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The type of GnRH analogue used during controlled ovarian stimulation influences early embryo developmental kinetics: a time-lapse study☆

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ABSTRACT

Objective: To explore if the GnRH analogue used for controlled ovarian stimulation (COS) and the ovulation triggering factor (GnRH agonist + hCG triggering versus GnRH antagonist + GnRH agonist triggering) affect embryo development and kinetics.
Study design: In a retrospective cohort study in the Instituto Valenciano de Infertilidad (IVI) Alicante and the Instituto Universitario-IVI Valencia, Spain, 2817 embryos deriving from 400 couples undergoing oocyte donation were analysed. After controlled ovarian stimulation and IVF/intracytoplamic sperm injection, the timing of embryonic cleavages was assessed by a video time-lapse system. The results were analysed using Student's *t* test for comparison of timings (hours) and Chi-squared test for comparison of proportions. A p-value < 0.05 was considered to be statistically significant.
Results: Embryos from cycles co-treated with GnRH antagonist + GnRH agonist ($n = 2101$) cleaved faster than embryos deriving from patients co-treated with GnRH agonist + hCG ($n = 716$): these differences were significant at the first stages of development but they disappeared as long as the embryo developed. Assessing embryo quality in terms of morphokinetic characteristics, we did not find significant differences between the two groups.
Conclusion(s): By adopting a time-lapse video system, we can suggest that the type of protocol used for controlled ovarian stimulation influences embryo kinetics of development but these variations are not reflected in embryo quality.

1. Introduction

Medications that restore or therapeutically modify the ovulatory process have been used for decades in the management of menstrual cycle disorders and for the treatment of infertility. For 25 years, ovarian stimulation has been applied with the aim of increasing the number of oocytes in order to compensate for inefficiencies of the vitro fertilization (IVF) procedure, enabling the selection of one or more embryos for transfer [1]. Current drugs used for ovarian stimulation, however, represent concern for clinicians, despite the larger number of oocytes obtained, mainly due to studies that have demonstrated the high treatment burden, risks and costs of this approach and a negative impact of ovarian stimulation on oocyte development [2–4]. Oocyte quality and the developmental potential of an embryo are clearly correlated and it may be assumed that the follicle is capable of profoundly influencing the quality of the oocyte obtained at ovulation, and as a result, the quality of the embryo obtained [5,6] There is also evidence that the ovary resists ovarian stimulation, at least in animal studies, by decreasing the quality of the oocytes it produces, bringing back into perspective the importance of tailoring the hormonal stimulation protocol [7].

A key step in assisted reproductive techniques (ART) is the assessment of oocyte and embryo viability to determine the embryo(s) most likely to implant. Current embryo assessment strategy in clinical settings largely relies on embryo morphology and cleavage rates, and although these systems have been successful improving pregnancy rates, their precision is far from ideal as they are based on the visual information obtained by the embryologist and thus subject to inter/intraobserver variance [8]. In contrast, automated image analysis may add objectivity to the process of embryo selection and, consequently, lead to an improvement in the implantation rates seen after IVF. In contrast

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to a daily check of embryo development, a time-lapse system offers specific benefits such as the possibility to determine the length of a cell cycle, enabling a more authentic and dynamic view of the embryo. By integrating morphological and kinetic criteria, we recently described a correlation between the length of the embryo cleavages and the subsequent implantation potential, thus identifying an optimal timing range for each embryo division [9].

By adopting the new time-lapse technology, in the present study we aimed to explore if controlled ovarian stimulation in terms of pituitary desensitization and ovulation triggering (GnRH agonist and hCG triggering versus GnRH antagonist and GnRH agonist triggering) affects embryo developmental kinetics.

2. Materials and methods

A retrospective cohort study was performed, analyzing embryos from 400 couples undergoing oocyte donation from July 2009 to July 2011. The study was conducted at the Instituto Valenciano de Infertilidad IVI in Valencia ($n = 278$) and Alicante ($n = 122$). The study complies with the Spanish law governing ART (14/2006).

2.1. Ovarian stimulation in oocyte donors

Oocyte donors were healthy women between 18 and 35 years old, with regular menstrual cycles, no family history of hereditary or chromosomal diseases, normal karyotype, body max index (BMI) 19–29 kg/m^2 and without the presence of any sexually transmitted diseases [10]. The present study included only donors from whom at least eight metaphase II oocytes were obtained after retrieval and denudation, thus excluding donors with low and moderate responses.

All donors received 1–2 months' treatment with oral contraceptives for cycle synchronization prior stimulation. Two stimulation protocols were compared:

(1) We performed a long GnRH agonist protocol ($n = 103$) consisting of subcutaneous (s.c.) injections of GnRH agonist leuprolide 0.5 mg/day (Procrin, Abbot Laboratories, Madrid, Spain) commencing on the 21st day of the previous cycle; once down-regulation was achieved, the leuprolide dose was reduced to 0.25 mg/day and stimulation with recombinant FSH (Gonal-F®; Serono, Madrid, Spain; Puregon®; MSD, Madrid, Spain), 150–300 UI was started with once daily s.c. doses. Human chorionic gonadotrophin (hCG) (Ovitrelle®, Serono Laboratories, Madrid, Spain) 10,000 UI was administered subcutaneously for final oocyte maturation when at least three follicles reached a mean size of $\geq$18 mm.
(2) We applied a GnRH antagonist protocol ($n = 297$) in which 150–300 UI/day s.c. of recombinant FSH (Gonal-F®; Serono, Madrid, Spain; Puregon®; MSD, Madrid, Spain) was used for stimulation from cycle day 2–3. When the leading follicle had reached a size of 14 mm a GnRH antagonist 0.25 mg was administered once daily s.c. (Cetrotide; Serono, Madrid, Spain). A single dose of triptoreline 0.2 ml was administered intramuscularly (Decapeptyl®; Ipsen Pharma, Barcelona, España) for final oocyte maturation when at least three follicles reached a mean size of $\geq$18 mm.

2.2. Recipient's endometrial preparation

The protocol for hormone therapy for oocyte recipients has been previously described [11]. Briefly, a baseline transvaginal scan was carried out before down-regulation to ensure the uterus was normal. For all recipients who were still cycling, down-regulation was performed using an intramuscular dose of 3.75 mg triptorelin (Decapeptyl®; Ipsen Pharma, Barcelona, Spain) in the midluteal phase of the previous cycle. Hormone therapy was initiated on days 1–3 of the following cycle, and doses of estradiol valerate (Progynova®; Schering-Plough, Madrid, Spain) were increased as follows: 2 mg/day for the first eight days of treatment, 4 mg/day for the following three days, and at least 6 mg/day until the pregnancy test. On day 15, an ultrasound scan was performed to evaluate endometrial growth. On the day after donation, 800 mg/day of micronized intravaginal progesterone (Progeffik; Effik Laboratories, Madrid, Spain) was added to the regimen.

In the current study only one recipient per donor was included: although some donors provide oocytes to more than one recipient, any recipient included in this study shared oocytes from the same donor.

2.3. Oocyte retrieval, fertilization and embryo culture

Transvaginal oocyte retrieval was performed under sedation 36 h after hCG under ultrasound guidance in both groups. After follicle aspiration, oocytes were kept in culture at 37.4 °C and 6.0% CO_2 until ICSI, which was performed using an Olympus IX7 microscope. Once injected, oocytes were placed in individual wells of a pre-equilibrated EmbryoSlide (EmbryoSlide®, Unisense FertiliTech, Aarhus, Denmark).

Embryos were cultured (Global® medium, Life-Global®, Canada) and evaluated morphologically 48 and 72 h after sperm injection. The parameters evaluated included cell number, symmetry and granularity, as well as type and percentage of fragmentation, presence of multinucleated blastomeres and degree of compaction, as previously described [12]. Human blastocysts were scored on day 5 (120 h after sperm injection) according to the expansion of the blastocoel cavity and the number and integrity of the inner cell mass (ICM) and trophoectoderm cells (TE).

Transfer was performed on day 3 (91.9% of the patients) or day 5 (8.1% of the patients) of development, depending on embryo evolution. Nevertheless, all embryos were selected on the basis of their morphological scoring on days 2 and 3 and according to the blastocyst quality on day 5; thus data from embryo kinetics were not used in the embryo selection process. We include only treatments with embryo transfer.

2.4. Time-lapse instrument

By means of a time-lapse system (Embryoscope, Unisense Fertilitech, Aarhus, Denmark), we determined the timing of a number of developmental parameters including cleavage timings from a zygote to a 9-cell embryo ($t2$–$t9$); time to formation of morulae (M), appearance of the blastocoel cavity (B) and time taken to complete maximal blastocyst expansion (EB). We also determined some variables related to duration of cell cycles, namely: second cell cycle (cc2) is the duration of the time as a 2-blastomere ($t3 - t2$); cc3 is the time to pass from a 3-blastomere embryo to a 5-blastomere embryo ($t5 - t3$); second synchrony ($s2$) is the duration of division from 2-blastomere embryo to 4-blastomere embryo; $s3$, third cell cycle ($t4 - t3$); length of the second embryo cleavage ($t4 - t2$); and finally, the third embryo cleavage ($t8 - t4$).

We considered "optimal" embryos, those with the highest probability to implant, as fulfilling the following kinetic values: $t5$, 48.8–56.6 h; $s2 \leq 0.76$ h; and cc2 ≤ 11.9 h. Embryos with cell division within these ranges have at least a 10% higher chance of implanting compared to embryos out of these intervals [9].

2.5. Morphokinetic categories

The hierarchical classification procedure proposed [9] starts with a morphological screening of all embryos to eliminate