Does ICSI require acrosomal disruption? 
An ultrastructural study

Takumi Takeuchi, Liliana T. Colombero, Queenie V. Neri, Zev Rosenwaks and Gianpiero D. Palermo

The Center for Reproductive Medicine and Infertility, Weill Medical College of Cornell University, 505 East 70th Street, HT-336, New York, NY 10021, USA

1To whom correspondence should be addressed. E-mail: gdpalerm@med.cornell.edu

BACKGROUND: Aggressive immobilization of sperm prior to ICSI significantly improves fertilization rates, but the mechanism of this effect is not yet clear. This study was performed in order to assess the characteristics of mechanically immobilized human sperm by transmission electron microscope (TEM).

METHODS: Sperm obtained from ejaculated semen samples from three different donors were immobilized in a standard manner for ICSI. They were then injected into the perivitelline space of mouse oocytes in order to be able to locate them by TEM. Intact motile sperm injected subzona1ly served as controls (n = 160). Finally, the ‘carrier’ oocytes were fixed and processed for TEM.

RESULTS: A total of 300 sperm were mechanically immobilized and inserted into the perivitelline space of mouse oocytes. Ultrathin sections revealed consistent alterations in the acrosomal region including disruption of the plasma membrane, and disruption, vesiculation or even loss of the acrosome. Thus, all of the sperm assessed had undergone some disorganization of the head, in contrast to a majority of control sperm.

CONCLUSIONS: Immobilization of sperm for ICSI by compressing and rolling the sperm tails induces a variable disruption and sometimes loss of the acrosome. This could well be a reason for the higher success rates when ICSI is performed using immobilized sperm.

Key words: acrosome reaction/human sperm/ICSI/mechanical immobilization/transmission electron microscopy

Introduction

In order to fertilize, mammalian sperm first need to undergo capacitation in the female tract (Austin, 1951; Chang, 1951). Capacitation involves modifications in the sperm plasma membrane, which lead to hyperactivation and permit the acrosome reaction. The latter involves multiple fusions between the outer acrosome membrane and the overlying sperm plasma membrane, enabling the soluble contents of the acrosome to leak out through the fenestrated membranes (Barros et al., 1967), and coincidentally it prepares the surface over the equatorial segment for its fusogenic role (Yanagimachi, 1994).

ICSI bypasses the events involved in physiological sperm penetration of the oocyte, and requires no specific pretreatment of sperm other than immobilization (Palermo et al., 1992, 1995; Vanderzwalmen et al., 1996). However, aggressive immobilization by compressing the tail prior to injection significantly improves ICSI fertilization rates (Fishel et al., 1995; Gerris et al., 1995; Van den Bergh et al., 1995). Although the mechanism of this beneficial effect is not yet clear, there is indirect evidence that such immobilization causes changes in the sperm permeability (Dozortsev et al., 1995a), and that it may possibly induce changes leading to acrosomal disruption (Fishel et al., 1995; Palermo et al., 1996). Nevertheless, there has been no report on the ultrastructural state of sperm immobilized for ICSI.

In this study, we have analysed membrane integrity and acrosomal characteristics of immobilized sperm using transmission electron microscopy (TEM).

Materials and methods

Sperm preparation and immobilization procedure

Ejaculated semen samples obtained from consenting men with normozoospermia (under the Internal Review Board No. 0696-389) were processed by density gradient centrifugation as routinely performed for ICSI (Palermo et al., 1995). Semen parameters from each individual donor are summarized in Table I. Immediately before immobilization, 1 μl of the final sperm suspension (~1×10⁶/ml) was placed in a 4 μl droplet of 10% polyvinylpyrrolidone (w/v) (PVP; Sigma Chemicals, USA) in human tubal fluid (HTF) medium buffered with HEPES medium (H-HTF; Irvine Scientific, USA) in the centre of a Petri dish (Palermo et al., 1995). For immobilization, motile sperm viewed under an inverted microscope at ×400 magnification (Olympus IX-70; New York/New Jersey Scientific Inc., USA) were positioned perpendicularly to an ICSI pipette. This was used to gently compress and roll the principal piece of the sperm tail in a repeti1ve...
manner until it became permanently kinked or convoluted (Palermo et al., 1996).

Subzonal insertion of sperm into mouse oocytes

Mouse oocytes were collected from B6D2F1 females and the subzonal insertion procedure was carried out as previously described (Palermo et al., 1991). To allow their study in the TEM, ~60 immobilized sperm were aspirated into an ICSI needle and injected together into the perivitelline space of oocytes, which acted as containers for these sperm (Figure 1). Motile (control) sperm were injected subzonally into oocytes in the same manner. The time required for sperm immobilization and oocyte injection ranged between 10 and 15 min.

Sample processing for TEM

Immediately after subzonal injection, each injected oocyte was fixed for 1 h in 2% glutaraldehyde (Electron Microscopy Sciences, USA) in 0.1 mol/l cacodylate buffer at room temperature and overnight at 4°C. They were then washed in buffer, exposed to 2% osmium tetroxide (Electron Microscopy Sciences) for 1 h, dehydrated through increasing concentrations of ethanol up to 100%, and embedded in Spurr’s resin (Electron Microscopy Sciences). Thick plastic sections were cut and stained with Toluidine Blue in borate buffer. When sperm were found, ultrathin sections were cut, stained with uranyl acetate and lead citrate, and studied in a JEOL 100S TEM.

Results

A total of 300 sperm from three different individuals were mechanically immobilized and inserted subzona1ly into five mouse oocytes (Figure 1, ~60 sperm per oocyte). In addition, 160 motile sperm were injected similarly under the zona pellucida of mouse oocytes as controls. We evaluated 22 immobilized sperm originating from donor A, 11 from B, and 19 from C, while 7, 9 and 10 control sperm were examined respectively for each of the donors. Thus, a total of 52 immobilized and 26 intact sperm was studied in the TEM.

Immobilized sperm all had alterations in the peri-acrosomal plasma membrane ranging from localized vesiculation to a complete disruption that allowed leakage of the soluble acrosomal content. While no changes in the chromatins were observed, some sperm even exhibited a complete loss of the acrosome. Each spermatozoon fell into one of four ultrastructural categories: (A) acrosome-intact (Figure 2a); (B) swelling of the acrosomal matrix (Figure 2b); (C) variable disruption of sperm head membranes and/or vesicle formation within the acrosomal matrix (Figure 2c); (D) loss of the carapace and content of the acrosome (Figure 2d).

Since the proportions of each group were similar among the three different individuals, the data were combined (Table II).

No immobilized sperm had an intact acrosome: 86.5% had undergone disruption of the acrosome or it had disappeared completely (group C + D), and 13.5% were in the initial stages of the disruption (group B). In contrast, the majority of non-immobilized cells (73.1%) were intact, with most of the remainder (23.1%; group B) showing some morphological change (Table II).

Discussion

Immobilization of sperm immediately before the ICSI procedure is one key to its consistent success (Fishel et al., 1995; Gerris et al., 1995; Van den Bergh et al., 1995; Palermo et al., 1996), but why is not clear. Most evidence suggests that this might depend on changes in the sperm plasma membrane (Dozortsev et al., 1995a), and our findings support this view. Sperm membrane permeabilization may help to expose the sperm nucleus to the ooplasm, facilitating male pronucleus formation (Maleszewski, 1990; Yanagimachi, 1998). Removal of the sperm plasma membrane also seems to be necessary for oocyte activation. Indeed, calcium oscillations and subsequent
oocyte activation appear to be induced after intracytoplasmic injection by a spermatozoon-associated oocyte activating factor(s) (Dozortsev et al., 1995b; Palermo et al., 1997), which can exert its effect only after damage or removal of this membrane (Swann et al., 1994).

While calcium oscillations are certainly responsible for oocyte activation, these oscillations are believed to be induced by a sperm cytosolic factor(s) (Swann, 1990; Palermo et al., 1997). Although the oscillogenic molecule is presumably a soluble polypeptide released into the ooplasm at the time of gamete fusion, its location in the sperm head has not yet been clearly defined (Parrington et al., 1996; Wolny et al., 1999). However, it is considered to reside in the perinuclear theca (PT) (Kimura et al., 1998). It has been suggested that exposure of the PT to the ooplasm and its dissociation are linked to the beginning of calcium oscillations (Sutovsky et al., 1997; Kimura et al., 1998), although this is not always a requirement (Knott et al., 2003). Sperm of globozoospermic patients, which typically lack a PT and are considered unable to fertilize oocytes without assisted activation (Battaglia et al., 1997; Rybouchkin et al., 1997) have on occasion been able to do so (Lundin et al., 1994; Liu et al., 1995). The fact that mechanically immobilized sperm often displayed some modification of the PT (Figure 2b and c) suggests that exposure of the PT might be a contributing factor in the relative success of immobilized sperm during ICSI (Knott et al., 2003).

The status of the acrosome after ICSI has been the subject of debate (Lacham-Kaplan and Trounson, 1995; Sathananthan et al., 1997). The present study demonstrates that immobilization elicits changes in the plasma membrane and acrosome, since these were always disrupted to varying degrees in immobilized sperm, in contrast to the control population. Since acrosomal disruption can sometimes occur spontaneously, it was not surprising to find this in some 25% of the sperm in the control group as well (Lee et al., 1997).

One problem in trying to perform ultrastructural studies on a small number of sperm is finding them in the TEM. Recently Cohen et al. (1997) used empty zonae pellucidae for cryopreservation of single human sperm, and we have followed this novel approach here, using oocytes as ‘carriers’ to aid in localization of the treated sperm cells in the TEM.

While sperm immobilization is usefully performed with the ICSI needle, immobilization by piezo-pulses (Huang et al., 1996) has also been practised, and some investigators have obtained identical fertilization rates using sperm exposed to a non-contact diode laser (Montag et al., 2000; Ebner et al., 2001). These authors noted that use of the diode laser reduces micromanipulation time, that the magnification of the laser

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Figure 2. (a) Intact human sperm head. (b) Human sperm head showing local disruption of the plasma membrane and a scalloping of the acrosome. (c) Irregular swelling and decondensation of the acrosomal matrix and within which small circular vesicles are evident. (d) Human sperm heads that have lost their acrosomes (TEM × 19 000).
objective allows for better evaluation of sperm morphology, and that its use eliminates the need for a viscous medium (e.g. PVP), which could be potentially toxic for the gametes. However, not only are laser and piezo systems more costly but the classical technique used here may be performed by any ICSI-trained embryologist with no extra equipment.

In conclusion, the present study demonstrates that mechanical sperm immobilization induces changes in the acrosome and sperm head plasma membrane, providing a likely explanation for the higher success rates obtained when ICSI is performed using immobilized sperm.

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References


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