Factors affecting the acrosome reaction in human spermatozoa*

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Summary. Large pieces of human cumulus oophorus were exposed for 20–30 min to washed spermatozoa or to spermatozoa recovered after a swim-up procedure, and then fixed for electron microscopy. Spermatozoa of both populations penetrated deeply into the cumulus within that time, and none of 48 observed clearly had undergone an acrosome reaction (AR).

As measured by fluorescence microscopy, an AR rate of 12% in spermatozoa obtained at 4 h following a swim-up increased to about 25% in samples incubated in culture dishes for ~20 h. However, this latter AR rate was no different in the presence or absence of a cumulus/oocyte complex, and was only moderately greater in 50% follicular fluid. Nor was it affected to any degree by the absence of calcium or by a low (26°C) temperature, both of which are regulators of the physiological AR in other species. By contrast, a clear dose-related enhancement of the AR by the calcium ionophore A23187 was almost completely Ca²⁺-dependent.

We conclude that the human cumulus oophorus does not rapidly induce an AR in spermatozoa capacitated in vitro and, unlike the situation in some other mammals, that washed human spermatozoa do not first require a period of capacitation in order to penetrate it. The results also point to the likelihood that ARs monitored in free-swimming human spermatozoa are for the most part spurious or artefactual, and they show that in-vitro AR rates in such populations do not parallel their fertilizing ability.

Keywords: man; spermatozoa; acrosome reaction; cumulus oophorus; capacitation; calcium

Introduction

To achieve fertilization, mammalian spermatozoa must first undergo an acrosome reaction (AR), which depends on the occurrence of capacitation, and is characterized initially by point fusions between the outer acrosomal and plasma membranes (Barros et al., 1967). The term ‘acrosome reaction’ generally refers not only to the membrane fusion stage, but also to subsequent loss of the reacted elements. The latter state is often designated ‘complete’ in reference to the reaction, and these usages need to be kept in mind.

The natural trigger that evokes the AR during mammalian fertilization and the functional relevance of reacting spermatozoa in different situations are currently topics of considerable interest and uncertainty. In mammals, the events related to the AR have been studied most intensively in the mouse by using in-vitro systems. In these conditions, the mouse AR occurs at the zona surface (Saling & Storey, 1979), apparently stimulated by a zona glycoprotein designated ZP3 (Bleil & Wassarman, 1983), and zona-evoked reactions have been reported for some other species. However, in many studies a normal AR has been observed before any interactions with

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the zona pellucida. For example, in the Syrian hamster (Cummins & Yanagimachi, 1982, 1986; Yanagimachi & Phillips, 1984; Cherr et al., 1986; Corselli & Talbot, 1987), the Chinese hamster (Yanagimachi et al., 1983) and rabbit (Bedford, 1968, 1972), reacted cells are very common among spermatozoa within the cumulus oophorus. Observations on guinea-pig spermatozoa are conflicting. Huang et al. (1981) reported that guinea-pig spermatozoa bind not at all or poorly to the zona pellucida if a reaction has not already occurred. Myles et al. (1987), however, report that both intact and reacted spermatozoa may bind to the guinea-pig zona.

Some human spermatozoa allowed to adhere to the naked zona pellucida of immature oocytes began to react within minutes (Cross et al., 1988). When eggs are invested by cumulus oophorus, however, normally reacting spermatozoa can be found amongst the cumulus cells as well as on the zona (Dvorak et al., 1984; Pereda & Coppo, 1985; Chen & Sathananthan, 1986). Moreover, what appear to be ARs occur in human spermatozoa in the absence of any product of ovulation. An AR incidence of 20% has been reported for spermatozoa in capacitating situations or in in-vitro fertilization dishes (Plachot et al., 1984; Wolf et al., 1985; Van Kooij et al., 1985; Stock & Fraser, 1987) and this may be greater at 36 h (Mortimer et al., 1989). Plachot et al. (1984) found no effect of the presence of a cumulus/oocyte complex, but other groups report that the AR rate is enhanced by the presence of human cumulus/oocyte complexes (Tesari, 1985) or of follicular fluid (Suarez et al., 1986; Sitteri et al., 1988; Stock et al., 1989), one active factor in which could possibly be progesterone (Osman et al., 1989). Since both acrosome-reacted and intact human spermatozoa may bind to the zona (Morales et al., 1989) it is therefore not clear whether physiological ARs occur at the surface of the human zona pellucida, within the cumulus oophorus, or even beforehand in some cells.

The present study was undertaken to investigate further the significance of the AR occurring in vitro in human spermatozoa and the relevance of various factors that may affect it. We emphasize that the studies were conducted for the most part with ‘swim-up’ spermatozoa, since it is likely that results with unselected ejaculate samples give an over-estimate of the AR rate (Overstreet & Cross, 1988).

**Materials and Methods**

*Incubation medium.* The basic medium used throughout this study was Human Tubal Fluid medium (HTF; Quinn et al., 1985). This contained 101.6 mm-NaCl, 4.69 mm-KCl, 2.04 mm-CaCl₂, 0.20 mm-MgSO₄, 0.37 mm-K₂HPO₄, 25 mm-NaHCO₃, 2.78 mm-glucose, 0.33 mm-pyruvate and 21.4 mm-lactate. The calcium-deficient medium was supplemented with NaCl to maintain osmolality. The medium also contained 100 i.u. penicillin, 50 μg streptomycin/ml, with 0.001% phenol red present as an indicator.

For incubation of spermatozoa and oocytes, Medium HTF was supplemented with 10% maternal or paternal serum first heated at 56°C for 30 min, or with 3 mg Plasmanate/ml (Cutter Biological, Berkeley, CA, USA). Plasmanate is a human plasma protein fraction containing approximately 88% albumin and 12% α- and β-globulins (Hink et al., 1957) that we have used with excellent results as an alternative medium supplement for human in-vitro fertilization and embryo culture.

*Sperm preparation.* Semen obtained by masturbation was allowed to liquefy for about 30 min before two washes in Medium HTF + Plasmanate (first wash 10 min at 300 g, in twice the volume; second wash 5 min at 300 g in 1 ml). The pellet of washed spermatozoa was then carefully layered beneath 1 ml Medium HTF + Plasmanate in a 5-ml plastic tube, the tubes were loosely capped and incubated at an angle in 5% CO₂ at 37-3°C. In one experiment the incubation temperature was 26°C. After 2-3 h, a population of highly motile spermatozoa (the ‘swim-up’ sample: > 85% motile) was recovered from the upper layer.

Except for the determination of effects of A23187 (see below), spermatozoa alone, or spermatozoa and oocytes together, were incubated in 0.9 ml medium in the central well of an organ-culture dish (43037, Falcon Plastics), with a further 3-5 ml in the outer well. For each experimental treatment, 5 such dishes and a variable number of in-vitro fertilization dishes were each inseminated with 20 μl of the ‘swim-up’ sample, containing between 100 000 and 150 000 spermatozoa. After incubation for 16 h in 5% CO₂ at 37-3°C (26°C for studies of temperature effects), the sperm suspensions were concentrated at 500 g for 5 min and a 45 μl sample was then removed for assessment of acrosomal status (see below).

In experiments involving the calcium ionophore A23187 (4-bromo A23187: Sigma, St Louis, MO, USA), ionophore or vehicle (Medium HTF + 0.01% DMSO) was added to the swim-up spermatozoa. After 3 h at 37-3°C in 5%
CO₂ the suspension was centrifuged for 5 min at 500 g and a 45 µl sample removed for determination of acrosomal status (see below). Percentage motility was estimated in a phase-contrast microscope with a heated stage. For each estimation at least 100 spermatozoa were counted with the aid of an eyepiece graticule.

Assessment of acrosomal status. The acrosomal status of free-swimming spermatozoa was assessed using fluoresceinated *Pseudotsuga* sativum agglutinin (PSA; Vector Laboratories, Burlingame, CA, USA), as described by Cross et al. (1986). Here the term ‘acrosome reaction’ is used to describe the initial fusion phase and/or the loss of acrosomal content in viable spermatozoa.

Briefly, 0-05 µg of the supravital stain Hoechst 33258 (bisbenzimide; Sigma) in 5 µl phosphate buffered saline (PBS-Dulbecco’s; Gibco, Long Island, NY, USA) was mixed and incubated with a 45 µl sample of spermatozoa for 10 min. Spermatozoa were then washed free of dye by centrifugation (900 g, 10 min) through a 2% (w/v) solution of polyvinylpyrrolidone in PBS. The washed sperm pellet was then resuspended in 300 µl 95% ethanol and further incubated at 4°C for 30 min. This solution was air-dried onto a glass slide and stained with 2 µg FITC-conjugated PSA in 20 µl PBA for 9 min. Unbound lectin was gently washed off with distilled water. Spermatozoa were then scored for acrosomal status and viability using a Zeiss universal epifluorescence microscope with a ×40 neofluor lens. Spermatozoa with a nucleus labelled with Hoechst 33258 (H +ve) were classed as non-viable. Both H +ve and H −ve spermatozoa were considered to have an intact acrosome if the lectin binding was uniform over the entire acrosome. Patchy or peripherally stained H −ve spermatozoa were in the process of reacting. H +ve and H −ve spermatozoa with either no lectin bound, or only a slight binding at the equatorial segment, had completed the acrosome reaction and had shed the carapace of fused membranes (Cross et al., 1986).

Collection of cumulus oophorus and follicular fluid. Human oocyte/cumulus complexes and follicular fluid aspirates were obtained ~34 h after injection of 10 000 i.u. hCG (Profasi: Serono, Randolph, MA, USA) from patients for in-vitro fertilization first stimulated with clomiphene citrate (Serophene: Serono) and human menopausal gonadotrophin (Pergonal: Serono). The samples were aspirated via vaginal ultrasound-guided needles from preovulatory follicles. Follicular fluid collected with the oocytes was frozen and stored at −135°C.

Sperm/cumulus interaction: transmission electron microscopy. For any one ultrastructural analysis of sperm/cumulus interaction, most of a whole cumulus was quickly removed with needles from mature oocytes (82-6% of which were subsequently fertilized) within 3 h of their retrieval. Oocyte maturity was assessed according to the visual appearance of the cumulus oophorus and corona radiata. The cumulus was recovered only from samples previously washed twice in dishes containing Medium HTF + 6% Plasmanate, and was kept in Medium HTF + Plasmanate or Medium HTF + 10% heated serum. The cumulus oophorus was then incubated with 100 000–150 000 spermatozoa at 37-3°C in 1 ml Medium HTF + 10% serum (or +6% Plasmanate) in an organ culture dish for up to 20–30 min. The spermatozoa used had been subjected previously to a 3-h swim-up procedure or, in two experiments, were simply washed twice to remove seminal plasma. The cumulus oophorus was then fixed for 1–2 h in 2.5% glutaraldehyde buffered with 0.2 M-collidine at 4°C, rinsed in buffer, post-fixed in 1% OsO₄ and dehydrated in alcohol. Epon-embedded material was sectioned using a Reichert OmU3 ultratome, stained overnight in aqueous 3% uranyl acetate and examined with a Philips 300 electron microscope. Studies of the acrosomal status of free-swimming spermatozoa were performed on sperm pellets obtained by centrifugation in a microfuge for 15 sec, and processed as for cumulus oophorus.

Effects of follicular fluid on the acrosome reaction. Dishes containing 50% follicular fluid were inseminated with swim-up spermatozoa as described above. Spermatozoa were retrieved 16 h later, centrifuged at 500 g for 5 min and resuspended in 5 ml Medium HTF. To remove follicular fluid, which precipitates in ethanol and obscures sperm/lectin binding, spermatozoa were washed again. A 45 µl sample of this twice-washed sample was then removed and processed for determination of acrosomal status.

Statistical analysis. Statistical comparisons were made when relevant using the Wilcoxon matched pairs signed-ranks test. In other cases, the Mann–Whitney U test was employed.

Results

Acrosome reaction rate

In the swim-up sample, recovered at 4 h, 88% of living (H −ve) spermatozoa had intact acrosomes (Table 1). Incubation in the swim-up tube for a further 16 h at a concentration of between 12 and 30 × 10⁶/ml resulted in a modest but significant (*P* < 0.01) increase in the percentage of acrosome-reacted H −ve cells. Parallel incubations of swim-up spermatozoa at a concentration of about 0.1 × 10⁶/ml in culture dishes containing Medium HTF + Plasmanate led to some further increase in the mean reaction rate. However, the rate observed under similar conditions in the presence of cumulus/oocyte complexes (i.e. in the fertilization dishes) did not differ significantly
from that. The inclusion of 50% follicular fluid (100% follicular fluid depressed motility significantly) brought a further minor though significant \(P < 0.01\) enhancement of the AR rate (Table 1).

**Table 1. Effect of different incubation conditions on the acrosome reaction rate of human spermatozoa in vitro**

<table>
<thead>
<tr>
<th>Incubation conditions</th>
<th>No. of samples (1/ man)</th>
<th>% Semi-reacted</th>
<th>% Completely reacted</th>
<th>% Acrosome-intact</th>
</tr>
</thead>
<tbody>
<tr>
<td>*4 h swim-up; tube</td>
<td>18</td>
<td>61 ± 0.9</td>
<td>6.9 ± 0.7</td>
<td>88.0 ± 1.3^a</td>
</tr>
<tr>
<td>*20 h swim-up; tube</td>
<td>20</td>
<td>85 ± 0.7</td>
<td>11.3 ± 1.3</td>
<td>80.0 ± 1.8^b</td>
</tr>
<tr>
<td>*20 h fertilization dish</td>
<td>18</td>
<td>87 ± 1.5</td>
<td>16.7 ± 2.3</td>
<td>74.6 ± 2.6^c</td>
</tr>
<tr>
<td>†20 h fertilization dish +</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cumulus/oocyte</td>
<td>29</td>
<td>10.6 ± 1.0</td>
<td>13.8 ± 1.3</td>
<td>75.6 ± 1.7^c</td>
</tr>
<tr>
<td>†20 h fertilization dish +</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50% follicular fluid</td>
<td>12</td>
<td>12.7 ± 1.6</td>
<td>22.0 ± 3.2</td>
<td>65.6 ± 3.6^d</td>
</tr>
</tbody>
</table>

Values are mean ± s.e.m.
*In medium HTF + Plasmanate (3 mg/ml).
†In medium HTF + 10% serum or Plasmanate.
Values with different superscripts are significantly different: \(P < 0.01\).

As expected, the ionophore A23187 significantly enhanced the AR rates in culture dishes in a concentration-dependent manner. A 3-h incubation in the presence of 1, 5 and 10 mM-A23187 brought a significant \(P < 0.01\) increase in the percentage of reacted spermatozoa over that in controls in the presence of vehicle alone (Table 2). In the presence of 1 mM-A23187, percentage motility after 3 h did not differ significantly from that of controls. However, a concentration of 5 mM- and 10 mM-A23187 brought a significant reduction in percentage motility, with levels reduced respectively to 77.1% and 30.2% of the control levels (Table 2). The stimulatory effects of A23187 on the acrosome reaction were entirely dependent upon the presence of calcium in the medium. Thus, 5 mM-A23187 in complete medium significantly \(P < 0.01\) increased the percentage of reacted H — ve spermatozoa. By contrast, this concentration of ionophore had no effect in the absence of calcium, when compared with control spermatozoa incubated in the calcium-deficient medium (Table 3).

**Table 2. Effect of different concentrations of the calcium ionophore A23187 on the acrosome reaction rate of human spermatozoa in vitro**

<table>
<thead>
<tr>
<th>Incubation conditions</th>
<th>% Semi-reacted</th>
<th>% Completely reacted</th>
<th>% Acrosome-intact</th>
<th>% Motility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Medium HTF)</td>
<td>3.5 ± 0.8</td>
<td>5.4 ± 1.0</td>
<td>91.1 ± 1.5^a</td>
<td></td>
</tr>
<tr>
<td>1.0 µM-A23187</td>
<td>9.9 ± 2.4</td>
<td>12.8 ± 3.3</td>
<td>77.4 ± 6.5^b</td>
<td>100.3 ± 4.5</td>
</tr>
<tr>
<td>5.0 µM-A23187</td>
<td>16.2 ± 5.2</td>
<td>25.3 ± 5.0</td>
<td>58.4 ± 6.7^c</td>
<td>77.1 ± 10.3</td>
</tr>
<tr>
<td>10.0 µM-A23187</td>
<td>11.0 ± 3.5</td>
<td>48.2 ± 7.1</td>
<td>40.8 ± 5.7^d</td>
<td>30.2 ± 13.3</td>
</tr>
</tbody>
</table>

Values are mean ± s.e.m. for 6 samples.
Values with different superscripts are significantly different: \(P < 0.01\).

Compared with samples in calcium-containing medium an absence of calcium in the medium also resulted in a swim-up of lower concentration, and a much poorer survival of motility at 16 h in culture dishes. However, there was only a minor difference in the AR rates achieved at 4 h in the calcium-deficient and calcium-containing swim-up samples and at 20 h in the respective culture dishes (Table 4).
Table 3. Effect on the acrosome reaction rate of human spermatozoa of 5 µM-A23187 with or without Ca\(^{2+}\) in the culture medium

<table>
<thead>
<tr>
<th>Incubation conditions</th>
<th>% Semi-reacted</th>
<th>% Completely reacted</th>
<th>% Acrosome-intact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium HTF + vehicle</td>
<td>4.5 ± 0.6</td>
<td>8.5 ± 1.0</td>
<td>87.0 ± 1.1(^a)</td>
</tr>
<tr>
<td>Medium HTF + A23187</td>
<td>7.0 ± 1.8</td>
<td>32.2 ± 4.9</td>
<td>60.8 ± 6.2(^b)</td>
</tr>
<tr>
<td>Medium HTF + vehicle - Ca(^{2+})</td>
<td>5.5 ± 1.3</td>
<td>4.5 ± 0.3</td>
<td>89.8 ± 1.0(^a)</td>
</tr>
<tr>
<td>Medium HTF - Ca(^{2+}) + A23187</td>
<td>4.5 ± 1.0</td>
<td>7.8 ± 1.7</td>
<td>87.8 ± 1.5(^a)</td>
</tr>
</tbody>
</table>

Values are mean ± s.e.m. for 4 samples. Values with different superscripts are significantly different: \(P < 0.01\).

Table 4. Influence of Ca\(^{2+}\) on the acrosome reaction rate of human spermatozoa

<table>
<thead>
<tr>
<th>Incubation conditions</th>
<th>% Semi-reacted</th>
<th>% Completely reacted</th>
<th>% Acrosome-intact</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 h swim-up + Ca(^{2+})</td>
<td>6.5 ± 1.5</td>
<td>5.7 ± 0.6</td>
<td>87.8 ± 1.7</td>
</tr>
<tr>
<td>4 h swim-up - Ca(^{2+})</td>
<td>7.6 ± 3.5</td>
<td>9.2 ± 5.7</td>
<td>83.2 ± 7.8</td>
</tr>
<tr>
<td>20 h in culture dish + Ca(^{2+})</td>
<td>14.2 ± 3.2</td>
<td>13.0 ± 3.8</td>
<td>72.8 ± 4.0(^b)</td>
</tr>
<tr>
<td>20 h in culture dish - Ca(^{2+})</td>
<td>7.7 ± 1.8</td>
<td>11.2 ± 3.9</td>
<td>81.2 ± 3.9(^b)</td>
</tr>
</tbody>
</table>

Values are mean ± s.e.m. for 6 samples. Values with different superscripts are significantly different: \(P < 0.01\).

Finally, incubation in 5% CO\(_2\) in air at a lower temperature of 26°C had no significant effect on AR rates. The percentage of H−ve acrosome-intact, spermatozoa was the same in swim-up samples incubated at 26°C and in parallel samples incubated at 37°C, as was the percentage of 26°C and 37°C following overnight incubation for 20 h (Table 5).

Table 5. Effect of incubation temperature on the acrosome reaction rate of human spermatozoa

<table>
<thead>
<tr>
<th>Incubation conditions</th>
<th>No. of samples (1/man)</th>
<th>% Semi-reacted</th>
<th>% Completely reacted</th>
<th>% Acrosome-intact</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 h swim-up at 37°C</td>
<td>4</td>
<td>4.8 ± 0.8</td>
<td>5.5 ± 1.9</td>
<td>89.8 ± 2.0</td>
</tr>
<tr>
<td>4 h swim-up at 26°C</td>
<td>4</td>
<td>5.5 ± 1.0</td>
<td>5.8 ± 1.1</td>
<td>88.8 ± 1.8</td>
</tr>
<tr>
<td>20 h in culture dish at 37°C</td>
<td>9</td>
<td>5.0 ± 0.9</td>
<td>15.0 ± 2.6</td>
<td>80.0 ± 2.9</td>
</tr>
<tr>
<td>20 h in culture dish at 26°C</td>
<td>9</td>
<td>7.9 ± 1.2</td>
<td>12.5 ± 1.8</td>
<td>79.4 ± 2.4</td>
</tr>
</tbody>
</table>

Values are mean ± s.e.m.

Spermatozoa within the cumulus oophorus

In two experiments, large pieces of intact cumulus of mature appearance were incubated for 20 min with uncapacitated spermatozoa taken immediately following two brief washes with no swim-up. Upon examination of thick sections in the light microscope, spermatozoa were found deep within the cumulus cell mass (see Fig. 1).
Fig. 1. Intact human cumulus oophorus fixed and embedded 20 min after its exposure to ejaculated spermatozoa which had been washed twice briefly to remove seminal plasma. Several spermatozoa are interspersed among cumulus cells. Toluidine blue, × 950.

In four cases, spermatozoa taken from the swim-up after 3-5 h were incubated for between 20 and 30 min with cumulus masses. A total of 53 spermatozoa were found deep within the cumulus. Of these, 2 were being phagocytosed and the acrosomal status of a further 3 was impossible to determine. However, the acrosomes were intact (Fig. 2) in all of the remaining 48 spermatozoa.

Discussion

The present study reports human sperm acrosome reaction (AR) patterns in vitro that in general compare to those reported by workers in several other laboratories. However, the implications of and conclusions from the data are somewhat different.

A significant observation is the finding that still-intact spermatozoa, obtained after a swim-up or even those only washed briefly after ejaculation, are able readily to penetrate deeply into organized cumulus oophorus within 30 min. Human spermatozoa therefore do not need to react in order to traverse cumulus of a quality comparable to that presented to spermatozoa at ovulation. Moreover, while it is possible that a small percentage are in this respect already capacitated, or reach this state very quickly after washing, human ejaculated spermatozoa do not seem to need to undergo a formal capacitation period after ejaculation in order to penetrate the cumulus.

Such behaviour of human spermatozoa is of interest because of previous findings in other mammals. Talbot et al. (1985) have shown that protozoa as well as frog and sea-urchin spermatozoa can penetrate the hamster cumulus, but capacitation appears to be important for penetration of hamster cumulus by hamster spermatozoa. Detailed observations using moderate sperm numbers (Corselli & Talbot, 1987) reveal that most uncapacitated hamster spermatozoa fail to penetrate beyond the periphery of the cumulus in vitro, and that none reaches the zona surface. By contrast, intact capacitated spermatozoa and those with modified (reacting) acrosomes were common within the cumulus and penetrated readily to the zona surface. Capacitation also facilitates the passage of
rabbit spermatozoa through the cumulus vestment in the environment of the oviduct (Overstreet & Bedford, 1974)—essentially the conclusion of Austin (1960) who readily observed moving spermatozoa within cumulus of several species after natural mating, whereas mature epididymal or ejaculated spermatozoa could not penetrate it in vitro.

In the present study no swim-up spermatozoa penetrating the cumulus underwent even the initial stages of an AR within 20–30 min. Since this is a population that can immediately begin to fertilize eggs in vitro, some spermatozoa at least are functionally capacitated. We conclude, therefore, that the human cumulus does not rapidly induce the AR in such spermatozoa. Chen & Sathananthan (1986) found that only 10–30% of human spermatozoa reaching the zona of cumulus-invested oocytes had reacted to some degree 1 h after insemination, and that at 2–3 h many spermatozoa were undergoing the AR within the cumulus as well as on the zona surface, the latter being an observation similar in principle to that of Sathananthan et al. (1982) and Dvorak et al. (1984).

Taken together, such TEM observations suggest that the first human spermatozoa to penetrate the cumulus probably react at the zona surface, and that only after $\geq 1$ h do significant numbers begin to react before reaching the zona, within the cumulus matrix. Such late responses within cumulus seem not merely to reflect 'false' reactions in what could perhaps be moribund spermatozoa, because the reactions within the cumulus (Chen & Sathananthan, 1986), as well as those on the zona (Cross et al., 1988) generally follow the classical fusion pattern described by Barros et al. (1967). Moreover, the AR rates achieved by 3 h within the cumulus (Chen & Sathananthan, 1986) far exceed those reported for free-swimming spermatozoa at 16–20 h in capacitating conditions (Table 1; and other studies). This implies some dependent relationship between the cumulus/oocyte complex and the spermatozoa penetrating it.
The present study raises doubts about the relevance of the ARs observed in free-swimming human sperm populations. In one sense our results agree with those of other groups, since AR rates of about 10% and 20% were obtained after incubations of about 4 and 20 h, respectively. A major problem, however, is the fact that elements known to regulate the 'physiological' reaction had little effect on the incidence of the AR in the present study. There was a significant dose-dependent enhancement of the AR in response to the calcium ionophore A23187 (Table 2), as noted previously by others (Aitken et al., 1984; Tesarik, 1985), and this was clearly calcium-dependent (Table 3). On the other hand, although an influx of Ca\(^{2+}\) is required to initiate 'true' ARs in all the systems examined (Yanagimachi & Usui, 1974; Fraser, 1987; Thomas & Meizel, 1988), the AR rates at 4 h and 20 h were little different in media with and without Ca\(^{2+}\) (Table 4), although the absence of Ca\(^{2+}\) was reflected strikingly in a reduction of the quality of sperm motility. Similarly, a lower temperature, which inhibits or delays the in-vitro development of an AR in spermatozoa of guinea-pig and hamster, respectively (Mahi & Yanagimachi, 1973; Fleming & Kuehl, 1985), had no effect on the incidence of the human AR after 4 or 20 h in-vitro (Table 5). Finally, in accord with Plachot et al. (1984), the AR rate in free spermatozoa in the medium was not affected by the presence of a cumulus/oocyte complex, and only marginally by a concentration of 50% follicular fluid.

Since factors known to regulate the physiological AR generally had so little effect on our measurements, it is possible that a majority of the spontaneous reactions scored here (and in other similar studies of human spermatozoa) were spurious or artefactual events in what may be inferior sperm cells. Because the acrosome was disrupted and reactions were scored in viable cells, some of these 'reacted' spermatozoa may be capable of fusion, and spermatozoa incorporated by zona-free hamster eggs in fertility tests may fall into this category. However, there was no relationship here between AR rate and fertilization of the normal human oocyte in-vitro and it is difficult to agree with the view (Topfer-Petersen et al., 1985) that the kinetics of the human acrosome reaction in-vitro offer a useful indicator of fertilizing ability.

While it is likely that we and others have not been measuring a true AR in spontaneously reacting populations, this material has been the basis for a suggestion that the acrosome reaction in man involves novel mechanisms, i.e. that this does not initially involve point fusions between plasma and outer acrosomal membranes, but rather a vesiculation of the acrosome alone (Nagae et al., 1986; Stock & Fraser, 1987). Taken together, the present results and the conventional mode in spermatozoa reacting on the zona (Chen & Sathananthan, 1986; Cross et al., 1988), within cumulus (Chen & Sathananthan, 1986) or induced by a specific fraction of follicular fluid (Yudin et al., 1988) suggest that the unusual alternative mode proposed for human spermatozoa may be based on spurious events in a sub-population of little relevance for fertilizing ability.

In conclusion it appears that ejaculated human spermatozoa behave in certain respects rather differently from mature epididymal/ejaculated spermatozoa of some common experimental animals. Some can penetrate cumulus oophorus immediately in an intact state without the benefit of a capacitation period. Furthermore, whereas a majority of guinea-pig, mouse and hamster spermatozoa undergo a normal AR progressively over 4–6 h in capacitating conditions in-vitro (Yanagimachi & Usui, 1974; Ward & Storey, 1984; Cuasnícu & Bedford, 1989), even the relatively low AR rates measured in free-swimming human sperm populations may, for the most part, represent spurious or artefactual events. Collectively, the evidence begins to suggest that the physiological AR in human spermatozoa is to be tied to an unusual degree to initiating factors intimately associated with the intact cumulus/oocyte complex.

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