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ARTICLE

Effect of co-incubation of oocytes with a decreasing number of spermatozoa on embryo quality

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Abstract Shortened exposure of oocytes to spermatozoa has been reported to improve embryo quality. This technique requires extra manipulation of gametes in order to remove oocytes from the spermatozoa. This study presents a fertilization method that does not require additional manipulation and interference of oocytes during fertilization. To determine the benefits of this method, a prospective controlled study using sibling oocytes was conducted. The oocytes of patients were randomly allocated to study and control groups. Fertilization rates, embryo cleavage rates, day-3 embryo morphology and clinical pregnancy rates were compared between the two groups. The normal fertilization rates (2PN) of the two groups were comparable. The percentage of usable embryos (transferred plus cryopreserved embryos) was significantly higher in the treatment group compared with the control group (66.9 ± 23.3% versus 57.6 ± 26.7%; $P = 0.03$). The mean embryo quality score of the treatment group was higher than the control group (18.3 ± 4.8 versus 15.2 ± 5.1; $P = 0.02$). The results of this study demonstrated that this method can improve embryo quality, but further studies with additional IVF patients are needed to confirm the beneficial effects.

KEYWORDS: embryo quality, fertilization, gamete co-incubation, IVF, short insemination, sibling oocytes

Introduction

Under physiological conditions there are several hundred spermatozoa present within the ampulla. Contrary to in-vivo conditions, excessive numbers of spermatozoa are co-incubated with oocytes to enhance fertilization *in vitro*, in which up to 500,000 motile spermatozoa/ml per oocyte are used (Fiorentino et al., 1994; Trounson, 1994; Trounson et al., 1994). This high concentration of spermatozoa is not necessary after a spermatozoon has pen-

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etrated the zona pellucida and has fused with the oolemma and might even negatively affect embryos by producing high concentrations of reactive oxygen species (Aitken and Clarkson, 1987; Aitken et al., 1989). Decreasing the negative effects of these spermatozoa while at the same time enhancing fertilization is important so as to achieve good-quality embryos and a high pregnancy rate for conventional IVF patients.

The short insemination method has been reported to decrease the detrimental effects of spermatozoa on oocytes through the reduction of the co-incubation time of gametes, from 16–19 h to 1–4 h (Gianaroli et al., 1996; Kattera and Chen, 2003). For short insemination methods, oocytes are removed from insemination dishes, washed several times and placed into fresh medium after 1–4 h co-incubation to eliminate spermatozoa during culture. This procedure usually takes 2–5 min per patient outside a CO_2 incubator, depending on the number of oocytes inseminated.

Some studies have shown enhanced embryo quality and a superior pregnancy rate (Dirnfeld et al., 1999; Gianaroli, 1996; Kattera and Chen, 2003), while others have described an equivalent embryo morphology or pregnancy rate compared with overnight incubation (Barraud-Lange et al., 2008; Coskun et al., 1998; Lundqvist et al., 2001). Although the different concentrations of spermatozoa and insemination methods might explain these conflicting results, additional manipulation of oocytes outside of incubators and the sharp changes in the micro-environment of oocytes during the early stages of fertilization might have masked the beneficial effects of the short insemination methods currently used by IVF centres.

This study proposes another fertilization method to decrease the negative effects of an excessive number of spermatozoa on oocytes by incubating oocytes with a decreasing number of spermatozoa during the fertilization process. To decrease the incubating spermatozoa, fertilization wells were specially designed in which sperm concentrations surrounding the oocytes would gradually decrease. Unlike short insemination methods, the present method did not require manipulation of gametes and would thus prevent possible interference of oocytes during the fertilization process.

To test the beneficial effects of this new fertilization method compared with the traditional fertilization method of co-incubating oocytes with a fixed number of spermatozoa, patients were used as their own control by dividing sibling oocytes between the study and control groups, and the fertilization rate, embryo quality and clinical pregnancy rate were compared between the two groups.

Materials and methods

Design and fabrication of insemination dishes

Four-well dishes (Nunc; Thermo Fisher Scientific, Kamstrupvej, Denmark) were used for oocyte fertilization. Holes were made between two wells to connect the wells to the large inner depression of the 4-well dish, while the other two wells remained intact; specifically, two wells per 4-well dish were randomly chosen and the red-hot needlepoint of a 2-ml syringe (BD Company, Franklin Lakes, NJ, USA) was used to quickly pierce the interior wall. The holes were made towards the centre well and close to the bottom of the dish (**Figure 1**). There were six types of 4-well dishes in the present study according to different combinations of drilled wells, such as holes between well 1 and well 2, or well 2 and well 4, etc. Every patient got a type of 4-well dishes for oocyte insemination according to random numbers generated by computer. After sterilization, each well of the 4-well dish was filled with 1 ml HTF medium (Sage In vitro Fertilization, Cooper Surgical Company, Trumbull, CT, USA) and the central depression between wells was also filled with 4 ml of HTF medium to make the medium of each well appear to be at the same level (**Figure 1**). The wells were not covered in oil. Any air bubbles residing in the holes after filling with medium and blocking the communication of medium and spermatozoa were carefully blown off using Pasteur pipettes. The 4-well dish was ready for use after being equilibrated overnight in a CO_2 incubator.

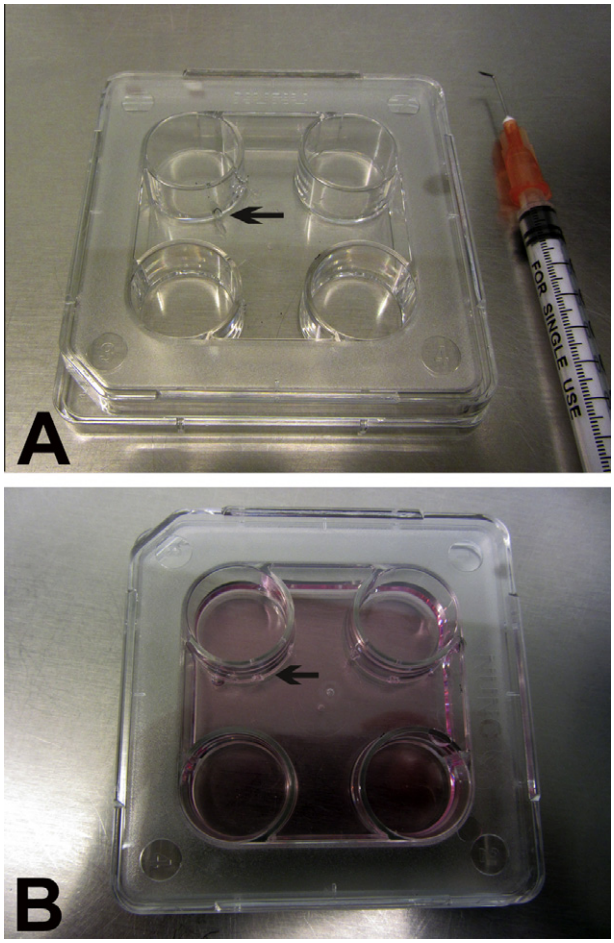

Figure 1 Design and fabrication of fertilization dishes. (A) Holes were made in two wells of a 4-well dish, which were designed to point towards the centre and be close to the bottom of the dish (arrow). The holes were made using the red-hot needle of a 2-ml syringe to quickly pierce the interior wall. (B) After sterilization, each well was filled with 1 ml HTF medium and the centre part was covered with 4 ml HTF medium to try to make the medium of each well appear to be at the same level.