Oocyte activation after intracytoplasmic injection of mature and immature sperm cells

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The inactivation of metaphase-promoting factor (MPF), leading to reactivation of the oocyte cell cycle after fertilization, is one of the most important results of oocyte activation in the case of intracytoplasmic injection of mature or immature sperm cells. Oocyte activation is a cell signalling event that is likely to imply receptors at the oocyte plasma membrane and a signal transduction pathway involving calcium and protein phosphorylation/dephosphorylation. The typical calcium signal during oocyte activation in mammals takes the form of calcium oscillations. Intracytoplasmic sperm injection (ICSI) is associated with a slightly different pattern of calcium oscillations in comparison with normal fertilization. Available data suggest that sperm cytosolic factor(s) play a role of oscillator, modulating the properties of the oocyte’s intracellular calcium stores, whereas the role of trigger, normally realized by sperm–oocyte interactions at the level of their respective cell surfaces, can be supplemented in the conditions of ICSI by an artificial calcium influx generated by the procedure itself. Delayed onset and an abnormal form of the oocyte activation-promoting calcium signal are the two known molecular abnormalities of human oocyte activation; they can lead to fertilization failure and are also suspected to be at the origin of various embryo abnormalities. The impact of oocyte activation abnormalities on future development increases when oocytes are fertilized with immature sperm cells (spermatids) because the chromatin of these cells is less protected than sperm chromatin against a rapid action of the oocyte’s MPF. If oocyte stimulation by the sperm calcium oscillation-promoting activity, which first appears at the round spermatid stage of human spermatogenesis, is insufficient to cause a rapid inactivation of MPF, premature condensation of spermatid chromatids may lead to aneuploidy.

Key words: fertilization/intracytoplasmic sperm injection/oocyte activation/spermatid/spermatocyte

Introduction

Following the first reported human pregnancies and births obtained after fertilization of oocytes by intracytoplasmic sperm injection (ICSI; Palermo et al., 1992) and the conclusive demonstration of the unusually high efficiency of ICSI in terms of fertilization and pregnancy rates (Van Steirteghem et al., 1993), this method has been increasingly used in the treatment of infertility, including cases in which spermatozoa have to be recovered from the epididymis or the testis and thus have not completed the process of epididymal maturation (Silber, 1994; Devroey et al., 1996). Pregnancies and births have also been obtained in mice after transfer of embryos developing from oocytes electropulsed or injected with round spermatids (Ogura et al., 1994; Kimura and Yanagimachi, 1995a) or secondary spermatocytes (Kimura and Yanagimachi, 1995b) and in humans after transfer of embryos developing from oocytes injected with elongated (Fishel et al., 1995a, 1996) and round (Tesarik et al., 1995a, 1996) spermatids (reviewed in Tesarik, 1996a).

The problem of oocyte activation is of paramount importance to all these techniques of micromanipu-
lation-assisted reproduction. In fact, the failure of oocyte activation appears to be the main cause of fertilization failure after ICSI (Sousa and Tesarik, 1994). Moreover, the events occurring during oocyte activation are believed to condition embryo quality for many subsequent cell cycles (Swann and Ozil, 1994), and irregularities of these events, that may occur after ICSI, are suspected to be the cause of different embryonic abnormalities, including chromosomal ones (Tesarik, 1995). This paper outlines the current understanding of the cellular and molecular mechanisms involved in oocyte activation and focuses on those elements that are likely to be affected by factors associated with the intra-ooplasmic injection technique and with the use of immature sperm cells for fertilization.

Mechanism of oocyte activation
The mature human oocyte has its cell cycle blocked at the metaphase of the second meiotic division (metaphase II). By analogy with other mammalian species, the metaphase II block of human oocytes is supposed to be maintained by persisting high activities of metaphase-promoting factor (MPF). From the functional point of view, this factor is the same as that controlling the progression of the cell cycle in somatic cells, in which its activity changes periodically to drive cell nuclei to metaphase during each mitotic division. Consequently, any nucleus introduced into a metaphase II oocyte will be driven to metaphase unless MPF has been inactivated. This MPF inactivation is one of the principal events of oocyte activation which unblocks the cell cycle of the future zygote, allows the female nucleus to complete the second meiotic division and protects the male nucleus from entering metaphase prematurely in response to the oocyte’s MPF. The triggering of the cortical reaction is another consequence of oocyte activation which, under normal conditions when the zona pellucida is not severed, is important in setting in motion a polyspermy-preventing mechanism. The fact that progression of preimplantation embryonic development can be modified by artificially manipulating the conditions of oocyte activation (Swann and Ozil, 1994) suggests that, in addition to MPF inactivation and the cortical reaction, many other cellular functions are influenced by events occurring during oocyte activation.

There is no doubt that physiological oocyte activation is induced by the spermatozoon at fertilization. However, oocytes can also be activated by a number of artificial physical and chemical stimuli unrelated to spermatozoa (parthenogenetic activation). Of course, oocyte activation produced by such unnatural stimuli will often be abnormal or incomplete. Whether or not such phenomena should still be referred to as oocyte activation will thus depend on the outcome measures followed in each experimental study.

The mechanism whereby the spermatozoon induces oocyte activation can be studied at different levels. One of the most important questions relating to this mechanism is how the spermatozoon generates the immediate triggering stimulus which starts the whole cascade of reactions ultimately leading to oocyte activation. How this introductory stimulus is subsequently transduced via intracellular signalling pathways towards the corresponding effectors is another fundamental question. Finally, it is crucial to know how these signalling pathways operate in space and time to ensure an ordered stimulation of different targets within the oocyte.

Triggering stimulus
Experimental studies performed in various animal species suggest that the spermatozoon triggers oocyte activation by acting at a receptor or several receptors on the oocyte plasma membrane; these receptors appear to be coupled to a G-protein or to a protein tyrosine kinase (Jaffe, 1990; Miyazaki et al., 1990; Ciapa and Epel, 1991; Foltz et al., 1993; Moore et al., 1993; Abassi and Foltz, 1994; Shilling et al., 1994; Iwao and Fujimura, 1996). A sperm receptor has been characterized in sea urchin eggs as a transmembrane glycoprotein sharing some sequence similarity with a heat-shock protein, hsp70 (Foltz et al., 1993). A protein capable of triggering electrical responses in eggs similar to those occurring at fertilization was isolated from Urechis sperm acrosomes (Gould and Stephano, 1987). In mammals, no candidate protein for this function has as yet been identified. However, the plasma membrane of mammalian oocytes contains the adhesion molecules integrins.
Immature sperm and oocyte activation

(Tarone et al., 1993), whereas the posterior head region of mammalian spermatooza (the region by means of which the first tight contact between the sperm and oocyte plasma membranes is established during fertilization) bears an integral plasma membrane glycoprotein termed fertilin (Primakoff et al., 1987; Blobel et al., 1992) whose β-subunit contains an integrin-binding domain (Wolfsberg et al., 1993; Primakoff et al., 1987; Blobel et al., 1992). Moreover, human sperm-oocyte binding has been shown to be inhibited by immunobeads coated with peptides containing the RGD (Arg–Gly–Asp) sequence known to serve as ligands for integrins (Fusi et al., 1992). Since integrins mediate transmembrane signal transduction in many cell types (Yamada and Miyamoto, 1995), sometimes involving a mobilization of intracellular calcium (Shankar et al., 1993) which also is a dominant feature of oocyte activation (see below), integrins present on the oocyte surface may be stimulated by sperm fertilin and are thus good candidates for oocyte activation-triggering receptors. Oocyte activation accompanied by intracellular calcium release could also be induced by RGD-containing peptides in Xenopus (Iwao and Fujimura, 1996).

The recent development of ICSI, and especially the finding that calcium responses similar to those observed during normal fertilization (Taylor et al., 1993) can be obtained after ICSI (Tesarak et al., 1994), has often been interpreted as arguing against the surface-receptor hypothesis of oocyte activation triggering and in favour of the so-called soluble-sperm-factor hypothesis. In fact, it was shown as early as the mid-1980s that sea urchin oocytes can be activated by direct intracytoplasmic injection of a soluble sperm extract (Dale et al., 1985), without any previous contact between the gamete surfaces. The possibility of activating oocytes by injecting soluble sperm extracts has subsequently been confirmed in various mammalian species (Swann, 1990, 1994) including the human (Homa and Swann, 1994; Dozortsev et al., 1995a). A highly conserved protein capable of carrying out this action in different mammalian species has been characterized (Parrington et al., 1996). The existence in spermatozoa of a soluble oocyte-activating factor explains why essentially normal oocyte activation events can be achieved after ICSI although the eventual function of oocyte surface receptors is obviously by-passed. However, the current experience with ICSI does not imply that such receptors play no role in human oocyte activation occurring in situations other than ICSI. On the contrary, the observations that some oocytes fail to activate after ICSI although the injected spermatozoon has completely disintegrated and thus released all its cytoplasmic contents into the ooplasm (Sousa and Tesarak, 1994) and that such oocytes can still be activated by artificially increasing their calcium load with an ionophore (Tesarak and Sousa, 1995a), suggest that the oocyte activation process after ICSI is unusually vulnerable and may not follow exactly the same pattern as the physiological oocyte activation involving the gamete surface interaction. The lack of surface receptor participation in the oocyte activation events after ICSI can also provide an explanation for the observation that the calcium responses recorded during oocyte activation after ICSI are slightly different from those observed when oocytes are fertilized by subzonal insemination, which does not bypass the gamete surface contact and interaction (Tesarak and Sousa, 1994). However, the reason for this difference may be related to different rates and locations of release of the sperm soluble factor in the two situations. Taken together, the data on human oocyte activation that are available, suggest that the fertilizing spermatozoon activates the oocyte both by acting at a receptor on the oocyte surface and by releasing a soluble factor into the ooplasm, although the latter can eventually assume the totality of the task, provided that it is aided by artificial stimuli brought about, intentionally or unintentionally, by the ICSI procedure itself (see below).

**Signal transduction pathways**

In all animal species studied so far, the oocyte-activating signal uses calcium as a second messenger. In mammalian oocytes, the source of calcium participating in these signalling events is predominantly intracellular, although calcium influx from the extracellular space is also involved (Swann and Ozil, 1994). Releasable calcium is stored in cells within calcium stores (presumably mainly endoplasmic reticulum) within which it is accumu-
lated against a concentration gradient through an energy-consuming action of a calcium pump and from which it can be released along the existing concentration gradient without an energy requirement by opening calcium channels in the calcium store membrane. Two types of calcium-store calcium channels are known: one of them is a receptor for inositol 1,4,5-trisphosphate (IP$_3$) and the other is a receptor for ryanodine. In addition to the plant alkaloid ryanodine, the ryanodine receptor/calcium channel also opens in response to a physiologically occurring ligand, cyclic ADP-ribose. Both types of channels can open as a result of an increase in the calcium concentration outside the stores in their immediate vicinity; this phenomenon is known as calcium-induced calcium release (CICR). The emptying of a calcium store usually generates a signal that is conveyed to the plasma membrane, leading to the opening of its own calcium channels and resulting in calcium influx; this phenomenon is known as capacitative calcium entry.

The current data about the type and function of calcium stores in mammalian oocytes are somewhat contradictory. There is little doubt that IP$_3$-sensitive stores are present in oocytes (Miyazaki et al., 1993; Kline and Kline, 1994; Fissore et al., 1995; Miyazaki, 1995). On the other hand, ryanodine-sensitive stores have been detected by some workers (Swann, 1992; Ayabe et al., 1995; Yue et al., 1995; Sousa et al., 1996a) but not by others (Miyazaki et al., 1993; Kline and Kline, 1994; Fissore et al., 1995). The presence of ryanodine-sensitive calcium stores in oocytes and their role in the oocyte activation mechanism have been recently demonstrated in humans (Sousa et al., 1996a), and these calcium stores have been suggested to participate, together with IP$_3$-sensitive stores, in a two-store mechanism of calcium oscillations (Tesarik et al., 1995b; Tesarik and Sousa, 1996).

How the fertilizing spermatozoon induces the release of calcium from oocyte intracellular stores is still a matter of debate. It is possible that the spermatozoon acts directly on only one type of calcium store within the oocyte, so that the resulting calcium discharge activates CICR from the other type of store. Data obtained with hamster and mouse oocytes suggest that the sperm action at the oocyte surface leads to the activation of a G-protein (Miyazaki, 1988; Moore et al., 1993). It can be postulated that the G-protein-mediated activation of phosphoinositide-specific phospholipase C leads to hydrolysis of plasma membrane phosphoinositides thus yielding IP$_3$ which, in its turn, will release calcium from the IP$_3$-sensitive stores followed by CICR from the ryanodine-sensitive stores. In agreement with this hypothesis, ryanodine-insensitive, but thimerosal- and presumably IP$_3$-sensitive, calcium stores have been detected in the cortical region of human oocytes (Sousa et al., 1996a). Diacylglycerol, another product of the phosphoinositide breakdown, may then activate protein kinase C (PKC), which is supposed to be involved in the down-regulation of the IP$_3$-mediated calcium release mechanism, and thus facilitates the subsequent return of the intracellular free calcium concentration ([Ca$^{2+}$]$_i$) to the basal level (Swann et al., 1989; Miyazaki et al., 1990). This sequence of events may underlie the sharp transient [Ca$^{2+}$]$_i$ increase referred to as a calcium spike. Notwithstanding, other mechanisms involving oocyte plasma membrane receptors and their ligands, sperm soluble factors, IP$_3$-sensitive calcium stores and ryanodine-sensitive calcium stores also may come into play. The observations that the injection of sperm extracts into oocytes can induce a rapid [Ca$^{2+}$]$_i$ increase (Swann, 1990, 1994) do not mean that soluble sperm factors are the only physiological oocyte-activation triggers. In fact, it appears difficult to determine an exact dosage of the sperm factors injected in the form of sperm soluble extracts. Consequently, there is still some doubt as to whether the dose of sperm cytosolic factors required for the rapid response observed after the injection of sperm extracts can actually be delivered by a single spermatozoon at fertilization. Moreover, the microinjection technique used for the deposition of sperm extracts into oocytes may itself modify the oocyte response to the injected factors. These uncertainties do not question the role of such factors in oocyte activation but warn against simplifications that lay too much emphasis on only one aspect of the oocyte-activation mechanism and ignore its probable complexity.

Relatively little is known about how the calcium signal is transduced to downstream elements of
Metaphase arrest

MPF (cyclin/cdc2)  CSF (c-mos product)

Sperm
Calcium

Metaphase arrest exit

MPF (cyclin/cdc2)  CSF (c-mos product)

CAM kinase II  PKC

Figure 1. Schematic representation of the probable mechanism by which sperm-induced oocyte activation drives the oocyte to exit from metaphase II arrest. The metaphase II arrest is maintained by persisting high activities of metaphase-promoting factor (MPF; consisting of the regulatory component termed cyclin and the cyclin-dependent kinase cdc2) and cytostatic factor (CSF) that is a product of the c-mos proto-oncogene. The calcium signal generated by the fertilizing spermatozoon activates protein kinases, of which calmodulin-dependent protein kinase II (CAM kinase II) and protein kinase C (PKC) have been suggested as the most likely candidates. The kinase activation is then postulated to lead to phosphorylation of MPF and CSF and their subsequent inactivation (for a more detailed review, see Swann and Ozil, 1994).

Spatiotemporal characteristics

Unlike fish, echinoderms and frogs, in which the fertilizing spermatozoon induces a single transient \([\text{Ca}^{2+}]_i\) increase, the oocyte-activation signal in mammals and some other species consists of a series of repetitive short calcium spikes known as calcium oscillations. In human oocytes, the sperm-induced calcium oscillations can last for up to 5–6 h (Taylor et al., 1993; Sousa et al., 1996b); the frequency with which individual calcium spikes appear ranges between one spike every 2 min to one every 35 min and is highly variable from oocyte to oocyte but relatively stable in a single oocyte during the whole calcium oscillation period (Taylor et al., 1993; Tesarik and Sousa, 1994; Tesarik et al., 1995b; Sousa et al., 1996b). However, in most mammalian species studied so far (reviewed in Swann and Ozil, 1994) including the human (Tesarik and Sousa, 1994), the very early \([\text{Ca}^{2+}]_i\) reaction of oocytes to the fertilizing spermatozoon shows a unique pattern that is slightly different from the bulk of the calcium oscillation period; in human oocytes, this early calcium reaction is characterized by an initial group of three to six rapidly occurring calcium spikes which is then followed by lower-frequency spikes for the rest of the calcium oscillation period (Tesarik and Sousa, 1994). The sperm-induced calcium oscillations of mammalian oocytes are believed to bear a unique, frequency-encoded signal that is necessary for the complete oocyte-activation process (Vitullo and Ozil, 1992; Ozil and Swann, 1995). In agreement with this hypothesis, incomplete activation with a failure of polar body extrusion was observed after artificial induction of a single \([\text{Ca}^{2+}]_i\) increase in oocytes of the protostome worm Cerebratulus lacteus that, similarly to mammalian oocytes, also require calcium oscillations for complete activation (Stricker, 1996).

In view of the long duration of the sperm-induced calcium oscillations in human oocytes, it
is clear that the fertilizing spermatozoon must deliver to the oocyte not only the immediate trigger to induce the oscillations but also a message modifying the properties of one or several of the main elements involved in the regulation of calcium homeostasis (calcium pumps and channels in the plasma and calcium store membranes) to facilitate that kind of calcium exchanges between individual compartments that are required for the maintenance of calcium oscillations. We may use the term ‘oscillator’ for this latter message. The consideration of the trigger and oscillator as two distinct physiological entities is based on the observations that human ICSI can sometimes lead to the development of a single monotonous sperm-dependent \([Ca^{2+}]_i\) increase (Tesarik et al., 1994) or bring oocytes into a condition in which they are ready to develop calcium oscillations but do not do so unless stimulated with an additional, ionophore-induced \([Ca^{2+}]_i\) increase (Tesarik and Testart, 1994). The former situation can be explained by an isolated action of the trigger without the oscillator, whereas the latter results from the action of the oscillator without the natural trigger. Sperm cytosolic factors, among which oscillin (Parrington et al., 1996) is the only one identified as yet, are the most probable candidates for the oscillator function.

Experimental data obtained with human oocytes (Sousa et al., 1996a) suggest that calcium oscillations are maintained by periodic release and resorption of calcium from IP\(_3\)-sensitive stores, localized predominantly in the oocyte cortex and subcortex, and ryanodine-sensitive stores localized mainly in the central ooplasm (Tesarik and Sousa, 1996), thus conforming to a previously formulated two-store calcium oscillation model (Tesarik et al., 1995b). An alternative two-store model for calcium oscillations in mammalian oocytes does not involve the ryanodine-sensitive calcium stores but assumes the existence of two functionally distinct types of IP\(_3\)-sensitive calcium store, one responsible for calcium entry and the other giving the regenerative spike (Berridge, 1996). According to the former model, the ability of the oocyte to maintain calcium oscillations depends on the differential sensitivity of the two types of calcium stores to CICR, such that an initial limited calcium release from the cortical stores (presumably sensitive to IP\(_3\)) acts as a detonator for CICR from the central, ryanodine-sensitive stores from which most of the calcium released during each calcium spike originates. Because the cortical stores have a higher sensitivity to CICR than the central ones, the zone of increased \([Ca^{2+}]_i\) propagates from the initial focus in the cortex around the oocyte periphery, with the likely participation of the capacitative calcium entry, before extending to the central ooplasm where, on the other hand, \([Ca^{2+}]_i\) remains elevated for the longest time at the end of each calcium spike (Tesarik et al., 1995b). The latter model (Berridge, 1996) can be expected to work in a similar way but the ryanodine-sensitive calcium stores are substituted by a unique type of IP\(_3\)-sensitive calcium store. The sensitivity to CICR of one or both of the oocyte’s calcium stores may be modified by the sperm-derived oscillator, as suggested by the unique pattern of the spatial propagation of sperm-induced calcium waves compared to those induced by artificial stimuli such as thimerosal or ryanodine (Tesarik et al., 1995b; Sousa et al., 1996a).

Phosphorylation by PKC has been suggested to be part of the mechanism controlling the characteristics of the ryanodine-sensitive calcium stores in human oocytes because a sustained stimulation of PKC inhibits sperm-induced calcium oscillations and prevents any calcium response of oocytes to ryanodine, but not to thimerosal, whereas inhibition of PKC interferes with the return of \([Ca^{2+}]_i\) to basal values during the sperm- and ryanodine-induced calcium oscillations, but not during the thimerosal-induced calcium oscillations (Sousa et al., 1996c).

**Particular questions relating to oocyte activation after intracytoplasmic injection of mature and immature sperm cells**

**Skipping gamete surface interaction: does it make a difference?**

The experience with ICSI indicates that injected spermatozoa can activate oocytes completely and induce calcium oscillations similar to those seen during normal fertilization. Using the preceding vocabulary, we can say that, in the absence of sperm-derived triggers that probably require an
Immature sperm and oocyte activation

Interaction between the gamete surfaces to act, the sperm-derived oscillators can be sufficient to support oocyte activation provided this process is triggered by an independent stimulus. It is very likely that the calcium influx produced by the ICSI procedure itself can serve as such an auxiliary stimulus (Tesarik and Sousa, 1995b), although it is insufficient to trigger oocyte activation in the absence of spermatozoa (Tesarik et al., 1994). The amount of calcium entering the oocyte is proportional to the degree of ooplasmic aspiration during the ICSI procedure (Tesarik and Sousa, 1995b). The degree of ooplasmic aspiration is difficult to quantify objectively, and even the degree of calcium influx without intentional ooplasmic aspiration is likely to vary from laboratory to laboratory as a result of differences in the quality of the injection needle and in the technical aspects of the oocyte plasma membrane disruption. This explains why fertilization results were related to the degree of aspiration in some (Palermo et al., 1995; Tesarik and Sousa, 1995b) but not all (Mansour et al., 1996) laboratories. Oocyte activation after ICSI also works better when spermatozoa are mechanically damaged by the microinjection needle just before ICSI (Dozortsev et al., 1995b; Fishel et al., 1995b; Van den Bergh et al., 1995) to facilitate sperm disintegration in the ooplasm and the release of oocyte-activating factors. In addition to the truncation of the typical initial sperm-induced calcium signal, which is common with ICSI (see above), two types of more severe oocyte-activation abnormalities have been reported: an unusual delay in the beginning of the sperm-induced calcium oscillations and substitution of calcium oscillations with a long non-oscillatory \([Ca^{2+}]_i\) increase (Tesarik et al., 1994; Tesarik and Testart, 1994). By analogy with other animal species, such abnormalities may cause serious developmental consequences. For instance, failure of the second polar body extrusion, leading to the formation of tripolar zygotes after ICSI, may result from an abnormal calcium signal, similar to that described in protostome worms (Eckberg and Miller, 1995; Stricker, 1996). Moreover, the delayed onset of the oocyte-activation signal is likely to lead to a delayed or incomplete inactivation of MPF (Tesarik, 1996b) which, in its turn, can cause abnormalities of the second meiotic anaphase leading to chromosomal abnormalities in the future embryo (Tesarik, 1995). Diverse cytological abnormalities, involving sperm aster formation, pronuclear development, spindle formation and cell division, were observed in rhesus monkey oocytes fertilized by ICSI and were suggested to be at least partly due to abnormalities of calcium signalling (Hewitson et al., 1996). Even though all of these relationships remain hypothetical in the human, they deserve attention, particularly in view of the recently published data about the incidence of chromosomal abnormalities after ICSI (In’t Veld et al., 1995).

Use of immature sperm cells: the significance of calcium oscillations revisited

The generally accepted view that the oscillatory character of the sperm-induced calcium signal is required for the optimal efficiency of the fertilization process in mammals, in terms of the developmental potential of the resulting embryo, has been challenged by recent reports of relatively elevated birth rates after fertilization of mouse oocytes with isolated nuclei of round spermatids and secondary spermatocytes (Kimura and Yanagimachi, 1995a,b). These findings mean that either calcium oscillations can be induced in oocytes by factors associated with spermatid and spermatocyte nuclei, or the oscillatory character of the sperm-induced calcium signal is not important for normal oocyte activation, at least in the mouse. The preliminary data with regard to spermatid conception in humans do not raise a similar dilemma. In fact, the three childbirths reported so far after spermatid conception in humans (Tesarik et al., 1995a, 1996; Fishel et al., 1995a, 1996) were all obtained after injection of whole spermatids into oocytes, not just their isolated nuclei. Moreover, the injection of human spermatids, but not primary and secondary spermatocytes, into human oocytes sensitizes the calcium response mechanism of the oocytes in a way very similar to the injection of mature spermatozoa (Sousa et al., 1996d). It should also be noted that a limited amount of ooplasm accompanied the spermatid and spermatocyte nuclei injected into the oocytes in the above mouse experiments, and this might be enough to sensitize the oocytes for
J. Tesarik

the development of calcium oscillations. To answer these questions, an analysis of $[Ca^{2+}]$, following injection of spermatid and spermatocyte nuclei into mouse and rabbit oocytes is needed.

Testicular spermatozoa may also be slightly different from ejaculated spermatozoa with regard to the activity or releasability of oocyte-activating factors because the former appear to require a more aggressive mechanical disruption before ICSI than the latter to achieve optimal fertilization results (Palermo et al., 1996).

Co-ordination of the effects of oocyte activation on the male and the female nucleus: a newly emerging problem

One of the reported abnormalities of oocyte activation following ICSI is its delayed onset (Tesarik et al., 1994), although such a delay has not always been observed (Nagy et al., 1994). Consequently, the male nucleus can be exposed for some time to MPF that is not inactivated until the oocyte is activated (Figure 1). A short exposure of male gamete nuclei to active MPF is necessary even for normal fertilization with mature spermatozoa, since Borsuk (1991) has shown that the sperm nuclear membrane breakdown in the ooplasm is a MPF-dependent event, whereas the subsequent pronuclear development is MPF-independent. This observation underscores the importance of a fine co-ordination between the exposure of the male nucleus to oocyte cytoplasmic factors and the progression of oocyte activation. Unlike mature spermatozoa, whose chromatin is highly condensed and stabilized by specific nuclear proteins, the protamines, so that the oocyte MPF is unable to drive sperm nuclei to metaphase prematurely, in developing spermatids the process of histone/protamine exchange is still ongoing. Spermatid nuclei are thus much more sensitive to untimely oocyte activation events than nuclei of mature spermatozoa (Tesarik, 1996b). If MPF is allowed to drive spermatid nuclei to metaphase, the consequences for future embryo development are disastrous because the prematurely condensed chromatids bypass the S-phase, associate with microtubules and can be extruded from the oocyte in an uncontrolled manner as soon as the metaphase II block is overcome by subsequent oocyte activation (Kimura and Yanagimachi, 1995a). This situation leads inevitably to the development of aneuploidy (Table I). Exactly the same mechanism was used with success for chromosomal reduction of secondary spermatocytes injected into mouse metaphase II oocytes, resulting in the development of diploid zygotes (Kimura and Yanagimachi, 1995b).

On the other hand, a premature onset of oocyte activation, while the male nucleus is still absent or not fully accessible to ooplasmic factors, can cause situations in which the male nucleus receives a truncated developmental message as compared to the female nucleus. This might result in a developmental imbalance between the two nuclei (Table I). It remains to be determined whether such an imbalance is the cause of the nuclear abnormalities, such as the persistence of an unusually small male pronucleus (Ogura et al., 1994; Tesarik and Mendoza, 1996) or the prolonged nuclear syngamy (Tesarik and Mendoza, 1996) observed in zygotes developing from spermatid-fertilized oocytes. Anyway, these examples show, first, that artificial manipulation of oocyte activation will be needed to increase the efficiency of fertilization with sperm precursor cells and, second, that the nature of this manipulation will depend strictly on the stage of the sperm precursor cell that is used. Further improvements to the success rates of human fertilization using spermatids can thus be expected to result from a better understanding of the relationship between the spermatid developmental stage, nuclear maturity, the activity and stability of the oocyte-activating factors, and the time needed for spermatid nucleus exposure to ooplasmic factors after the injection. Based on

<table>
<thead>
<tr>
<th>Type of sperm cell injected</th>
<th>Beginning of activation</th>
<th>Type of diploid zygote development</th>
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<tr>
<td>Spermatocyte II</td>
<td>Triploidy</td>
<td>Ageing</td>
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<tr>
<td>Spermatid</td>
<td>Pronuclear asynchrony</td>
<td>PCC, aneuploidy</td>
</tr>
<tr>
<td>Spermatozoon</td>
<td>Pronuclear asynchrony</td>
<td>Ageing</td>
</tr>
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</table>

PCC = premature chromosomal condensation.

Table I. Consequences of inadequate timing of oocyte activation after injection of mature and immature sperm cells

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this knowledge, it will hopefully be possible to
determine the optimal combination of spermatid
plasma membrane destabilization before injection,
dergree of ooplasmic aspiration during the injection
and the timing of eventual auxiliary treatments to
boost oocyte activation (ionophore, electroactiva-
tion, etc.).

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