ORIGINAL ARTICLE: ASSISTED REPRODUCTION

Characterization of the injection funnel during intracytoplasmic sperm injection reflects cytoplasmic maturity of the oocyte

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Objective: To quantify cytoplasmic maturity on the basis of intracytoplasmic sperm injection (ICSI) injection funnel manifestation and to evaluate influence factors of the latter.
Design: Prospective study.
Setting: Private fertility center.
Patient(s): A total of 31 patients with good ovarian response.
Intervention(s): Mature and immature oocytes were injected intracytoplasmatically. Formation and persistence of an injection funnel was documented and measured.
Main Outcome Measure(s): ICSI funnel size, persistence of injection funnel, rates of degeneration and fertilization, embryo quality.
Result(s): Funnel volume in germinal vesicle stage oocytes (prophase I [PI]) was significantly smaller than that of metaphase I (MI) and MII oocytes. Immature eggs (PI, MI) almost never showed a persistent funnel 2–4 minutes after ICSI, whereas in MII eggs the funnel was still observable in 35% (117/334) of the cases. Uni- and multivariate analysis revealed that pipette type and stimulation protocol significantly influenced appearance of injection funnel. Funnel volume in oocytes that fertilized regularly was significantly higher compared with three–polar body and degenerated oocytes.
Conclusion(s): Oocyte maturation within the follicle is closely associated with a remarkable change in cytoplasm viscosity from an aqueous to a more viscous subtype. Precise evaluation of the injection funnel may help to explain deviations from expected ICSI outcome and could also assist in optimizing controlled ovarian hyperstimulation. (Fertil Steril® 2016;■:■–■. ©2016 by American Society for Reproductive Medicine.)
Key Words: Injection funnel, ICSI, ICSI pipette, cytoplasmic maturity, controlled ovarian hyperstimulation

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During folliculogenesis oocytes undergo a considerable growth phase (from 40 μm to 120 μm) in which molecules and cell organelles are produced that are crucial for the later development of the preimplantation embryo. This period is followed by a maturation phase at ovulation comprising modifications of the chromosomal complement as well as rearrangements of cytoplasmic components that are fundamental for the achievement of developmental competence (1), in other words, both nuclear and cytoplasmic maturation are finalized.

This growth and maturation of human eggs in fact parallels the differentiation of the follicle and is mutually interdependent with its development (2). Consequently, the granulosa cells surrounding the oocyte, also known as cumulus cells (CC), are of utmost importance to the ovum throughout folliculogenesis. Communication between the oocyte and the somatic cells within the same follicle occurs via paracrine and gap-junctional signaling (3, 4). To be more precise, around ovulation a bidirectional communication axis is established (5). While CCs provide the oocyte with metabolic support and

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provide signaling molecules assisting resumption of meiosis, oocyte-secreted factors play an important role in the differentiation of different granulosa cell lineages (6).

In controlled ovarian hyperstimulation, however, the situation in the follicle is not comparable to the in vivo scenario, because it is aimed to obtain a higher number of oocytes, thus increasing the risk that not all gametes will show the same developmental competence. One possible explanation for this observed heterogeneity is the simultaneous presence of follicles with altered blood supply and, as a consequence, with different levels of vascularization and subsequent hypoxia (7). There is evidence that in such cases oocytes could be affected by a decoupling of nuclear and cytoplasmic maturation processes.

Indeed, data from in vitro–matured ova indicate that cytoplasmic maturation may be dissociated from nuclear maturation. This would mean that although resumption of meiosis is achieved, maturation of cytoplasm is still impaired (8).

It has been hypothesized that a potential deficiency in cytoplasmic maturity could be reflected at the light microscope level by the presence of cytoplasmic dysmorphisms such as cytoplasmic inclusions, vacuoles, or clusters of the smooth endoplasmic reticulum (9). In addition to cytoplasmic features, differences in cytoplasmic viscosity have been observed. Indeed, it could be shown that deviations in ooplasmic viscosity can result in the restraint of cell organelles and pronuclei (10).

There is still a lack of markers allowing for identification of changes in cytoplasmic viscosity, although it has been reported that granular areas are more viscous than the surrounding cytoplasm (11). Viscosity of the cytoplasm has been found to correlate with the potential of oocytes to restore their spherical shape after intracytoplasmic sperm injection (ICSI) (10). It has been suggested that oocytes with a less viscous cytoplasm show a higher tendency to restore their shape owing to a higher intracellular pressure.

However, those authors (10) failed to focus on technical aspects of the injection procedure and its possible effect on the ooplasm, and, more importantly, they did not quantify the injection funnel at all. Therefore, the present prospective study was set up to accurately measure the amount of cytoplasm that is harmed during ICSI and to correlate it with further outcome. Three injection tools were used to calculate the actual influence of this technical aspect.

MATERIALS AND METHODS

The present study was carried out at Zentrum für IVF und Reproduktionsmedizin, Amedes Deutsche Klinik, Bad Münder (Institutional Review Board approval 14-KI-07). Within the 5-month study period, a total of 31 patients were prospectively included after they had given informed consents.

The main focus was to recruit patients with a higher number of oocytes, which would allow splitting of the gametes between the pipette types to be examined. Consequently, the mean antimüllerian hormone value of the cohort (3.8 ± 2.7 ng/mL) was of good prognosis (12). Female patients averaged 34.1 ± 3.2 years (range 28–38 years) of age. The present patient population represented an everyday composition, with four patients each suffering from bilateral occlusion of the tubes and polycystic ovary syndrome (12.9%), and seven having moderate endometriosis (22.6%). Apart from one case of unexplained infertility (3.2%), all other couples showed a pure male-factor indication (48.4%). However, sperm quality was affected in all patients (96.8%) except one (unexplained infertility). No cases of microsurgical epididymal sperm aspiration/testicular sperm extraction were included.

In the majority of cycles (67.7%), controlled ovarian hyperstimulation was done according to an antagonist protocol, whereas ten patients (32.3%) were down-regulated with the use of a long protocol (13). In all cases, stimulation was done with the use of market recombinant or highly purified urinary products. Once the lead follicle reached 20 mm and the associated E_2 levels appeared to be reasonable, ovulation was induced with the use of 10,000 IU hCG. Thirty-six hours later, ovarian puncture was performed transvaginally in an ultrasound-guided mode. The harvested cumulus-oocyte complexes (COC) were kept in human tubal fluid medium (Life Global) for later use. Agonist as well as antagonist cycles had a similar ($P=.46$) rate of metaphase II (MII) oocytes (93.7% vs. 91.5%).

In parallel to COC collection, ejaculate (after abstinence of 3–5 days) was collected in a sterile semen jar. With addition of 300 μL trypsin (Serva), liquefaction of the ejaculate was induced. Sperm analysis then was done according to the World Health Organization (14) with the use of a computer-aided semen analysis system (Medealab Casa; MTG). Subsequently, ejaculate was processed with the use of a simple swim-up procedure (15).

Immediately preceding ICSI (16), COCs were incubated in 80 IU hyaluronidase (Serva) for 15 seconds to facilitate subsequent mechanical denudation. It should be highlighted that this process was performed exactly 38 hours (36 hours after hCG plus 2 hours of rest) after induction of ovulation to avoid differences in oocyte maturation (17). At this phase, oocytes were checked for their state of maturation, and MII gametes were split into three groups. Randomization was performed under an inverted microscope so that the operator was blinded for oocyte morphology. Each subgroup of mature oocytes thereafter (not more than 1 hour after denudation) was injected with the use of a different microtool produced by the same manufacturer (Cook Medical). All pipettes used are commercially available products and have well defined bevels to aid zona pellucida puncture. The associated spikes were never manipulated before use. Although the bend-to-tip length is the same for all three pipette types, they differ in inner and outer diameters. The following glass tools were used:

- Type A (small): inner diameter 4.7 μm, outer diameter 6 μm.
- Type B (intermediate): inner diameter 5 μm, outer diameter 7 μm.
- Type C (large): inner diameter 5.5 μm, outer diameter 7 μm.

For better visualization of the effect of these dimensions on the degree of manipulation, it should be mentioned that the volumes of cytoplasm that are aspirated during the ICSI process for pipette types A–C are 1.73 pL, 1.96 pL, and 2.38 pL, respectively (provided that the ooplasm level within the