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ARTICLE

Culture of human oocytes with granulocyte-macrophage colony-stimulating factor has no effect on embryonic chromosomal constitution

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Inge Agerholm has been laboratory director at the Fertility Clinic at Brædstrup Hospital, Brædstrup Denmark, since 1996. In 2007 she obtained a PhD degree from University of Aarhus. The thesis described the relation between morphology and chromosome constitution of human embryos. She is currently a board member of the Danish Fertility Society and the Nordic IVF Laboratory Society as well as a National Representative in ESHRE. Her main interest is early embryo development and selection together with aneuploidy in human embryos.

Abstract The effect on ploidy rate in donated human oocytes after in-vitro culture with recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF; 2 ng/ml) from fertilization until day 3 was examined in a multicentre, prospective placebo-controlled and double-blinded study including 73 women donating 86 oocytes. The primary endpoint was to investigate the chromosomal constitution of human embryos (fluorescence in-situ hybridization analysis for chromosomes 13, 16, 18, 21, 22, X and Y) cultured with or without GM-CSF. The secondary endpoints were number of top-quality embryos (TQE) and number of normally developed embryos evaluated morphologically on day 3. The cytogenetic analyses demonstrated non-inferiority and therefore the chromosomal constitution of human embryos cultured *in vitro* in the presence of 2 ng/ml GM-CSF was no worse than the control group cultured without GM-CSF. In-vitro culture of human embryos in the presence of 2 ng/ml GM-CSF resulted in 34.8% (8/23) uniformly normal embryos. Culture without 2 ng/ml GM-CSF resulted in 33.3% (9/27) uniformly normal embryos. A trend towards a higher number of TQE in the test group was observed; however, due to lack of TQE in the control group, this was considered a random finding.

KEYWORDS: chromosomes, cytokines, embryonic development, FISH analysis, GM-CSF, granulocyte-macrophage colony stimulating factor

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Introduction

Besides being a haematopoietic cytokine that acts as a key regulator of host defence and response to external insult and injury, granulocyte-macrophage colony-stimulating factor (GM-CSF) is also believed to play an important role in preimplantation embryo development and regulation of placental morphogenesis (Robertson, 2007). In humans, the expression of GM-CSF has been demonstrated in endometrial tissue (Giacomini et al., 1995), in Fallopian tubes (Zhao and Chegini, 1994), in theca cells surrounding large follicles and in ovarian luteal cells (Zhao et al., 1995), in follicular fluid and granulosa-lutein cell culture (Jasper et al., 1996), in placenta (Berkowitz et al., 1990) and in maternal decidual tissue (Dudley et al., 1990). The synthesis of GM-CSF in the uterus and Fallopian tubes is primarily localized to the oestrogen-primed luminal and glandular epithelial cells. Several studies indicate that synthesis is cycle dependent and thus regulated by ovarian steroids (Robertson et al., 1996). Two studies found the highest level of GM-CSF expression in endometrial epithelial cells in the secretory phase (Sharpe-Timms et al., 1994; Zhao and Chegini, 1999), while Giacomini et al. (1995) could not reproduce this finding. However, Zhao and Chegini (1994) demonstrated that the expression of GM-CSF in the epithelial cells of the Fallopian tube, primarily in the ampullary and isthmus region, was considerably higher during mid—late follicular and early—mid secretory phase. The concentration of uterine GM-CSF rises in mice after exposure to semen and remains high for the first few days after conception. At the time of embryo implantation, the concentration declines due to an inhibitory effect of progesterone. After implantation, the production of GM-CSF is established in the early placenta and maintained throughout pregnancy together with synthesis in the maternal decidual tissue. (Robertson and Seamark, 1990; Robertson et al., 1992; Robertson, 2005, 2007). The GM-CSF receptor has been detected from the fertilized oocyte through to blastocyst stage in both mice and humans (Sjöblom et al., 2002).

The importance of GM-CSF is illustrated by studies of mice with a null mutation in the GM-CSF gene. These mice have impaired reproductive capacity with a higher rate of fetal loss in late gestation and a higher mortality rate during early post-natal period. Male pups especially seem most susceptible to being lost. Surviving pups are smaller and for male pups the growth impairment continues into adulthood. (Robertson, 2007; Seymour et al., 1997).

Studies of in-vitro culturing of mouse embryos in the presence of recombinant murine GM-CSF have shown to be associated with increased glucose uptake and reduced apoptosis (Robertson et al., 2001). The maximum effect of GM-CSF on mouse blastocysts regarding hatching and implantation stages was found at a concentration of 2 ng/ml (Robertson et al., 2001). A large follow-up study on mouse embryos has shown that the addition of this concentration of GM-CSF to IVF culture media improved the implantation rate and alleviated deficiencies in placental structure and fetal growth, including to some degree the post-natal growth pattern when compared with culture media without GM-CSF (Sjöblom et al., 2005).

In-vitro culturing of human embryos in the presence of 2 ng/ml recombinant GM-CSF has demonstrated an accelerated embryo development, a two-fold increase in the proportion of early cleavage embryos that develop to blastocyst stage, an increased viable inner cell mass combined with a reduction in apoptotic nuclei, increased in-vitro hatching and embryo adhesion to extracellular matrix-coated culture dish. These favourable effects of GM-CSF were demonstrated in two different culture media systems (IVF-50/S2 and G1.2/G2.2) (Sjöblom et al., 1999, 2002).

Based on these studies, it is believed that GM-CSF secretion into the uterus and Fallopian tubes has an important physiological role and that the presence of recombinant GM-CSF to IVF culture media will improve the competence of the embryo/blastocyst to implant (Robertson, 2007). But before this cytokine is added to standard IVF culture media and used in human in-vitro fertilization programmes where the embryos/blastocysts are transferred to the women, it is important to verify that GM-CSF does not have any negative influence on the chromosomal constitution of the human embryo.

The purpose of this study was to address the effect on ploidy rate of exposing donated human oocytes to recombinant human GM-CSF (2 ng/ml) during in-vitro culture from fertilization until the day-3 embryo stage. The primary endpoint was to investigate the chromosomal constitution of human embryos cultured with or without GM-CSF by performing fluorescence in-situ hybridization (FISH) analysis for chromosomes 13, 16, 18, 21, 22, X and Y. The secondary endpoints were number of top-quality embryos (TQE) and number of normally developed embryos evaluated morphologically on day 3.

Materials and methods

Trial design

The design was a multicentre prospectively randomized, placebo-controlled and double-blinded in-vitro study with two parallel groups for non-inferiority evaluation of in-vitro effect on ploidy rate and efficacy with regards to embryo development. The aim of the study was to assess the effect of recombinant human GM-CSF (2 ng/ml) on selected chromosomes in human embryos, when added to standard culture media. Two Danish centres (Rigshospitalet, Copenhagen, Denmark and Braedstrup Hospital Denmark) participated in the study, which was conducted from January 2006 until July 2006. For each centre, a randomization list and sealed envelopes were prepared. The oocytes were randomized 50:50 between the blinded media (medium A and medium B). The randomization lists were prepared using the computer program www.randomization.com (accessed 21/12/09), and kept by sponsor.

Patients

The inclusion criteria were indication for IVF or intracytoplasmic sperm injection (ICSI) treatment, female age between 25 and 37 years (both inclusive) and regular menstrual cycles (21—35 days, both inclusive). Furthermore, a minimum of six aspirated oocytes was required for the patient's own treatment after donation.