Impact of follicular-fluid meiosis-activating sterol in an albumin-based formulation on the incidence of human pre-embryos with chromosome abnormalities

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Objective: To evaluate the effect of adding follicular-fluid meiosis-activating sterol (FF-MAS) in a novel 0.2% recombinant human albumin–based formulation to cumulus-enclosed oocytes on chromosomal status and development of pre-embryos.
Design: Multicenter, prospective, randomized, open (double-blind for vehicle and FF-MAS groups), four parallel groups, controlled trial.
Setting: Four public IVF clinics in Denmark.
Patient(s): Two hundred eighteen women undergoing IVF donated 483 oocytes.
Intervention(s): Follicle-stimulating hormone/hCG-primed cumulus-enclosed oocytes randomized to 4 hours of exposure to medium with 1 or 10 μmol/L of FF-MAS dissolved in 0.2% recombinant human albumin, medium with 0.2% recombinant human albumin (vehicle control), or medium alone (control) before insemination.
Main Outcome Measure(s): Primary endpoint: incidence of human pre-embryos with chromosomal abnormalities. Secondary endpoint: fertilization rate, cleavage rate, and pre-embryo quality assessed after 68 hours of culture.
Result(s): At pre-embryo level, the overall abnormality rates in the control, vehicle control, and 1- and 10-μmol/L FF-MAS groups were 53%, 39%, 42%, 53%, respectively, and at blastomere level 49%, 44%, 44%, and 48%, respectively. After 20 and 26 hours, the fertilization rates were between 67% and 71% in all groups. No differences in the cleavage rates were observed.
Conclusion(s): The concentrations of FF-MAS in a novel 0.2% recombinant human albumin–based formulation of FF-MAS did not increase the risk of chromosomal abnormalities in pre-embryos or blastomeres. No statistically significant differences in fertilization rate, cleavage rate, or number of good quality pre-embryos were found among the four groups. (Fertil Steril® 2005;84(Suppl 2):1269–76. ©2005 by American Society for Reproductive Medicine.)

Key Words: Chromosomal abnormality, follicular-fluid meiosis-activating sterol, FF-MAS, recombinant human albumin, human, oocyte

Previous studies have shown that follicular-fluid meiosis-activating sterol (FF-MAS), which is believed to be secreted by the cumulus cells in response to FSH stimulation and the LH surge, plays an important role in the control of oocyte meiosis in mammals (1–3). Human FF-MAS has been synthetically manufactured in a 0.2% ethanol formulation (Novo Nordisk) but only used for research purposes (4, 5). The addition of FF-MAS to culture media has been shown to induce resumption of meiosis in mouse oocytes (cumulus enclosed as well as denuded) in vitro in a dose-dependent manner (6), an increased rate of fertilization and development of pre-embryos (7), and improvement of the cytoplasmic maturation (8, 9). Further, normal spindle and chromosome alignment in mouse oocytes has been found after exposure to FF-MAS (8, 10), together with a possible protection from precocious chromosome segregation in mice oocytes. Recently, Marin Bivens et al. (11, 12) have shown that both FF-MAS and a synthetic analogue of FF-MAS improved mouse oocyte quality by promoting nuclear as well as cytoplasmic maturation in vitro.

Grøndahl et al. (13) found a significantly higher frequency of fully matured metaphase II human oocytes after in vitro maturation for 30 hours with 20 μmol/L FF-MAS compared to spontaneously matured oocytes in women with polycystic ovarian syndrome. Additionally, Cavilla et al. (14) demonstrated that MAS (10 or 30 μmol/L) had positive effects on immature oocytes retrieved from unstimulated patients with polycystic ovaries (in this study the diagnoses were based on the ultrasound appearance of the ovaries combined with a history of oligo- or amenorrhoea and blood hormone measurements) and stimulated patients undergoing ICSI.

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However, in a recent study by Bergh et al. (15) the addition of relatively high doses of FF-MAS (5 and 20 μmol/L) in a 0.2% ethanol formulation to donated denuded mature FSH/hCG-primed human oocytes 4 hours before and 20 hours after insemination had detrimental effects on the incidence of chromosome abnormalities in the pre-embryos as well as on the cleavage rate and quality of pre-embryos. There are several possible explanations for this, including the fact that the oocytes were denuded (e.g., absence of cumulus cells), the timing of FF-MAS exposure (both before and after insemination), and/or the exposure time to FF-MAS. An inert control (water for injection) as well as an ethanol 0.2% vehicle control was included in the study, and no negative effect on the incidence of chromosome abnormalities was observed in the ethanol group.

In another study by Loft et al. (16), donated human cumulus-enclosed FSH/hCG-primed oocytes were exposed to 0.1, 1, or 10 μmol/L of FF-MAS in a 0.2% ethanol formulation for 4 hours before insemination. Again two control groups were included: water for injection and ethanol 0.2%. Unexpectedly, this study showed that the incidence of chromosome abnormalities in the blastomeres was increased in the ethanol 0.2% control group and the FF-MAS groups, when compared with the inert control group. No negative effect of FF-MAS was observed and the detrimental effect was attributed to the ethanol vehicle.

As a consequence of these results, the formulation of FF-MAS was changed from the ethanol-based to a recombinant human 0.2% albumin–based formulation.

The primary endpoint of this safety study was to evaluate the effect of adding one of two different concentrations of FF-MAS (1 or 10 μmol/L) in a novel recombinant human albumin–based formulation to cumulus-enclosed FSH/hCG-primed human oocytes on the incidence of pre-embryos with chromosome abnormalities. The rate of fertilization and cleavage together with the quality of the human pre-embryos were secondary endpoints.

MATERIALS AND METHODS

Trial Design

The study was designed as a phase 1, multicenter, prospectively randomized, open (double-blind for the vehicle control and active groups), four parallel groups, and controlled in vitro study. The design of this safety and efficacy study was identical to the two previously conducted FF-MAS studies, described in detail by Loft et al. (16) and Bergh et al. (15). A randomization list and corresponding sealed codes with the vial numbers were prepared by Clinical Trials Logistics, Novo Nordisk, Copenhagen, Denmark, and the code was only broken after completion of the study. An independent data monitoring board (Quintiles, Copenhagen, Denmark) monitored the trial. Four Danish hospitals participated in this study, which was conducted from May 2002 to December 2002.

Patients

Criteria for inclusion were indication for IVF or ICSI, female aged 25–37 years, and regular menstrual cycles (21–35 days). A minimum of six oocytes should be available to the couple after donation. No donated pre-embryos entering the trial were transferred to the patients. Exclusion criteria were medical conditions or genetic disorders prohibiting IVF/ICSI or interfering with the interpretation of the results and the use of any investigational drug within 30 days before oocyte retrieval.

Two hundred eighteen patients donated 483 oocytes. Forty-five percent of the women donated one oocyte, 32% two, and 22% three or more oocytes. Demographic characteristics of the patients according to trial site are shown in Table 1. Four women failed to meet the age inclusion criteria (two were 24 years and two 38 years old at the date of inclusion). All four violations were considered minor, and all women were considered compliant with the protocol and included in the data analyses. The mean age of the women was 31.4 (±3.3) years (range, 24–38 years). Apart from fewer smokers at site 4, there were no major differences among the sites. The majority of the women had been infertile for 2–5 years (77%), and male factor together with tubal factor were the most frequent reasons for infertility (44% and 31%, respectively). Of the study population, 64% had never undergone infertility treatment and 52% had never been pregnant. Fewer than 14% of the women had delivered live babies and 18% reported miscarriages, 7% ectopics, and 8% terminations.

Treatment and Donation

Patients were stimulated using long protocols (e.g., down-regulation in the luteal phase with GnRH agonists) (Synarela; Pharmacia, Copenhagen, Denmark; or Suprefact; Aventis Pharma, Hørsholm, Denmark) for at least 14 days, or short protocols using GnRH antagonists when the leading follicle was approximately 14 mm (Orgalutran; Organon, Skovlunde, Denmark; or Cetrotide; Serono, Copenhagen, Denmark). Patients were stimulated with recombinant FSH (Puregon; Organon; or Gonal-F; Serono) with an average daily dose from 100 to 300 IU of hCG (10,000 IU; Profasi; Serono; or Pregnyl; Organon) was administrated when at least 3 follicles of 17 mm were registered by ultrasound. Oocyte aspiration was performed according to the normal procedure of each clinic. Depending on the number of oocytes the patients wished to donate, the selection procedures were: the third aspirated oocyte from the first ovary, the third aspirated oocyte from the second ovary, and an oocyte randomly chosen among the remaining oocytes. The time interval from aspiration to randomization by treatment group was less than 90 minutes. The oocytes were inseminated with sperm from a single donor whose fertility had been proven by documented paternity and whose cells had been shown to exhibit a normal karyotype. (Cryos International Sperm Bank, Aarhus, Denmark).