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ARTICLE

Spindle and chromosome configurations of human oocytes matured *in vitro* in two different culture media

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Abstract In-vitro maturation can have deleterious effects on spindle formation and proper chromosome alignment in human oocytes and can be profoundly affected by culture conditions. This study compared the spindle presence and location with the maturation rate of germinal vesicle (GV) oocytes cultured in two different media: G1.2 and G1.2 supplemented with follicle-stimulating hormone, human chorionic gonadotrophin and 17β-oestradiol. A total of 304 oocytes were retrieved from 101 women undergoing IVF treatment with intracytoplasmic sperm injection. Spindle presence was recorded using the Polscope. Spindle morphology was evaluated with immunocytological staining for α-tubulin and chromatin. Twenty-one in-vitro matured oocytes with the presence of spindle and ten of their corresponding polar bodies (PB) were also assessed for aneuploidy. A significantly increased maturation rate (69.7%) was observed after 24 h in the supplemented culture media compared with the G1.2 media (56.6%; $P < 0.05$). The proportions of metaphase II (MII) oocytes with spindle presence and abnormal spindle morphology were similar in the two culture media. Also, 76.9% of MII and 70% of PB had chromosomal abnormalities. In conclusion, supplementing culture media may increase the oocyte maturation rate *in vitro*, but does not necessarily indicate the presence of a birefringent spindle, or normal spindle and chromosomal alignment.

KEYWORDS: culture medium, FISH, immunostaining, oocyte maturation, polscope, spindles

Introduction

In-vitro maturation (IVM) of human oocytes was first demonstrated by Edwards in 1965 (Edwards, 1965), preceding by more than a decade his first successful IVF attempt in 1978 (Steptoe and Edwards, 1978). Since then, IVF has followed different pathways, which have led it far from the necessity of IVM. Assisted reproduction techniques

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became more effective and the stimulation protocols provided many alternatives using either gonadotrophin-releasing hormone (GnRH) agonists for pituitary suppression in long or short protocols (Kerin, 1989), or GnRH antagonists (in order to prevent premature LH surge) which provide a patient-friendly solution (Al-Inany et al., 2006).

Lately there is increasing demand for less drug-oriented, lower risk, less expensive and more patient-friendly approaches to assisted reproduction, embracing gentle stimulation protocols and IVM of oocytes. Extensive research plus the latest pregnancies successfully achieved in Canada after IVM (Al-Sunaidi et al., 2007) suggest the usefulness and the need for improvement of this technique.

This renewed interest is of great importance for women with polycystic ovary syndrome who are classified as high responders to ovarian stimulation protocols. Their vigorous response to stimulation may lead to ovarian hyperstimulation syndrome, which in its severe forms leads to cycle cancellation, hospitalization and, in rare cases, death (Enskog et al., 1999; Rizk, 1995). IVM seems to be a viable alternative for these women, since the milder stimulation protocol required in this procedure is expected to significantly reduce the risk of ovarian hyperstimulation syndrome.

However, if the outcome measures in the specific subgroup should become comparable to classic protocols, then it is understandable that they could soon encompass a wider population of women undergoing IVF, since protocols including IVM would lead to milder and more simplified ovarian stimulation protocols and a reduction in the cost of treatment (Jurema and Nogueira, 2006). There are two other scenarios where improvement of IVM technology will prove beneficial. The first encompasses the salvage of immature oocytes for IVF (intracytoplasmic sperm injection; ICSI) after standard stimulation and the second involves fertility preservation with the cryopreservation of oocytes matured *in vitro*. The potential of this technology to restore fertility in women anticipating sterility secondary to cancer treatment is an important and exciting prospect (Elizur et al., 2007; Huang et al., 2008).

Recent clinical results from in-vitro matured human oocytes are promising (Chian et al., 2003). However, the developmental and implantation potential of IVM oocytes have been reported to be low (Mikkelsen et al., 1999). It has been suggested that the reduced developmental potential in human oocytes matured *in vitro* may be attributable to sub-optimal culture conditions, incomplete oocyte growth or abnormal cytoplasmic maturation and distortion of spindle apparatus (Moor et al., 1998). Disruption of spindle morphology leads to chromosome abnormalities that are reported to be high in human oocytes, especially among those matured *in vitro* (Gras et al., 1992; Racowsky and Kaufman, 1992).

A major technological innovation that gave a tremendous boost to IVM studies is the Polscope, which allows the non-invasive study of spindles during the maturation of oocytes (Liu et al., 2000). It was established that the spindle images obtained with the Polscope in living human oocytes are coordinate with those in fixed oocytes as imaged by confocal microscopy (Wang and Keefe, 2002). By using the Polscope, it was found that the presence of a birefringent meiotic spindle in human oocytes could predict a higher embryonic developmental competence (Moon et al., 2003) and a higher fertilization rate (Wang et al., 2001a,b). However, the relative position of the spindle within the oocyte did not appear to influence the developmental potential of embryos (Moon et al., 2003).

The most important factor affecting oocyte maturation *in vitro* is the culture conditions and specifically the composition of the culture media. Although numerous data have been accumulated from animal studies, the current rationale for choosing a specific medium for IVM of immature oocytes appears to stem largely from adapting methods developed from culturing other cell types (Chian et al., 2003). The most common culture media used in IVM are G1, IVF20 and CCM supplied by Vitrolife (Goterborg, Sweden) and Medicult (Mollehaven, Denmark). The composition of culture media used successfully for maturation of human oocytes is surprisingly similar to that originally developed for maturation of oocytes in follicle culture *in vitro*. The presence of follicle support cells in culture is necessary for the gonadotrophin-mediated response required to mature oocytes *in vitro*. Gonadotrophin concentration and the sequence of follicle-stimulating hormone (FSH) and FSH/LH exposure may be important for human oocytes, particularly those not exposed to the gonadotrophin surge *in vivo* (Trounson et al., 2001).

The aim of the present study was to compare the maturation rate of germinal vesicle (GV) oocytes cultured in two different media and to record the spindle presence, location (deviation angle) and morphology as well as the chromosome abnormalities.

Materials and methods

Source of oocytes

The study was approved by the local institutional review board. A total of 304 oocytes at the GV stage were retrieved from 101 consenting patients undergoing the ICSI programme in the study centre between January and December 2006. The patients' ages ranged from 29 to 43 years, with a mean ± SD of 33.2 ± 2.5 years. Patients were allocated to the study when a minimum of 10 metaphase II (MII) oocytes and two oocytes at the GV stage were obtained after retrieval. **Table 1** summarizes the aetiology of infertility of patients included in the study and the clinical outcome of their stimulation cycles.

Monitoring and oocyte preparation

Ovarian stimulation was achieved by a long down-regulation with a GnRH-agonist and recombinant FSH, either with Gonal-F (Merck Serono Europe, London) or Puregon (Organon, Oss, Holland) to induce follicular growth. The mean ± SD of the starting dose of recombinant FSH was 345 ± 134 (IU) and for total dose was 3150 ± 950 (IU) during stimulation cycles. When three leading follicles were >18 mm in diameter, 10,000 IU human chorionic gonadotrophin (HCG) (Pregnyl, Organon) was administered, 36 h before oocyte collection. Oocyte–cumulus-complexes were obtained via ultrasound-guided transvaginal aspiration.

Cumulus and corona cells were removed from oocytes by exposure to HEPES-buffered medium (Sage IVF, Thumbull, CT, USA) containing 40 IU/ml hyaluronidase (IV-S, H4272;