Hyper-osmotic condition enhances protein tyrosine phosphorylation and zona pellucida binding capacity of human sperm

D.Y.Liu1,5, G.N.Clarke3 and H.W.G.Baker1,2,4

1Department of Obstetrics and Gynecology, University of Melbourne, 2Reproductive Services and 3Andrology Laboratory, Royal Women’s Hospital and 4Melbourne IVF, Melbourne, Australia
5To whom correspondence should be addressed. E-mail: dyl@unimelb.edu.au

BACKGROUND: The aim of this study was to determine the effect of culture medium osmolality, in the range known to occur in the male and female reproductive tracts, on human sperm tyrosine phosphorylation and sperm–zona pellucida (ZP) interaction in vitro. METHODS: Motile sperm (2 × 10⁶), selected by swim-up from semen of normozoospermic men with normal sperm–ZP binding, were incubated with or without four oocytes in 1 ml human tubal fluid (HTF) medium with different osmolalities (150, 200, 280, 350, 400 mOsm/kg) adjusted by variation of the NaCl concentration. After 2 h incubation, the number of sperm bound to the four ZP was examined, sperm motility and velocities were assessed by Hamilton–Thorn Motility Analyzer (IVOS 10) and sperm tyrosine phosphorylation was assessed by both western immunoblotting and immunofluorescence with an anti-phosphotyrosine monoclonal antibody (PY20). The effect of hyper-osmolality (400 mOsm/kg) on the ZP-induced acrosome reaction (AR) was also determined. RESULTS: Incubation of human sperm in hyper-osmotic medium significantly increased tyrosine phosphorylation and the number of sperm bound to the ZP. In contrast, hypo-osmotic medium significantly decreased both tyrosine phosphorylation and sperm–ZP binding. Medium with high osmolality (400 mOsm/kg) significantly reduced the ZP-induced AR. Both hypo- and hyper-osmotic media significantly decreased average sperm percentage progressive motility and velocities. CONCLUSION: Incubation of human sperm in hyper-osmotic media was associated with significantly increased tyrosine phosphorylation and ZP-binding ability but severely reduced the ZP-induced AR.

Key words: capacitation/osmolality/sperm–zona pellucida interaction/tyrosine phosphorylation

Introduction

Human seminal plasma has high osmolality (300 to ∼400 mOsm/kg) compared with human serum or female reproductive tract fluid (280–300 mOsm/kg; Makler et al., 1981; Polak and Daunter, 1984; Rossato et al., 2002). A recent report by Cooper et al. (2005) showed that osmolality increases during 30 min incubation after ejaculation. Earlier studies had shown that seminal osmolality continues to increase for up to 6 h post-ejaculation due to enzymatic breakdown of proteins, peptides and choline compounds (Hofmann and Karlas, 1973). Another study reported a negative relationship between seminal osmolality and sperm progressive motility (Rossato et al., 2002). Therefore, during the process of human fertilization either in vivo or in vitro, sperm will encounter significant variations in osmotic conditions. Such marked changes in osmolality may have some impact on plasma membranes which may affect sperm fertilizing ability (Bielfeld et al., 1993; Rossato et al., 1996).

Previous research has clearly shown that osmolality has a significant impact on sperm motility, capacitation, calcium influx and the acrosome reaction (AR) in vitro (Mahi and Yanagimachi, 1973; Aitken et al., 1983; Rossato et al., 1996). Treatment of sperm with medium of relatively low osmolality induces plasma membrane swelling which leads to stimulation of calcium influx and the AR of human sperm in vitro (Rossato et al., 1996). On the other hand, hyper-osmotic conditions significantly inhibit both spontaneous and calcium ionophore-induced AR (Bielfeld et al., 1993). It is also reported that hyper-osmolality inhibits cortical granule exocytosis in sea-urchin oocytes (Merkle and Chandler, 1989).

In standard IVF procedures, it was reported that hyper-osmotic medium (345 mOsm/kg) significantly reduced human sperm fertilizing ability compared with medium with osmolality of either 285 mOsm/kg or 315 mOsm/kg (Dumoulin et al., 1990). However, with ICSI procedures, a recent study showed that mouse sperm preserved in a medium with very high osmolality (800 mOsm/kg) at 4°C were able to produce normal fertilization and embryonic development, and live birth of healthy offspring after ICSI (Van Thuan, 2005).
Overall evidence suggests that osmotic conditions play an important role in sperm fertilizing ability in vitro and simple changes of osmolality of medium may significantly impact on sperm function. One of the most important sperm functions is the ability to interact with the zona pellucida (ZP): sperm must be able to bind to the ZP, undergo the AR on the surface of ZP and penetrate the ZP before fertilization takes place (Yanagimachi, 1994). A significant correlation between phosphorylation and binding to the ZP in vitro has been found with human sperm (Sakkas et al., 2003). The aim of this study was to determine the effect of culture medium osmolality on human sperm protein tyrosine phosphorylation, sperm–ZP binding and the ZP-induced AR in vitro.

Materials and methods

Chemicals and culture medium

Human tubal fluid (HTF) with osmolality adjusted by variation of NaCl concentration was prepared with the following final values: 150, 200, 280, 350 and 400 mOsm/kg determined by freezing point depression using Advanced DigiMatic Osmometer Model 3D II (Advanced Instrument Inc., Needham Heights, MA, USA). The final concentrations of NaCl in these different osmotic media (150, 200, 280, 350 and 400 mOsm/kg) were 12, 42, 101, 132 and 165 mmol/l respectively. In some experiments, sucrose (115 mmol/l) was used instead of NaCl to increase the osmolality of standard HTF medium up to 400 mOsm/kg. Repeated measurements of osmolality for the same media by osmometer was within ±1%. The pH of the medium was 7.4–7.6 and was supplemented with 5% heat-inactivated human serum. Pisum sativum agglutinin (PSA) conjugated with fluorescein isothiocyanate (FITC), anti-mouse IgG-FITC and phosophotyrosine antibody inhibitor, a solution of α-phospho-L-tyrosine conjugated to BSA (specific inhibitor of the reactivity of anti-phosphotyrosine antibody) were purchased from Sigma Chemical Company (St Louis, MO, USA). Human serum and Anti-phosphotyrosine monoclonal antibody (PY20, mouse IgG) labelled with FITC were obtained from ICN Pharmaceuticals Inc (Costa Mesa, CA, USA). Polyvinylidene difluoride (PVDF) membrane was purchased from Pall Life Sciences (Australia). Horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG was obtained from Zymed Laboratories (San Francisco, CA, USA). A chemiluminescence ECL kit was obtained from Amersham Biosciences UK Ltd, (Little Chalfont, Buckinghamshire, UK).

Human oocytes

Oocytes which showed no evidence of two or three pronuclei or cleavage at 48–60 h after insemination or immature oocytes (with germinal vesicles) in a clinical IVF programme were used for the ZP-induced AR test. Oocytes with any remaining cumulus and corona cells or sperm bound to the ZP from the IVF insemination were removed by repeated aspiration of the oocyte using a fine glass pipette with an inner diameter (120 μm) slightly smaller than the oocyte diameter (Liu and Baker, 1994, 1996). Degenerate, activated or morphologically abnormal oocytes as well as oocytes with >10 sperm penetrating the ZP were not used for the test. All the oocytes were obtained on day 3 of insemination and the oocytes were pooled from several patients and used for the test on the same day or kept in the 5% CO₂ in air incubator and used within next 3 days.

Sperm samples and preparation

Semen samples were obtained by masturbation after 3–5 days abstinence from normozoospermic men who had sperm counts >20×10⁶/ml, progressive motility >30% and variable normal sperm morphology. Sperm count and motility were performed after liquefaction and within 1 h of collection of semen, according to World Health Organization (1999) procedures. Sperm morphology was assessed on Shorr-stained smears under oil immersion with magnification ×1000 and bright-field illumination. Percentage normal sperm morphology was assessed according to strict criteria (Kruger et al., 1988; World Health Organization, 1999).

Motile sperm were selected by swim-up technique as follows: 0.3 ml of semen was carefully added to the bottom of a test tube (12×75 mm) containing 0.7 ml standard HTF (280 mOsm/kg) supplemented with 5% heat-inactivated human serum. Care was taken to avoid bubbles and not disturb the interface between semen and the medium. After incubation for 1 h, 0.5 ml of the top layer of the medium containing motile sperm was aspirated. The motile sperm suspension was then centrifuged at 1000 g for 5 min, the supernatant removed and the sperm pellet washed again with 1 ml fresh HTF by centrifugation at 1000 g for 5 min. The washed sperm pellet was resuspended with serum-supplemented standard HTF to a motile sperm concentration of 4×10⁶/ml for subsequent experiments. Only sperm samples with >90% progressive motility after swim-up preparation were used for the study. Viability of sperm was assessed by counting 200 sperm using Eosin Y exclusion (World Health Organization, 1999).

All patients signed consent forms permitting use of their gametes (unfertilized oocytes and sperm samples) for research. The Royal Women’s Hospital Research and Ethics Committees approved the project.

Sperm–ZP interaction test

Motile sperm (2×10⁶ in 1 ml medium) selected by swim-up were incubated with four oocytes in media with different osmolarities in 4-well culture plates (Nunc, Rosilde, Denmark) for 2 h at 37°C in 5% CO₂ in air. After incubation, each group of four oocytes was transferred to phosphate-buffered saline (PBS) containing 2 mg/ml bovine serum albumin (BSA; Commonwealth Serum Laboratory, Parkville, Victoria, Australia). After incubation the oocytes were flushed several times to dislodge loosely adherent sperm using a pipette approximately twice the diameter of the oocyte (250 μm) in three separate wells containing 0.5 ml PBS with 0.2% BSA. The sperm which were tightly bound to all the four ZP/test were removed by repeated aspiration using a narrow-bore pipette with diameter slightly smaller than the oocyte (120 μm) in a small volume (~5 μl) of PBS on a slide, then smeared in a limited area (~4×4 mm) which was marked with a diamond pen to help find the sperm under the microscope for counting and the AR assessment (Liu and Baker, 1996; Liu et al., 2001). Because all semen samples were from normozoospermic men with normal sperm–ZP binding, there were >100 sperm bound/ZP. Therefore, removing all tightly bound sperm on a slide would allow accurate counting of the number of sperm bound to the ZP as described previously (Liu et al., 2003). The total number of sperm bound to all the four ZP per test was used for statistical analysis.

Assessment of acrosome status of sperm bound to the ZP

The sperm bound to the ZP were removed and smeared on a glass slide as described above. The AR of ZP-bound sperm was assessed using PSA–FITC as described previously (Cross et al., 1986; Liu and Baker, 1996). Sperm smears were fixed in 95% ethanol for 30 min after air-drying and then stained using 25 μg/ml PSA–FITC in PBS for 2 h at 4°C. The slides were washed and mounted with distilled water and 200 sperm per sample were counted with a fluorescence microscope using excitation wavelengths of 450–490 nm and a magnification of ×400. When more than half the head of a sperm was brightly and uniformly fluorescent, the acrosome was considered intact. Sperm with a fluorescence band at the equatorial segment or without fluorescence (a rare pattern) were considered acrosome-reacted.
**Assessment of sperm motility, velocity and movement characteristics**

After 2 h incubation of sperm in medium with various osmolality values, sperm motility, velocity (VSL, straight line velocity; VCL, curvilinear velocity) and other movement characteristics (LIN, linearity and STR, straightness) were measured by Hamilton–Thorn Motility Analyzer (IVOS 10, 60 Hz; Hamilton–Thorn Research, Danvers, MA, USA). For this study, total motility or progressive motility was defined as the percentage of sperm with VAP >7.5 or >25 μm/s respectively. Because the sperm concentration was only 2×10⁶/ml when the sperm were incubated in culture medium, the sperm concentration was adjusted to ∼20×10⁶/ml by centrifugation at 800 g for 5 min and resuspended in 100 μl of the same medium. A sample of 5 μl was placed in a Microcell (20 μm depth) for assessment of motility and velocities. For each sperm sample, the average of six (five to seven) fields with a total of >400 sperm was assessed.

**Immunofluorescent detection of tyrosine phosphorylation in human sperm**

Motile sperm (2×10⁶/ml) selected by swim-up were incubated for 2 h in HTF medium with various osmolarities in standard culture conditions. After incubation, sperm were washed with 10 ml normal saline and the sperm pellet resuspended in ∼20 μl saline and 5 μl smeared on a glass slide. After drying in air, the smear was fixed in 90% ethanol for 30 min to permeabilize plasma membranes. Tyrosine phosphorylation of sperm tails was assessed by direct immunofluorescence using the monoclonal antibody PY-20 (ICN Pharmaceuticals Inc., Costa Mesa, CA, USA) labelled with FITC. The antibody is diluted 1:300 in PBS containing 10 mg/ml BSA. For each smear, 50 μl of diluted PY-20 is added to cover the smear (∼10×10 mm) which was then incubated for 4 h in a humidified box at 37°C. After incubation, the slide was washed and mounted with distilled water and covered with a coverslip (22×22 mm). Sperm tails with positive binding of PY-20 antibody had bright fluorescence and the percentage of sperm with positive stain (tyrosine phosphorylated sperm) was determined by scoring 400 sperm for each sample using a combination of light and fluorescent microscopy (Figure 1). Antibody specificity was confirmed by control tests demonstrating that phosphotyrosine anti-body inhibitor (a solution of o-phospho-L-tyrosine conjugated to BSA, 10 μg/ml) could block the binding of PY20-FITC on the sperm tails. In addition, mouse IgG-FITC used as an extra control for non-specific binding did not show positive fluorescence on any of the sperm.

**Western immunoblotting**

Western immunoblotting for tyrosine phosphorylation was performed using protein extracts from 2×10⁶ sperm incubated in media with various osmolarities. The protein bands were visualized using a chemiluminescence detection system (Bio-Rad, Hercules, CA, USA). Antibody specificity was confirmed by control tests demonstrating that phosphotyrosine antibody inhibitor (a solution of o-phospho-L-tyrosine conjugated to BSA, 10 μg/ml) could block the binding of PY20-FITC on the sperm tails. In addition, mouse IgG-FITC used as an extra control for non-specific binding did not show positive fluorescence on any of the sperm.

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**Figure 1.** Effect of iso-osmotic (280 mOsm/kg **A** and **B**) and hyper-osmotic (400 mOsm/kg **C** and **D**) conditions on tyrosine phosphorylation of sperm from one subject determined with PY20-FITC. (**A** and **C**) Light microscopy showing all sperm. (**B** and **D**) Fluorescence microscopy showing bright fluorescence on two (**B**) and four (**D**) sperm tails.
osmolalities as described above using the method of Buffone et al. (2005). After 2 h incubation, sperm were pelleted and washed twice with PBS and then solubilized in 2% sodium dodecyl sulphate (SDS in 0.375 mol/l Tris, pH 6.8, 10% sucrose) for 5 min with vigorous mixing by vortex but without boiling and then centrifuged at 6000 g at 4°C for 5 min. The supernatants were recovered and stored at −20°C until required. Solubilized sperm proteins were diluted (1:1) with Laemmli (1970) sample buffer and then heated at 100°C for 5 min in a water bath before loading onto a 10% polyacrylamide gel. The proteins were separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) under denaturing conditions. For each lane of the gel, the protein extract from the equivalent of 2×10⁶ sperm was applied.

After SDS–PAGE, the proteins were transferred onto the PVDF membrane at a constant current of 50 mA for 90 min. To block non-specific binding sites, the membrane was first incubated with 5% skimmed milk powder dissolved in Tris-buffered saline (TBS, pH 7.6). After that, it was incubated for 2 h with the monoclonal anti-phosphotyrosine antibody PY20 diluted 1:5000 in blocking solution. After three 10 min washes with TBS, HRP-conjugated goat anti-mouse IgG diluted 1:5000 in blocking solution was added. Following a further 2 h incubation, the membrane was washed for 1 h in 0.05% Tween-20 in TBS, followed by a further three 10 min washes in TBS alone. Reactive bands were detected by enhanced chemiluminescence using the ECL kit.

To quantify changes in protein tyrosine phosphorylation of sperm from four subjects incubated in media with different osmolalities, rectangular boxes were drawn around the major bands on scanned digital images of ECL contact photographs of western immunoblots and adjusted optical densities for each lane were obtained.

Effect of hyper-osmotic conditions on tyrosine phosphorylation and ZP binding capacity of sperm in men with defective sperm–ZP binding

To determine if hyper-osmotic conditions could enhance tyrosine phosphorylation and ZP binding capacity of sperm from men with defective sperm–ZP binding, tyrosine phosphorylation and sperm–ZP binding tests were performed using both iso-osmotic (280 mOsm/k) and hyper-osmotic (400 mOsm/k) media on sperm samples from six men with defective sperm–ZP binding (normal: ≥40 sperm bound/ZP). All these men had sperm counts >20×10⁹/ml, progressive motility >30% and variable sperm morphology.

Statistical analysis

The significance of the differences for the number of sperm bound to the ZP, sperm motility and velocities, sperm tyrosine phosphorylation between medium with various osmolalities were examined by non-parametric two-way analysis of variance (Friedman test) for all the data and paired comparisons between any two osmolality results.

Results

As compared to sperm in iso-osmotic (280 mOsm/kg) medium, sperm in both hypo- (150–200 mOsm/kg) and hyper- (350–400 mOsm/kg) osmotic media had significantly reduced motility, velocities, movement characteristics: straightness and linearity, and viability (Figure 2). Sperm viability, progressive motility and velocities reduced more severely in media with lower osmolality (150 mOsm/kg) than media with higher osmolality (400 mOsm/kg). There were highly significant relationships between osmolality of medium and both tyrosine phosphorylation of
sperm detected by immunofluorescence (Figure 3) and western immunoblotting (Figure 4) and the number of sperm bound to the ZP of human oocytes (Figure 5). There were three major bands ~100 kDa in the immunoblots that increased with osmolality (Figure 4). Other faint bands were not measured. When sperm and oocytes were incubated in hyper-osmotic medium of 350 and 400 mOsm/kg, the number of sperm bound to the ZP was 2–3-fold higher than with sperm in iso-osmotic medium (Figure 5). Also, hyper-osmotic medium enhanced tyrosine phosphorylation of sperm 2-fold over the level observed on sperm incubated in iso-osmotic medium (Figures 3 and 4). Hypo-osmotic medium (150 and 200 mOsm/kg) severely impaired sperm–ZP binding and no sperm bound to the ZP when sperm and oocytes were incubated in medium with osmolality of 150 mOsm/kg. On the other hand, the ZP-induced AR was significantly lower in hyper-osmotic medium (400 mOsm/kg) than in iso-osmotic medium (280 mOsm/kg, Figure 6).

To confirm that the enhancement of sperm–ZP binding and tyrosine phosphorylation was due to high osmolality rather than to high concentrations of NaCl, we also used sucrose to adjust osmolality of standard HTF medium (280 mOsm/kg) up to 400 mOsm/kg. Hyper-osmotic medium (400 mOsm/kg) adjusted with either NaCl or sucrose gave similar enhancement of both sperm–ZP binding and tyrosine phosphorylation when compared with standard medium (280 mOsm/kg, Table I).

Figure 3. Relationship between osmolality of culture medium and sperm tyrosine phosphorylation (TP) after 2 h incubation detected by immunofluorescence with PY20-FITC. (A) Line graph showing individual results, and (B) bar graph of means with error bars of SEM, for 11 sperm samples from different men (all comparisons $P < 0.01$).

Figure 4. Relationship between osmolality of culture medium and sperm tyrosine phosphorylation after 2 h incubation detected by western immunoblotting of extracts from $2 \times 10^6$ sperm per lane. There are significant increases in tyrosine phosphorylation (assessed on the major bands of ~95–170 kDa) with increasing osmolality of medium. Bar graphs represent means with error bars of SEM for sperm samples from four different men (all comparisons $P < 0.05$).

Figure 5. Relationship between osmolality of culture medium and the number of sperm bound/four zona pellucida (ZP) after 2 h incubation (all comparisons $P = 0.005$). (A) Line graph showing individual results for eight sperm samples from different men. (B) Bar graph of means with error bars of SEM. There were zero sperm bound/four ZP with 150 mOsm/kg and an average of 37 sperm bound/four ZP with 200 Osm/kg.
Table I. Comparison of sperm–zona pellucida (ZP) binding and the percentage of tyrosine-phosphorylated sperm determined with PY20-FITC after sperm from four men were incubated in iso-osmotic (280 mOsm/kg) or hyper-osmotic (400 mOsm/kg) medium achieved by the addition of NaCl (Na) or sucrose (Su).

<table>
<thead>
<tr>
<th>Samples</th>
<th>No sperm bound/four ZPa</th>
<th>Phosphorylated sperm (%)b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>280 400-Na 400-Su</td>
<td>280 400-Na 400-Su</td>
</tr>
<tr>
<td>1</td>
<td>390 1521 1475</td>
<td>9 48 50</td>
</tr>
<tr>
<td>2</td>
<td>326 1011 1076</td>
<td>11 31 34</td>
</tr>
<tr>
<td>3</td>
<td>498 2154 2242</td>
<td>12 40 41</td>
</tr>
<tr>
<td>4</td>
<td>309 1214 1159</td>
<td>8 33 31</td>
</tr>
<tr>
<td>Mean</td>
<td>380 1475 1488</td>
<td>10 38 39</td>
</tr>
</tbody>
</table>

aP < 0.05 only for 280 versus either 400-Na or 400-Su.
bP < 0.05 only for 280 versus either 400-Na or 400-Su.

Interestingly, for sperm samples with defective sperm–ZP binding, hyper-osmotic medium also significantly increased tyrosine phosphorylation but did not enhance the number of sperm bound to the ZP (Table II). In other words, hyper-osmotic conditions promoted tyrosine phosphorylation of any sperm samples but only enhanced sperm–ZP binding capacity in those samples with normal sperm–ZP binding.

Discussion

The present study is apparently the first report that hyper-osmotic conditions (350 and 400 mOsm/kg) are associated with increased sperm tyrosine phosphorylation and binding to the ZP. There was a 2-fold increase in the percentage of tyrosine-phosphorylated sperm with hyper-osmotic media over that in standard (iso-osmotic) medium. Enhancement of sperm tyrosine phosphorylation by hyper-osmotic conditions was detected by both immunofluorescence and western immunoblotting. The number of sperm bound to the ZP tripled in hyper-osmotic media. The promotion of both ZP binding capacity and tyrosine phosphorylation of sperm by hyper-osmotic conditions further supports previous findings that tyrosine phosphorylation is related to sperm–ZP binding (Sakkas et al., 2003). We have recently confirmed that the proportion of sperm showing tyrosine phosphorylation on the tails as detected with PY20–FITC is strongly correlated with human sperm–ZP binding (D.Y.Liu et al., unpublished data).

In this study, we did not use medium with osmolality >400 mOsm/kg since it would severely reduce sperm motility which would then affect the sperm–ZP interaction test. The primary aim was to test the effect of osmolalities, in the range known to occur in the male and female reproductive tracts, on sperm–ZP interaction. Rossato et al. (2002) showed that sperm motility was nearly completely lost when sperm were exposed to medium with 600 mOsm/kg. Low sperm–ZP binding in hyper-osmotic (150 mOsm/kg) medium may be explained by severely reduced sperm motility or velocities. However, for 200 mOsm/kg osmolality medium, low sperm–ZP binding was not due to reduced sperm motility since results of sperm motility and velocities were very similar to those in hyper-osmotic (350 and 400 mOsm/kg) media. We confirmed that the increased sperm–ZP binding and tyrosine phosphorylation was due to high osmolality per se rather than to higher concentrations of NaCl, by obtaining similar results when sucrose was used instead of NaCl to increase osmolality to 400 mOsm/kg.

Interestingly, enhancement of sperm–ZP binding by hyper-osmotic conditions was limited to those sperm samples with initially normal sperm–ZP binding capacity. For sperm with defective ZP binding, hyper-osmotic conditions had no effect on the sperm–ZP binding capacity (Table II). Therefore, tyrosine phosphorylation may be an indirect indicator for sperm–ZP binding and may not be directly involved with the sperm receptors for the ZP. Simple enhancement of tyrosine phosphorylation alone by increasing osmolality of medium would not improve sperm–ZP binding capacity in men with defective sperm–ZP receptors.

Hyper-osmotic medium significantly reduced the ZP-induced AR. Previous studies showed that hyper-osmotic conditions reduced ionophore A23187-induced AR and calcium-influx.
(Bielfeld et al., 1993; Rossato et al., 1996). We have found that sperm tyrosine phosphorylation detected with PY20-FITC is not correlated with the human ZP-induced AR (D.Y.Liu et al., unpublished data). Patients with defective ZP-induced AR have sperm that are able to bind to the ZP but fail to undergo the ZP-induced AR (Liu and Baker, 1996). The fact that very few sperm bound to the ZP in hyper-osmotic conditions had undergone the AR further confirms that only acrosome-intact human sperm are able to initiate binding to the ZP. It is likely that hyper-osmotic conditions affect sperm plasma membranes and ion channels involved in the calcium influx which is required for the AR (Rossato et al., 1996). Human sperm have been shown to have osmo-sensitive calcium influx (Rossato et al., 1996).

During sperm capacitation, the sperm plasma membrane undergoes numerous biochemical and molecular changes, including removal of cholesterol which alters membrane fluidity, an increase in bicarbonate uptake, activation of adenyl cyclase and protein kinase A leading to an increase in protein tyrosine phosphorylation (Yanagimachi, 1994; Visconti and Kopf, 1998). Therefore, the level of tyrosine phosphorylation of sperm is considered a good marker for sperm capacitation. Although albumin, bicarbonate, calcium and glucose are considered to be the most important factors for promotion of sperm capacitation in vitro, other factors such as pH and osmolality are also important (Mahi and Yanagimachi, 1973; Visconti and Kopf, 1998; Umer and Sakkas, 2003). The present study clearly demonstrates that osmolality of medium significantly affects sperm tyrosine phosphorylation. Although it is not known how this occurs, it is possible that changes in osmolality that affect sperm volume regulation may alter the function of plasma membrane ion channels (Yeung et al., 2003). However, it is obvious that sperm capacitation in vitro may be different from capacitation under in vitro conditions.

In humans, osmolality is usually much higher in semen than in serum or cervical mucus. The physiological role of the higher osmolality of semen is currently poorly understood, but it is possible that hyper-osmotic conditions may promote sperm fertilizing ability and prevent a premature acrosome reaction. In cryopreservation of boar sperm, hyper-osmotic (420 mOsm/kg) conditions were found to yield higher motility and intact acrosomes of sperm after thawing (Zeng et al., 2001). Hyper-osmotic (800 mOsm/kg) medium at 4°C was also able to preserve mouse sperm, which, when used for ICSI, gave normal fertilization and live birth of healthy offspring (Van Thuan et al., 2005). This study also supports the observation that cryopreservation of human sperm in hyper-osmotic media is not detrimental to sperm fertilizing ability.

The general increase in tyrosine phosphorylation as measured in this study by immunofluorescence or immunoblotting is often used as a marker of sperm capacitation. These results show that the ZP-induced acrosome reaction can be dissociated from increased tyrosine phosphorylation and sperm–ZP binding under hyper-osmotic conditions. This provides another avenue for investigation of the regulation of sperm function during the fertilization process.

In conclusion, the osmolality of the culture medium plays an important role in sperm function. Hyper-osmotic conditions promote both sperm tyrosine phosphorylation and sperm–ZP binding, but severely inhibit the ZP-induced AR. Using hyper-osmotic conditions to increase tyrosine phosphorylation and sperm–ZP binding appears to have no therapeutic application because of the reduced ZP-induced AR. While changing the osmotic conditions may be not useful for clinical IVF, it will be useful for studying aspects of sperm function.

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References


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