New candidate genes to predict pregnancy outcome in single embryo transfer cycles when using cumulus cell gene expression

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Objective: To relate the gene expression in cumulus cells surrounding an oocyte to the potential of the oocyte, as evaluated by the embryo morphology (days 3 and 5) and pregnancy obtained in single-embryo transfer cycles.
Design: Retrospective analysis of individual human cumulus complexes using quantitative real-time polymerase chain reaction for 11 genes.
Setting: University hospital IVF center.
Patient(s): Thirty-three intracytoplasmic sperm injection patients, of which 16 were pregnant (4 biochemical and 12 live birth).
Intervention(s): Gene expression analysis in human cumulus complexes collected individually at pickup, allowing a correlation with the outcome of the corresponding oocyte. Multiparametric models were built for embryo morphology parameters and pregnancy prediction to find the most predictive genes.
Main Outcome Measure(s): Gene expression profile of 99 cumulus complexes for 11 genes.
Result(s): For embryo morphology prediction, TRPM7, ITPKA, STC2, CYP11A1, and HSD3B1 were often retained as informative. Models for pregnancy—biochemical or live birth—complemented or not with patient and cycle characteristics, always retained EFNB2 and CAMK1D together with STC1 or STC2. Positive and negative predictive values of the live birth models were >85%.
Conclusion(s): EFNB2 and CAMK1D are promising genes that could help to choose the embryo to transfer with the highest chance of a pregnancy. (Fertil Steril® 2012;98:432–9. ©2012 by American Society for Reproductive Medicine.)
Key Words: Human cumulus cell, gene expression, oocyte quality, pregnancy, live birth

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Multiple pregnancies still remain a major concern in assisted reproductive technology (ART) when replacing more than one embryo to increase the chance for pregnancy per cycle. Single-embryo transfers (SETs) avoid this risk. To improve the pregnancy chance per cycle when performing SET, more criteria to select the embryo with the highest implantation potential may be implemented. For several years now, cumulus cell (CC) gene expression has been investigated as a tool to predict the quality of the oocytes (1, 2) along with the morphologic criteria of the embryos. CC analysis is an obvious choice for a noninvasive analysis, because CCs are in direct contact with the oocyte and voided during the intracytoplasmic sperm injection (ICSI) procedure.

In a previous study, models were designed to predict embryo morphology features and clinical pregnancy with the use of gene expression in CCs that had been removed shortly before ICSI (3). The genes analyzed originated

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from previous experiments using Affymetrix arrays resulting in 500 genes related to pregnancy (unpublished data). Out of eight tested genes with quantitative polymerase chain reaction (PCR) (3), the four most informative genes were transient receptor potential cation channel, subfamily M, member 7 (TRPM7) and inositol-trisphosphate 3-kinase A (ITPKA) for better cleavage-stage embryo prediction and syndecan-4 (SDC4) and versican (VCAN) for pregnancy prediction. Those four genes together with seven newly chosen genes (also originating from the same array data) were retained to analyze cumulus complexes in the present study. The additional seven genes were: stanniocalcin-1 (STC1), stanniocalcin-2 (STC2), parathyroid hormone–like hormone (PTHLH), ephrin-B2 (EFNB2), cytochrome P450, family 11, subfamily A, polypeptide 1 (CYP11A1), hydroxy-delta5-steroid dehydrogenase, 3beta- and steroid delta-isomerase 1 (HSD3B1), and calcium/calmodulin-dependent protein kinase 1D (CAMK1D).

Stanniocalcin is a secreted glycoprotein hormone that was first described in bony fish, where it prevents hypercalcemia (4). In cultured rat granulosa cells, STC1 and STC2 decreased the FSH-induced progesterone production which was paired with a decrease in CYP11A1 and HSD3B (5, 6). In swine granulosa cell cultures, STC1 production increased when the granulosa cells were put in hypoxic conditions (7). The close association with steroidogenesis of both STCs led us to investigate CYP11A1 and HSD3B1 in the same sample set. CYP11A1 is responsible for the conversion of cholesterol to pregnenolone, the first and rate-limiting step in the synthesis of the steroid hormones (8). HSD3B transforms pregnenolone into progesterone (9). HSD3B1 has previously been described as a positive marker when comparing the expression of human follicular cells from an oocyte resulting in pregnancy with those from an oocyte giving an embryo arrested in development (10).

PTHLH is known to be important during the development of several organs, including the mammary glands (11). PTHLH is involved in lactation possibly by regulating the mobilization and transfer of calcium to the milk (12). It also plays a role in the development of hypercalcemia in patients with small cell carcinomas of the ovary (13). In porcine granulosa cell culture it was demonstrated that transforming growth factor (TGF) β1 could increase the PTHLH concentrations (14), and TGF-β1 family members such as GDF9 and BMP15 are known to help regulate CC function (15).

EFNB2 is a transmembrane protein that belongs to the largest subfamily of receptor protein–tyrosine kinases and has been implicated in mediating developmental events, especially in the nervous system and in erythropoiesis (16). EFNB2 expression was described in human granulosa cells mainly during luteinization (17). The EFNB2 receptor, EPHB2, was shown to increase in mice granulosa cells when injecting pregnant mare serum gonadotropin in wild-type mice but not in estrogen beta-receptor–null mice. This suggests a regulation of EPHB2 expression by FSH and the estrogen beta-receptor (18).

CAMK1D encodes a member of the Ca-/calmodulin–dependent protein kinase 1 subfamily of serine/threonine kinases. CAMK1D itself has never been reported in the ovary. Other members of the gene family have, however, been related to ovarian events such as oogenesis (CAMK1, CAMK2A, and CAMKG), folliculogenesis (CAMK4), ovulation (CAMK4 in granulosa (GC) and theca cells (TC)), and corpus luteum formation where it may serve as a Ca^{2+}-dependent effector mechanism to maintain basal CYP11A gene expression (CAMK4) (19–21).

Genes coding for diverse pathways in the cell (calcium, steroidogenesis, extracellular matrix formation, and TGF-β–related pathways) were chosen to enhance the predictive power of the models described hereafter and to avoid collinearity.

First, we evaluated the inter- and intrapatient variation for the 11 genes in CCs. Second, we explored the possibility to predict the most relevant morphologic features of day 3 and day 5 embryos with the 11 genes complemented with patient and cycle characteristics with the use of stepwise multiple regression analysis.

In a third multiparametric approach, models only allowing gene expression values were determined first for biochemical pregnancy (positive hCG value) and second only for live birth pregnancy. In the next step, patient and cycle characteristics were introduced in the models if they improved them significantly.

Finally, results were validated by randomly splitting the patients into two halves, with the first group building the model for pregnancy and the second group testing the predictive power of the polynomials.

MATERIALS AND METHODS

Patient Population

This study was approved by the Ethical Committee of Universitair Ziekenhuis Brussel, and patient consents were obtained. Consecutive patients were chosen based on the stimulation protocol and transfer strategy (SET). Patients underwent controlled ovarian hyperstimulation by administration of GnRH antagonist combined with recombinant FSH (Gonal-f, Serono [n = 5]; or Puregon, Shering-Plough [n = 28]). Follicular development was monitored by vaginal ultrasound. The endocrine profile was evaluated by analysis of serum E_2, P, FSH, and LH. A dose of 10,000 IU hCG was administered to induce final follicular maturation when at least three follicles 17 mm in diameter were observed by transvaginal ultrasound. Cumulus oocyte complexes (COCs) were aspirated 36 hours later. ICSI was performed as described previously (22), and embryo culture was performed in sequential media of Vitrolife G7. Out of the 33 patients, 13 had an embryo transfer on day 3 and 20 had a blastocyst transfer on day 5.

Infertility causes were: male factor (n = 18), female factor (ovulation disorder [n = 1], endometriosis [n = 1], and myomatosis [n = 1]), a combination of male and female factors (tubal pathology [n = 2] and PCO [n = 2]), and idiopathic (n = 8).

Out of the 33 patients, 16 were pregnant after SET (four were biochemical pregnancies, and 12 delivered a live born).

Collection of CCs

COCs were retrieved 36 hours after hCG and collection of the CCs was carried out as described earlier (3). Briefly: After washing the COCs at collection, oocytes were denuded in