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ARTICLE

A unique biological in-vivo model to evaluate follicular development during in-vitro maturation treatment

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Abstract The aim of this study was to identify the size in which the dominant follicle acquires the ability to produce a functional corpus luteum. This observational study includes 15 women with ovulatory cycles who underwent human chorionic gonadotrophin (HCG)-primed in-vitro maturation (IVM) treatments without embryo transfer. All patients received subcutaneous injection of HCG 10,000 IU 38 h prior to oocyte retrieval. Five to seven days following retrieval, serum concentrations of progesterone and oestradiol were measured along with ultrasound scan measuring the antral follicle count. Using receiver operating characteristic curves and the Youden index (*J*), this study clearly shows that the diameter of the dominant follicle at the time of the LH surge is a good predictor for its ability to form a progesterone-producing corpus luteum (area under the curve 0.94). These findings revealed that the dominant follicle develops the competence to form a corpus luteum, signified by the production of more than 10 nmol/l serum progesterone at 5–7 days from oocyte retrieval, as soon as it acquires a diameter of 10.5–12.0 mm. In addition, a new cohort of viable antral follicles can be identified as early as 5–7 days following IVM oocyte retrieval.

KEYWORDS: corpus luteum, dominant follicle, fertility preservation, folliculogenesis, IVM

Introduction

In-vitro maturation (IVM) of oocytes is an evolving technique, which was initially indicated for patients with polycystic ovaries (PCO) and polycystic ovary syndrome (PCOS) in order to avoid hormonal ovarian stimulation, thus eliminating the risk of ovarian hyperstimulation syndrome (OHSS). This technology is now being extended to various

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infertility and fertility preservation treatments (Elizur et al., 2008a,b).

IVM treatment in ovulatory women provides a unique opportunity to study follicular development and the characteristics of the dominant follicle. It has been suggested that the first observation of a dominant follicle by ultrasound scan occurs between menstrual cycle days 5 and 12, at a mean diameter of 11 mm (range 9–15) and that this correlates strongly with a clear rise in plasma oestradiol concentrations (van Santbrink et al., 1995). However, the size in which the dominant follicle acquires the ability to respond to the LH surge and to produce a progesterone-secreting corpus luteum is still to be determined.

This study was designed to estimate the size in which the dominant follicle acquires the ability to produce a progesterone-secreting corpus luteum, by using a unique biological in-vivo model of human chorionic gonadotrophin (HCG)-primed IVM treatments without hormonal luteal support.

Materials and methods

Patients

This study includes HCG-primed IVM cycles performed at the McGill Reproductive Centre, Montreal, Canada, in which no embryo transfer was scheduled. Treatments were undertaken for fertility preservation or as part of a clinical trial (Chian et al., 2008). Full details of this clinical trial have been published previously (Chian et al., 2008). Briefly, the aim of that study was to evaluate the obstetric outcomes with oocyte vitrification after IVM of immature oocytes. Therefore, immature oocytes were retrieved during an HCG-primed IVM cycle, matured in vitro and vitrified. One to two months later the vitrified oocytes were warmed and fertilized and the cleaved embryos transferred into the patient. All patients for fertility preservation ($n = 6$) were diagnosed with breast cancer. The clinical trial and fertility preservation treatments were approved by the hospital ethics committee and all women signed informed consent. At the study centre, patients are offered IVM treatment if they have an antral follicle count (AFC) of 20 or more. In addition, for this study only women with regular ovulatory menstrual cycles were included ($n = 15$).

IVM protocol

IVM procedure was performed as follows. The first ultrasound scan was scheduled between days 2 and 5 of the menstrual cycle. During this scan, the number and size (average of two perpendicular dimensions) of all antral follicles (dimension of 2–10 mm) were recorded along with the thickness of the endometrial lining (Tan et al., 2002). A second ultrasound scan was performed between days 6 and 10 of the cycle and, in addition to all the above-described measurements, the diameter of the largest follicle was recorded. All patients received one subcutaneous injection of 10,000 IU HCG (HCG; Profasi; Serono, Oakville, ON, Canada) 38 h prior to oocyte retrieval (Son et al., 2008). On the day of oocyte retrieval, a blood sample was withdrawn to measure serum oestradiol and progesterone concentrations. All visible follicles ($\geq$4 mm) were aspirated.

Mature oocytes on collection day were either inseminated on the same day by intracytoplasmic sperm injection (ICSI) or vitrified, while immature oocytes (germinal-vesicle and metaphase-I stage) were cultured in 1.0 ml of IVM medium (Cooper Surgical, CT, USA) supplemented with 75 mIU/ml of FSH and LH.

As no embryo transfer was scheduled, women did not receive any hormonal luteal support following oocyte retrieval and were invited for a follow-up visit 5–7 days following the procedure. An additional ultrasound scan was performed during this visit to measure the number and size (average of two perpendicular dimensions) of all visible antral follicles and blood was withdrawn to determine serum oestradiol and progesterone concentrations. All the ultrasound scans were performed by one of three fellowship trainees in reproductive endocrinology and infertility.

Serum oestradiol and progesterone concentrations were measured using the Centaur machine, an automated immunoassay instrument that uses chemiluminescence as the detection method.

Statistical analyses

Statistical analyses were performed using the Chi-squared, Fisher's exact, Mann–Whitney *U* or Student *t*-tests when appropriate. All *P*-values quoted are two-sided and values <0.05 indicate statistical significance. Analyses were performed using the StatDirect software.

Receiver operating characteristics (ROC) curves and the Youden index (*J*) were used to evaluate the effectiveness of the dominant follicle diameter in predicting the formation of a progesterone-producing corpus luteum in response to LH surge. A serum progesterone concentration >10 nmol/l 5–7 days after oocyte retrieval was considered indicative of luteal phase. *J* index ranges between 0 and 1, with values close to 1 indicating relatively high effectiveness of the biomarker and values close to 0 indicating limited effectiveness (Youden, 1950). The area under the curve (AUC) provides an objective measure of the discriminatory power of a screening test. Its values range between 0.5 (the classes are identical) and 1.0 (there is a threshold value that can achieve a perfect separation between the classes).

Results

Fifteen women were included in this study. Only women with regular ovulatory menstrual cycles were included. **Table 1** presents patients and cycle characteristics at the study cycle. It should be emphasized that between 5 and 7 days after oocyte retrieval, the mean ± SD AFC was 16.8 ± 5.3, which was more than 60% of the AFC prior to oocyte retrieval. In several cases which are not included in this study, second oocyte retrieval was performed 7 days following the first, and viable oocytes were obtained and fertilized.

Table 2 summarizes cycle characteristics on the day of oocyte retrieval and 5–7 days later. Interestingly, metaphase-II (MII)-stage oocytes were identified on the day of retrieval (in-vivo matured), even in cycles in which the largest follicle measured 7 mm in diameter. This suggests that