New insights into possible factors contributing to male subfertility

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Male subfertility contributes significantly to fertility problems in couples. Although semen analysis may identify abnormalities in sperm numbers, morphology and/or motility that might contribute to subfertility, in other instances the semen parameters may appear to be normal, but the spermatozoa might be dysfunctional. A number of endogenous and exogenous factors have now been identified that can significantly affect sperm function in vitro and it is possible that they may have similar effects in vivo. Some endogenous factors maintain the spermatozoa in a non-fertilizing state, to avoid them ‘burning out’ and losing fertility before they reach an oocyte, while others stimulate spermatozoa to become fertile and then hold them in a state of readiness to fertilize. Exogenous environmental molecules, referred to as xenobiotics, have been shown to continuously stimulate spermatozoa so that they become fertile quickly, but then ‘burn out’. Defects relating to the endogenous molecules could result in spermatozoa either never becoming fertile or becoming fertile too quickly and so losing fertilizing potential. By understanding the mechanisms involved in promoting sperm fertilizing ability, it may be possible to develop new therapeutic treatments to overcome such defects. (Reprod Med Biol 2005; 4: 45–52)

Key words: acrosome reaction, adenylyl cyclase, cAMP, capacitation, xenobiotics.

INTRODUCTION

It is now recognized that male subfertility is a significant contributor to infertility in couples.1,2 In many instances, semen analysis has revealed specific qualitative defects at the cellular level, such as morphological and/or motility abnormalities, and quantitative defects, such as the presence of only a few or even no spermatozoa in the ejaculate. In many instances, there is no obvious cause for these defects although there is now considerable evidence that genetic abnormalities may contribute significantly to problems in some men.

However, it is possible that defects might be much more subtle than those mentioned above, such as when the quantity of spermatozoa being produced is good but there may be functional defects not detected by a routine semen analysis. After all, such analyses just describe how many cells are present and whether they appear to meet ‘normal’ criteria for morphology and motility. This is descriptive information that does not really give any insight into the fertilizing potential of the spermatozoa. It seems likely that some men have fertility problems because their spermatozoa cannot complete functional maturation and so cannot interact successfully with an unfertilized oocyte. Current evidence indicates that spermatozoa come into contact with endogenous molecules that can bind to the cells and affect sperm function; some of these effectors have inhibitory effects, trying to keep the spermatozoa from becoming fertile, while others have stimulatory effects, causing the cells to ‘switch on’ physiologically to become fertile. There is also very recent evidence that molecules, present either in the environment or in substances ingested, may also have biologically important effects on sperm function.

DECAPACITATION FACTORS

It has been known for over 50 years that mammalian spermatozoa are non-fertilizing at the time they leave the male reproductive tract; they require a few to several hours, the length of time needed being species-dependent, in an appropriate environment during which time they acquire fertilizing potential. Essentially, the spermatozoa must complete the maturation events that were begun in the epididymis. Since these
cells have the capacity to fertilize an oocyte, they are said to have undergone ‘capacitation’. The molecular events that comprise capacitation are complex and still poorly understood, but it is clear that there are important changes to the sperm surface. These include the loss, unmasking or rearrangement of specific molecules. The ones that are lost during capacitation are often referred to as decapitation factors (DF) because their addition to capacitated, fertile sperm suspensions rapidly ‘decapitates’ the cells so that they become poorly fertile. However, with time, the DF will again be lost and the cells will regain their fertilizing ability. Thus capacitation is reversible.

For quite a long time, we have been studying a DF that is present on epididymal mouse spermatozoa. When cells are released into an appropriate culture medium in vitro, the DF will be lost or inactivated gradually, by mechanisms currently not understood, and the spermatozoa will capacitate and acquire fertilizing potential. We have shown that the DF molecules can be removed quite easily: gentle centrifugation of mouse spermatozoa and resuspension in fresh medium results in highly fertile cells, as demonstrated by fertilization in vitro. Conversely, if either crude DF (supernatant obtained from centrifuging uncapacitated cells) or purified DF is added to capacitated suspensions, the suspensions quickly become poorly fertile because the DF molecules have bound to the sperm surface and caused a reversal in the cells’ physiological status.

The mechanism of action of this DF appears to be the activation of a calmodulin-stimulated Ca\(^{2+}\)-ATPase. When DF is present, the calcium pump is activated and pumps Ca\(^{2+}\) out of the cell; low intracellular Ca\(^{2+}\) helps keep spermatozoa uncapacitated. As the DF is lost, the pump activity will decrease and the consequent rise in intracellular Ca\(^{2+}\) will promote capacitation.

Biochemical characterization of this DF has shown that it is an anionic protein of ∼40 kDa, stable to heating to 100°C and to proteolytic degradation at basic pH; this suggests that saccharide residues may contribute to its biological activity. More recent results have confirmed those findings and also revealed that the DF does not appear to bind directly to the sperm plasma membrane, but instead binds to a receptor (DF-R) that is inserted into the plasma membrane by means of a glycosylphosphatidylinositol (GPI) anchor. The presence of such a receptor was shown by incubating uncapacitated sperm suspensions in the presence of phosphatidylinositol-specific phospholipase C (PIC), an enzyme that can cleave GPI anchors. This resulted in the loss of DF, but more importantly, exogenous DF was no longer able to bind to these PIC-treated cells and so could not decapacitate them. Therefore, the reversibility of capacitation requires the presence of the DF-R; when DF is added to capacitated suspensions, it is effective because the DF-R is still present. It was also found that the binding of DF to its receptor involves fucose residues on the DF and complementary fucose binding sites on the receptor. When capacitated suspensions were incubated briefly in exogenous fucose prior to addition of DF, the DF was unable to bind and so unable to decapacitate the cells. Conversely, the addition of fucose to uncapacitated suspensions resulted in an acceleration of capacitation as a result of fucose displacement of the DF; suspensions treated in this way were significantly more fertile when evaluated in vitro and there was a higher incidence of polyspermy, indicating the presence of many capacitated cells (Table 1).

Although this work has focused on DF found on mouse spermatozoa, the same mechanism of action, regulation of Ca\(^{2+}\)-ATPase activity, appears to be involved in capacitation in other species as well. For example, inhibitors of calmodulin, which would interfere with calmodulin’s stimulation of the calcium pump, were shown to accelerate capacitation in both human and bull spermatozoa. Furthermore, purified mouse DF was shown to decapacitate human spermatozoa, while exogenous fucose accelerated capacitation in human sperm suspensions, suggesting that human spermatozoa have both DF and DF-R proteins that are structurally quite similar to those identified in mouse spermatozoa.

This DF falls into the category of molecules which act as a brake to inhibit or slow down the ‘switching on’ of mammalian spermatozoa. Such a delay may be

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fertilized oocytes/total oocytes (%)</th>
<th>Range (%)</th>
<th>Polyspermic oocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>95/189 (50.3)</td>
<td>(23–67)</td>
<td>2/95 (2.1)</td>
</tr>
<tr>
<td>5 mM fucose</td>
<td>255/343 (74.3)*</td>
<td>(53–95)</td>
<td>41/255 (16.1)</td>
</tr>
</tbody>
</table>

*P < 0.025 compared with control suspensions. Data are from reference 8 with permission.