administration of GnRH agonist for final oocyte maturation, which may cause corpus luteum dysfunction and decreased pregnancy rates, coadministration of hCG prevents the negative luteolytic effects of the GnRH agonist trigger, providing adequate luteal support and improving pregnancy rates.

The aim of this study was to investigate the messenger RNA (mRNA) expression of genes related to OHSS, gap junctions, and the epidermal growth factors, in GCs retrieved from patients undergoing COH for IVF with double trigger compared with the mRNA expression after triggering with hCG only. We chose to investigate expression of genes that have been shown previously to have a different mRNA expression after triggering with GnRH agonist compared with hCG (VEGF and PEDF), genes related to LH and FSH levels (LH and FSH receptors, LHR and FSHR) that may be different after double triggering compared with hCG, and genes that have been shown to be directly related to levels of FSH (epiregulin, amhiregulin,and conexin43).

MATERIALS AND METHODS

The study was approved by our local institutional review board committee, and written, informed consent was obtained from each participating subject. This study included 15 patients undergoing IVF because of different indications (male infertility, unexplained infertility, mechanical infertility, or preimplantation genetic diagnosis).

This was a prospective study. All 15 patients, during both of their subsequent cycles, were treated with a GnRH antagonist protocol, and ovarian stimulation with both hMG and recombinant FSH. Follicular growth was monitored by pelvic ultrasound and serum E_2 levels. Final maturation was induced by hCG (Ovitrelle 250 mg; Merck Serono) (group 1), and in the subsequent IVF cycle the same 15 patients were triggered with hCG plus GnRH agonist (Decapeptyl 0.2 mg; Ferring) (group 2), to improve the oocyte maturation and oocyte yield. Only patients with the two subsequent cycles within 1 year were included in the study. Oocyte retrieval was performed with transvaginal ultrasound–guided needle aspiration. The decision regarding triggering method was made by the treating physician independently.

The GCs were obtained at the time of oocyte retrieval for IVF procedures.

Expanded cumulus cells that surround metaphase II oocytes were obtained during the IVF/intracytoplasmic sperm injection (ICSI) protocol. Oocytes were denuded mechanically using needles before the ICSI procedure.

Mural GCs were collected from pooled follicular fluid, avoiding blood clots, and resuspended in phosphate-buffered saline. After allowing the cells to settle by gravity for a few minutes, the top medium was aspirated. This step was repeated two to three times until the medium was clear. The cells were centrifuged at $500 \times g$ for 5 minutes at room temperature, and the resulting pellets were snap-frozen and subjected to total RNA purification.

The method we used for the isolation of the GCs has been used in previous studies by us and by other authors (18–22).

Total RNA was extracted from cumulus cells and GCs by a Micro or Mini RNA Isolation I Kit (Zymo Research), respectively, according to the manufacturer's instructions. One microliter of RNA solution was used for reverse transcription with a high-capacity cDNA RT kit (Applied Biosystems) according to the manufacturer's instructions. A Fast SYBR Green PCR mix (Applied Biosystems) was used for the polymerase chain reaction (PCR) step. Amplification and detection were performed using the StepOnePlus real-time PCR system (Applied Biosystems) with the following profile: 1 cycle at 95°C for 20 seconds, 40 cycles each at 95°C for 3 seconds, and 60°C for 30 seconds. One microliter of complementary DNA was used per reaction in a total of 10-μL reaction volume. All samples were run in duplicates. The β-actin RNA was chosen as a suitable normalization control gene. The same quantitative real-time PCR protocol was used for all the genes analyzed. Results are expressed as fold change with respect to the experimental control. For primers details, see Supplemental Table 1 (available online).

The follicular fluid aspirated from one follicle per patient was analyzed for P and E_2 concentration on the day of triggering using an E_2 and a P ELISA kit (Enzo Life Sciences) according to the manufacturer's instructions.

Comparisons were performed using the paired two-tailed Student t test. A P value $<.05$ was considered statistically significant.

RESULTS

There were no differences between the two groups in terms of age, duration of COH, and the total dose of gonadotropins required for ovarian stimulation.