Kinetics of the early development of uniparental human haploid embryos

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Objective: To describe morphokinetically the early development of human haploid parthenotes and androgenotes and to compare them with euploid embryos.
Design: Experimental study of kinetics.
Setting: University-affiliated private fertility center.
Patient(s): Experimental haploid parthenotes and androgenotes.
Intervention(s): Kinetic study of early development (up to eight cells) of 8 parthenotes, 10 androgenotes, and 20 euploid embryos.
Main Outcome Measure(s): Timing of the first seven cleavages determined according to embryo origin, then calculation of the duration of the second and third cell cycles (cc2 and cc3) of whole embryos and individual cells.
Result(s): Parthenotes and androgenotes were experimentally produced by artificial oocyte activation and intracytoplasmic sperm injection of enucleated oocytes, respectively. Uniparental embryos having 6 to 10 cells were assessed for haploidy, their kinetics analyzed, retrospectively compared with euploid embryos. All the first seven cleavages occurred later in parthenotes than in both androgenotes and correctly fertilized embryos. The whole embryos and single cells showed that cc2 was longer in parthenotes than in both androgenotes and correctly fertilized embryos; cc3 was shorter in androgenotes than in both parthenotes and correctly fertilized embryos. The duration of cc2 versus cc3 was longer in correctly fertilized embryos and parthenotes than in androgenotes.
Conclusion(s): Parthenotes and androgenotes have different kinetics. The former have a longer cc2, and the latter a consistently shorter cc3 in comparison with correctly fertilized embryos. (Fertil Steril® 2016;105:1360–8. ©2016 by American Society for Reproductive Medicine.)
Key Words: Androgenote, haploid, human, morphokinetic, parthenote

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Uniparental (parthenogenetic and androgenetic) embryos, each with their unique set of maternal and paternal chromosomes, constitute a useful model for investigating the existence of developmental differences according to haploidy and parental origin of human embryos. Most previous studies of uniparental embryo development have employed diploid or diploidized parthenogenetic (or gynogenetic) and androgenetic embryos. Few have assessed purely and entirely hemizygous haploid uniparental embryos, which might represent the most feasible approximation to study unilaterally the constituents of the embryo.

Parthenogenesis involves artificial oocyte activation (AOA), which can be achieved by a wide range of physical and chemical stimuli that mimic the Ca^{2+} oscillations induced by the sperm during natural fertilization. Although human oocytes are successfully activated by such stimuli, the majority of parthenotes arrest early in their development (1–6). Nevertheless, a few studies have reported successful parthenogenetic blastocyst formation (7–12). Such contrasting reports with respect to the development of parthenotes might be due to varying oocyte quality, the effectiveness of the AOA protocol applied, or more probably the ploidy of the parthenotes, as reported in other mammalian species (13–19). After AOA based on ionomycin incubation alone (1, 4, 5, 20, 21) or in combination with a protein synthesis inhibitor such as puromycin (6, 20,22–27), most oocytes (60% to 75%) extrude the second polar body and form a single pronuclear structure with a DNA content that is compatible with a haploid set of chromosomes (4, 20, 21, 24, 26). However, a detailed description of

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in vitro development under these circumstances has not been provided to date, probably due to the difficulty in obtaining parthenotes beyond the four-cell (2, 6) or eight-cell (1, 4, 7) stage.

On the other hand, the developmental capacity of haploid androgenotes, produced by in vitro fertilization of enucleated metaphase 2 (MII) oocytes (19,28–30) or removal of the female pronucleus from the zygote (30–32), has been studied extensively in mice (28,33–39) and, to a lesser extent, in bovine (19), ovine (32), and porcine (40) species and in humans (30). Most of the studies in question have assessed the development of diploid androgenetic (diploidized monospermic or bispermic) embryos, but few have focused on that of haploid androgenotes [mice (28, 33, 34), bovine (19), human (30)].

Regardless of differences related to species and the methodology used to produce haploid androgenotes, the evidence to date confirms the poor capacity of these embryos for development. Research shows that most mouse haploid androgenotes cleave successfully but their development is arrested after the first few divisions, with only a few developing into blastocysts (28, 33, 34). That said, Kono et al. (28) reported that 60% and 11% of haploid androgenotes developed into eight-cell embryos and blastocysts, respectively. In another study, nearly 3% of bovine haploid androgenotes were found to progress to the compact morula stage, but only 1.8% developed into blastocysts (19). More limited developmental rates have been reported in humans (30); in the study in question, the percentage of androgenotes reaching the two- to eight-cell stage ranged from 65% to 90%, depending on the methodology used to produce them. Unfortunately, no further details were provided regarding their development.

In our present study, we describe for the first time the kinetic development of haploid human parthenotes and androgenotes from the one- to eight-cell stage using time-lapse monitoring. Moreover, by comparing retrospectively these embryos with correctly fertilized (biparental) embryos that become healthy babies (euploid embryos), we have determined how development differs according to haploidy and parental origin.

MATERIALS AND METHODS

This research was conducted at the Instituto Universitario IVI Valencia. All procedures and protocols for androgenote and parthenote production were approved by the institutional review board (0703-E-404-ME and 073-E-402-ME, respectively) and by the Spanish government (National Committee for Assisted Human Reproduction).

Oocyte Origin for Uniparental Embryo Production

For uniparental androgenote and parthenote production, MII oocytes were retrieved from healthy donors (aged between 18 and 35 years old) by follicular puncture and aspiration after a standard ovarian stimulation protocol. After cumulus removal, 60 mature oocytes were cryopreserved by vitrification according to the Cryotop method previously described by Kuwayama et al. (41), with slight modifications (42). In brief, oocytes were equilibrated in 7.5% (v/v) ethylene glycol and 7.5% (v/v) dimethyl sulfoxide in TCM199 medium with 20% synthetic serum substitute at room temperature for 15 minutes. They were then placed in the vitrification solution containing 15% ethylene glycol, 15% dimethyl sulfoxide, and 0.5 M sucrose. After 1 minute in this solution, oocytes were placed on the Cryotop strip and immediately plunged into sterile liquid nitrogen (Ceralyn Online; Air Liquid France). Cryopreserved oocytes were stored pending signed informed consent to use them for the current research purposes.

For warming, each Cryotop was removed from the liquid nitrogen and placed in 1.0 M sucrose in TCM199 20% synthetic serum substitute at 37°C. After 1 minute, the oocytes were placed in 0.5 M sucrose in TCM199 20% synthetic serum substitute at room temperature for 3 minutes. Finally, the oocytes were washed for 6 minutes in TCM199 20% synthetic serum substitute at room temperature before they were incubated in cleavage medium for 2 hours (42). The oocytes were assessed for survival, and those with a healthy cytoplasm appearance without signs of atresia or degeneration (91.7% vitrification survival rate) were used for haploid androgenote and parthenote production.

Haploid Parthenogenote Production

For parthenote production, 25 surviving warmed MII oocytes were artificially activated using a calcium ionophore (A23187) and puromycin incubation (24, 26, 27). In short, the oocytes were exposed for 5 minutes to A23187 (4 mM; Sigma-Aldrich) and were subsequently cultured for 5 hours in puromycin (10 μg/mL; Sigma-Aldrich). After the AOA protocol had been applied, the eggs were cultured in a time lapse-system in 25 μL of cleavage medium. After 16 to 20 hours of culture, the eggs were assessed for extrusion of the second polar body and number of pronuclei. Although several types of oocyte activation response have been reported (15–17, 26, 43), only parthenotes that had extruded the second polar body and had a single maternal pronucleus (n = 13; 52.0% of haploid parthenotes) were subsequently cultured in a time-lapse system for 3 additional days, as described herein.

Haploid Androgenote Production

Androgenote production was performed according to the procedure described by Kono et al. (28, 29), with some modifications. In short, the procedure involves enucleation of MII oocytes and subsequent intracytoplasmic sperm injection (ICSI).

For MII oocyte enucleation, 30 surviving, warmed oocytes were inspected for the presence of the meiotic spindle using a light microscope equipped with Octax PolarAid (Olympus) imaging software. Once detected, the spindle was removed by gentle aspiration through an ICSI pipette (Cook) according to the procedure described by Grau et al. (44). One hour later, absence of the spindle was confirmed in the 21 oocytes that had survived manipulation (70.0% successful enucleation rate). Ooplasts were then microinjected following the conventional ICSI procedure and cultured in an EmbryoSlide (Unisense Fertilitech) containing 25 μL of cleavage medium with 1.2 mL of overlay of mineral oil (Cook). After this