Hatching of in vitro fertilized human embryos is influenced by fertilization method

Kirstine Kirkegaard, M.D., Johnny Juhl Hindkjaer, M.Sc., and Hans Jakob Ingerslev, D.M.Sc.

Fertility Clinic, Aarhus University Hospital, Aarhus, Denmark

Objective: To describe hatching of human embryos and investigate differences in hatching between IVF and intracytoplasmic sperm injection (ICSI)–fertilized embryos with the use of time-lapse monitoring.
Design: Clinical observational study.
Setting: University-based fertility clinic.
Patient(s): From February 2011 to July 2012, 161 women consented to embryo culture in a time-lapse incubator until day 6 after oocyte retrieval. The mechanism of hatching was recorded and related to method of fertilization (ICSI or IVF) and clinical pregnancy outcome.
Intervention(s): IVF or ICSI.
Main Outcome Measure(s): Hatching pattern.
Result(s): A total of 430 IVF fertilized embryos from 62 patients and 594 ICSI-fertilized embryos from 99 patients were included. We observed spontanous hatching in 165 IVF embryos and 215 ICSI embryos. Two distinct mechanisms of hatching were observed. Type 1 was characterized by penetration of the zona pellucida (ZP) by small trophectoderm projections, whereas type 2 was preceded by a regular rupture of the ZP followed by extrusion of the blastocyst. Type of hatching was significantly different between IVF and ICSI embryos, with type 2 observed more often in IVF embryos than in ICSI embryos. Furthermore, IVF embryos escaped the ZP more readily than ICSI embryos. Regardless of the type of hatching, implantation rates were similar.
Conclusion(s): We describe two distinct mechanisms of in vitro hatching related to fertilization method and suggest that hatching pattern is associated with fertilization method. The hatching pattern has, however, no influence on future implantation.
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Key Words: In vitro fertilization, human, ICSI, hatching, time-lapse

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Escape of the mammalian embryo from the surrounding zona pellucida (ZP), referred to as hatching, is a prerequisite for subsequent implantation. Failure of the embryo to hatch has been proposed as a cause of unsuccessful implantation when treating infertile patients with in vitro fertilization (IVF). This has promoted the interest in the process of spontaneous hatching and the effect on implantation of assisted hatching (1). Knowledge of spontaneous hatching is mainly derived from nonhuman studies performed in vitro (2–8). The process is described as a continuous expansion of the blastocele, which leads to progressive global thinning and focal rupture of the ZP. The blastocyst subsequently herniates through the opening until complete extrusion of the embryo, leaving behind an empty but largely intact ZP. Repeated collapses and reexpansion of the blastocyst are often observed before hatching (4, 8–13). Hatching of human embryos in vitro has been studied less extensively but appears to follow a similar pattern (9, 14, 15). The details regarding the ZP rupture in vitro are largely unclarified. Both in animals and humans, small projections of specialized cellular trophectoderm (TE) through the ZP have been reported to precede extrusion of the embryo (3, 4, 6, 14). These projections have been suggested to induce the focal opening(s) through

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Reprint requests: Kirstine Kirkegaard, M.D., Fertility Clinic, Aarhus University Hospital, Brendstrupgaardsvej 100, 8200 Aarhus N, Denmark (E-mail: kirstine.kirkegaard@ki.au.dk).

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one of which the embryo escapes, presumably aided by TE proteinase secretion (16–19).

Interestingly, in vivo hatching appears to differ distinctively from the described in vitro hatching (20). In vivo hatching in hamsters and mice occurs earlier and within a narrower time frame than in vitro. The physiologic hatching process is characterized by gradual zona thinning that is seemingly independent of blastocyst expansion and which is followed by total loss of ZP (2, 3). Presumably, in vivo hatching involves ZP lysis, which is supported by the presence of uterine proteases during the period of zona loss (21). The commonly described in vitro hatching is therefore likely to represent a deviation from the physiologic process of hatching. The differences between in vivo and in vitro hatching may be accentuated by the varying steps in the IVF procedure, i.e., oocyte pick-up and oocyte and embryo handling with possible mechanical or chemical modifications of the ZP.

In vitro treatment often involves fertilization of the oocyte by intracytoplasmatic sperm injection (ICSI). ICSI introduces an early, artificial, mechanical breach, though small, in the ZP. Although concerns have been expressed addressing the potential implications of manipulating the ZP, the direct impact of ICSI on the process of hatching has not been studied. Accordingly, the purpose of the present study was to describe in vitro hatching of human embryos and to investigate whether the hatching process was affected by microinsemination.

MATERIALS AND METHODS

Study Design and Participants

The study was conducted at the Fertility Clinic, Aarhus University Hospital, from February 2011 to July 2012. Patients aged <38 years without endometriosis were offered participation and their embryos included if at least eight oocytes were retrieved. Eligible patients could contribute to the study with one treatment cycle only. In total, embryos from 161 patients were included.

Ethical Approval

Written informed consent was obtained from all of the participants. The Central Denmark Region Committees on Biomedical Research Ethics and the Danish Data Protection Agency approved the study. The study was registered at Clinicaltrials.gov with accession number NCT01139268.

In Vitro Fertilization, Embryo Culture, and Embryo Score

Patients underwent ovarian stimulation in either a long down-regulation protocol (Supracur; Hoechst) or a short antagonist protocol (Cetrotide; Serono) using urinary (Menopur; Ferring Pharmaceuticals) or recombinant (Puregon; MSD) FSH stimulation. FSH doses were administered according to a clinical evaluation and ovarian response of each patient. Patients received a dose of 10,000 IU hCG (Pregnyl; MSD) when at least three follicles measured ≥17 mm. Oocyte retrieval was conducted with the use of ultrasound-guided puncture of ovarian follicles 36 hours later and was followed by insemination with either standard IVF or microinsemination (ICSI) procedures according to treatment indications. IVF was performed by incubating one to six oocyte-cumulus complexes in 4-well plates with ~150,000 motile sperm in a conventional incubator (Galaxy R; RS Biotech, CM Scientific) at 37°C, 20% O_2, and 6% CO_2 for 1.5 hours, after which the sperm was washed away. Denudation was performed ~18 hours after fertilization and was followed by transfer of 2-pronuclei (2PN) zygotes to Embryoslides (Unisense Fertilitech) for further culturing in the Embryoscope (Unisense Fertilitech). In case of ICSI, sperm was resuspended in 10% polyvinylpyrrolidone (PVP; Cook) for a maximum of 15 minutes. A single spermatozoon was immobilized and injected with the head first into an oocyte by a micropipette (outer diameter 7 μm; Vitrolife Swemed) with the use of micromanipulators (Narishige). After insemination, the oocytes were placed in individual wells in a special culture slide (Embryoslide) in a tri-gas time-lapse incubator (Embryoscope) (22). Fertilization was checked in the Embryoscope for both IVF and ICSI-fertilized embryos, All embryos were cultured in sequential culture medium (Sydney IVF Fertilization/Cleavage/Blastocyst Medium; Cook) under oil at 37°C, 5% O_2, and 6% CO_2. Media change was performed in the mornings of days 3 and 5. In the morning of day 6, blastocysts were graded according to the Gardner criteria, in brief, based on expansion of the blastocele cavity (1–6) and number and cohesiveness of the inner cell mass (ICM) and TE (A–C) (23), and the embryo with the better morphology was selected for transfer. All transfers were single-embryo transfers. Supernumery embryos were vitrified after laser puncture. A TE biopsy was obtained from the blastocyst with the best morphology if more than one blastocyst of high quality had developed and the procedure was logistically feasible. In brief, an opening in the ZP was introduced by laser in the morning of day 5 after oocyte retrieval. After 4–5 hours of continued culture, a biopsy of 5–10 TE cells was performed if sufficient TE cells had herniated through the hole. Biochemical pregnancy rate was confirmed by serum β-hCG measurement 16 days after retrieval. Clinical pregnancy rate was registered as number of ongoing pregnancies per embryo transfer, based on presence of fetal heart beat (FHB) activity visualized by ultrasound 8 weeks after embryo transfer.

Time-Lapse Monitoring

Images were recorded every 20 minutes in 7 planes (15-μm intervals, 1,280 × 1,024 pixels, 3 pixels/μm, monochrome, 8-bit, <0.5 s per image, single 1-W red LED). Embryos with 2PN completing the first cleavage were annotated manually according to a previously described protocol (15). Hatching was defined as the first image where projections of TE cells were visible outside the ZP. Time points refer to the exact time that an image was recorded and are reported as hours after first cleavage, because exact time of fertilization is difficult to establish for IVF-inseminated embryos (24). The embryos were observed and time-lapse recordings obtained until the morning of day 6.