BRIEF COMMUNICATION

An alternative to PVP for slowing sperm prior to ICSI

B. Balaban¹, K. Lundin², J. M. Morrell³,*, H. Tjellström³,*, B. Urman¹ and P. V. Holmes³,4,*

¹American Hospital, Istanbul, Turkey, ²Reproductive Medicine, SU/Sahlgrenska, Gothenburg and ³NidaCon International, Gothenburg, Sweden

*To whom correspondence should be addressed. E-mail: paul@nidacon.com

BACKGROUND: There is a growing awareness of potential problems in exposing sperm to polyvinylpyrrolidone (PVP) to slow their motility, a procedure commonly used prior to ICSI. The study presented here evaluates an alternative product for slowing sperm motility, which contains hyaluronate, a substance found naturally in the reproductive tract. METHODS: Computerized sperm motility analysis was used to compare the motilities of sperm exposed to either a PVP-containing product (ICSI-100), or a hyaluronate-containing product (SpermCatch™), or control sperm resuspended in a sperm maintenance medium. A subjective assessment was made of the ease with which sperm could be isolated and be drawn into, and expelled from, an injection pipette after having their tails nicked. Sperm exposed to either ICSI-100 or SpermCatch were used for ICSI. Fertilization rate, zygote development, grading, and outcome of transfer were recorded for the two treatment groups. RESULTS: The hyaluronate-containing product slowed sperm motility sufficiently for the sperm to be captured in an injection pipette, was easy to draw into and expel from the pipette, prevented sperm sticking to plastic or glassware, and did not affect post-injection zygote development. Clinical pregnancy rates were similar for the two groups. CONCLUSIONS: This product represents an alternative to PVP for slowing sperm motility prior to ICSI.

Key words: hyaluronate/ICSI/PVP/slowing sperm motility

Introduction

Intracytoplasmic sperm injection (ICSI) requires the capture of an individual sperm in a glass pipette for injection into the oocyte. This procedure is facilitated by first immobilizing the sperm, which is accomplished by a variety of means, for example by laser (Montag et al., 2000; Ebner et al., 2001; 2002), or by sonication, freezing, detergent treatment and piezo-actuated pulses (reviewed by Mizuno et al., 2002). Probably the most widely practised method, since it does not require special equipment, is to reduce sperm motility by placing the sperm in a viscous medium prior to nicking the tail to immobilize the sperm completely (Van Steirteghem et al., 1993).

Previously, the only products commercially available for slowing sperm motility contained a synthetic plastic, polyvinylpyrrolidone (PVP). However, some PVP is injected into the oocyte along with the sperm and, as the PVP cannot diffuse out and is not digestible by lysosomal enzymes, it will remain in the oocyte for a prolonged period (Jean et al., 2001). To avoid any potential damaging effects of PVP, techniques have been developed which do not necessitate slowing sperm motility (Harari et al., 1995; Jean et al., 1996; 1997; Butler and Masson, 1997; Hlinka et al., 1998; Tsai et al., 2000). However, not everybody is able to crush the tails of sperm moving at their normal velocity.

Therefore, a physiological alternative to PVP has been sought for reducing sperm motility to facilitate capturing sperm for ICSI. Ideally the product should possess the following characteristics: (i) be sufficiently viscous, to slow sperm motility enough to aspirate them into an ICSI pipette; (ii) be sufficiently fluid, to facilitate ease of aspirating and dispensing the sperm and a little of the liquid into, and from, the pipette; (iii) be able to prevent sperm sticking to either the plastic culture dish or the glass ICSI pipette; and (iv) have no deleterious effects on post-ICSI zygote development.

This study was designed to evaluate SpermCatch™ (NidaCon International, Gothenburg, Sweden), a viscous liquid containing hyaluronate and human serum albumin, as a potential substitute for PVP. Since hyaluronate and human serum albumin are found naturally in the mammalian reproductive tract, this product may prove to be a physiological alternative to PVP which can be used in the clinic to reduce sperm motility. SpermCatch is manufactured according to current Good Manufacturing Practice (cGMP) by NidaCon International AB, an ISO-registered company, and is approved by medical authorities.

*J.M. Morrell, H. Tjellström, and P.V. Holmes are employees of NidaCon International, the manufacturer of SpermCatch medium.
Ejaculates from 11 healthy volunteers, whose semen parameters were considered 'normal' (World Health Organization, 1999) and whose fertility status was unknown, were prepared on PureSperm® density gradients by centrifugation at 300 g, to separate sperm into aliquots of the sperm preparations were added to equivalent volumes of SpermCatch, ICSI-100, or SpermAssist as a control (all materials were obtained from NidaCon International AB with the exception of gamete-holding medium). SpermTracker (Hobson Vision Ltd, Derbyshire, UK). A total of 500 million sperm from each donor was analysed per treatment group according to standard procedures (Holt et al., 1989).

The sperm were slowed by exposure to SpermCatch, immobilized by nicking their tail and drawn into an ICSI pipette. Sperm did not stick to the plastic culture dish or to the glass pipette. The hyaluronate-containing medium was sufficiently viscous to allow fine control during sperm aspiration into, and expulsion from, the injection pipette. Sperm did not stick to the plastic culture dish or to the glass pipette.

Results

Motility was modified immediately for sperm exposed to the hyaluronate-containing medium (SpermCatch) compared with sperm in gamete-holding medium alone. Progressive forward motility was decreased without any noticeable change in the lateral movement of the sperm head. Although the spermatozoa did not cease moving completely, it was easy to immobilize and catch them individually and draw them into an injection pipette. The hyaluronate-containing medium was sufficiently viscous to allow fine control during sperm aspiration into, and expulsion from, the injection pipette. Sperm did not stick to the plastic culture dish or to the glass pipette.

CASA was performed on sperm exposed to either the hyaluronate-containing medium or the PVP product (ICSI-100). These sperm had mean curvilinear velocities, mean velocity of the average path and mean straight line velocities which were significantly slower ($P < 0.001$ for all parameters) than those of control sperm diluted in sperm maintenance medium (Table I).

The rates of zygote cleavage and development after injection of the oocyte with sperm exposed to either the hyaluronate-containing medium or the PVP product are shown in Table II, together with the number of grade 1 and 2 embryos developing and transferred. The rates were not different between the two treatment groups. Apart from one pregnancy loss in each group, the remaining pregnancies proceeded to term. No genetic abnormality was observed in any of the offspring.

Discussion

Clinicians have become increasingly concerned about the use of PVP-containing products to slow sperm motility prior to ICSI (Jean et al., 2001). Dozortsev et al. (1995) reported that

Materials and methods

Table I. Mean velocities of sperm exposed to either hyaluronate-containing or PVP-containing products, compared with control sperm in sperm maintenance medium ($n = 11$ sperm donors)

<table>
<thead>
<tr>
<th></th>
<th>Curvilinear velocity (mean ± SD) (μm/s)</th>
<th>Velocity of the average path (mean ± SD) (μm/s)</th>
<th>Straight line velocity (mean ± SD) (μm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>88.4 ± 10.8</td>
<td>47.5 ± 5.1</td>
<td>36.7 ± 7.4</td>
</tr>
<tr>
<td>Hyaluronate</td>
<td>48.3 ± 5.5</td>
<td>29.2 ± 5.0</td>
<td>23.6 ± 3.8</td>
</tr>
<tr>
<td>PVP</td>
<td>42.2 ± 6.1</td>
<td>22.1 ± 6.1</td>
<td>15.1 ± 4.9</td>
</tr>
</tbody>
</table>

Table II. Proportion of oocytes cleaving and zygotes developing after ICSI with sperm exposed to hyaluronate-containing or PVP-containing products

<table>
<thead>
<tr>
<th></th>
<th>Hyaluronate</th>
<th>PVP</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. patients</td>
<td>48</td>
<td>44</td>
</tr>
<tr>
<td>No oocytes injected</td>
<td>499</td>
<td>449</td>
</tr>
<tr>
<td>No. with two pronuclei (% fertilization)</td>
<td>360 (72)</td>
<td>337 (75)</td>
</tr>
<tr>
<td>Cleavage</td>
<td>353 (98)</td>
<td>330 (97.9)</td>
</tr>
<tr>
<td>No. good embryos (% G1, G2)</td>
<td>226 (64)</td>
<td>211 (63.9)</td>
</tr>
<tr>
<td>No. G1, G2 transferred (% of total no. transferred)</td>
<td>127 (80.3)</td>
<td>113 (82.4)</td>
</tr>
<tr>
<td>No. embryos transferred (mean)</td>
<td>149 (3.1)</td>
<td>141 (3.2)</td>
</tr>
<tr>
<td>Clinical pregnancy rate (%)</td>
<td>20/48 (41.7)</td>
<td>19/44 (43.2)</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>27/149 (18.1)</td>
<td>27/141 (19.1)</td>
</tr>
<tr>
<td>Pregnancy outcome</td>
<td>7 twins, 13 singletons</td>
<td>8 twins, 11 singletons</td>
</tr>
<tr>
<td>Pregnancy loss</td>
<td>1 singleton</td>
<td>1 twin</td>
</tr>
</tbody>
</table>
PVP may interfere with sperm nucleus decondensation while Feichtinger et al. (1995) stated that they have always avoided the use of PVP. Detrimental effects on mouse zygote development were reported following exposure of the sperm to PVP (Mizuno et al., 2002). In contrast, bull zygotes were not affected by PVP (Motoshi et al., 1996), possibly reflecting an effect of either the source or concentration of PVP used for the procedure. Moreover, differential species susceptibility to the effects of PVP cannot be ruled out.

Some embryologists, concerned about the potential adverse effects of PVP, have developed modified techniques to capture sperm without slowing sperm motility (Harari et al., 1995; Hlinka et al., 1998). Others have reported that even fast-moving sperm can be caught by this method, with the added advantage that since the sperm is never aspirated into the needle, a minimal volume of fluid is expelled during sperm deposition in the oocyte (Hlinka et al., 1998). Others have reported crushing the sperm tail before aspirating the sperm into the tip of the ICSI pipette (Harari et al., 1995; Tsai et al., 2000). Jean et al. (2001) noted that training was sufficient to overcome any initial difficulties experienced in catching the sperm in the absence of PVP.

Although avoiding the use of exogenous substances altogether is probably the most desirable situation, sperm are not easy to tail-nick or catch without slowing their motility. Therefore, a physiological alternative to PVP is required. The new product, SpermCatch, which contains sodium hyaluronate, slowed sperm sufficiently for them to be caught, was easy to handle in the ICSI pipette and prevented sperm from sticking to the culture dish or to the pipette. Although sperm motility was somewhat faster in the hyaluronate-containing product than in the PVP-containing product, which necessitated some slight adaptations to the handling techniques on the part of the operator, it was not difficult to learn to use this product. Furthermore, since there was no difference in zygote development after ICSI, it would appear that the hyaluronate-containing product could be used in preference to PVP-containing products without detriment to the patient’s chances of becoming pregnant.

The observation that hyaluronic acid can modulate sperm motility is not new; for example, Zimmerman et al. (1994) reported that exposure of sperm to hyaluronic acid at 1 mg/ml caused a decrease in straight line velocity, amplitude of lateral head deviation and mean angular deviation compared with non-exposed sperm. Moreover, the suggestion that hyaluronic acid could be used to slow sperm motility prior to ICSI was advocated by Barak et al. (1999; 2001). These authors reported similar fertilization rates and pregnancy rates after ICSI with sperm exposed to either hyaluronic acid or PVP and recommended the use of hyaluronic acid as a physiological replacement for PVP.

The results reported here concur with those of Barak et al. (1999; 2001), in that sperm motility was effectively slowed by the hyaluronate-containing product, and zygote development after ICSI was not impaired. Furthermore, the hyaluronate-containing product was easy to handle in the ICSI pipette and prevented sperm from sticking to the culture dish or to the pipette. Therefore, it is concluded that SpermCatch represents an effective, physiological alternative to PVP for modulating sperm motility prior to aspirating a single sperm into an ICSI pipette.

References


