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TREATMENT OF SEVERE MALE INFERTILITY BY MICROMANIPULATION-ASSISTED FERTILIZATION: NEWS AND VIEWS

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1. ABSTRACT

Recent progress in cell micromanipulation has made it possible to achieve fertilization even with highly deficient male gametes, by injecting them directly to oocyte cytoplasm. This technique, known as intracytoplasmic sperm injection (ICSI), has proved efficient in most types of sperm pathology. However, ICSI fails when injected spermatozoa are not capable of supporting the process of oocyte activation or when they carry genetic abnormalities incompatible with normal embryonic development. Abnormalities of oocyte activation and the transmission of genetic anomalies to the progeny are also two major concerns relating to the clinical use of ICSI. The use of micromanipulation-assisted fertilization in cases of male germ cell maturation arrest, namely fertilization by round spermatid injection (ROSI), has brought preliminary clinical results, and the improvement of ROSI clinical efficacy remains a major challenge for future research.

2. EVOLUTION OF MICROMANIPULATION-ASSISTED FERTILIZATION TECHNIQUES AND THEIR USE IN HUMAN INFERTILITY MANAGEMENT

2.1.Rationale underlying the use of micromanipulation-assisted fertilization

Coinciding with the massive application of in vitro fertilization (IVF) in the treatment of human infertility in the 1980s, there was a rapid progress in the understanding of cellular interactions underlying human fertilization. Although the advent of IVF marked a tremendous progress in the evolution of assisted reproduction techniques, it was also noted how inefficient IVF is in terms of sperm cell economy. In fact oocyte-to-sperm ratios of 1:10,000 to 1:100,000 are necessary to succeed in IVF, which means that only 0.001-0.01% of spermatozoa co-incubated with oocytes will eventually be able to fertilize. Most of the

unsuccessful spermatozoa remain free in incubation medium, but many associate with the oocyte and remain blocked on, or within, its glycoprotein envelope, the zona pellucida which is known to be the main mechanical obstacle to sperm penetration into the oocyte. This was at the origin of the idea that IVF results in cases of abnormally low sperm count (oligozoospermia), defective sperm movement (asthenozoospermia) or abnormal sperm morphology (teratozoospermia) may be improved by using micromanipulation techniques to help spermatozoa penetrate the zona pellucida.

2.2. Increasing degree of assistance

Early attempts to assist sperm penetration into the oocyte by making holes in the zona pellucida (zona drilling, zona cutting) or by subzonal insemination (SUZI) did not fulfil the original expectations, mainly because of difficulties of mimicking the natural mechanism limiting the access of supernumerary spermatozoa to the oocyte (block to polyspermy). Under normal conditions, this mechanism is ensured by the structurally and functionally intact zona pellucida. Hence, the application of these techniques led to high frequencies of fertilization failure, on the one hand, and of polyspermic fertilization on the other hand, whereas the occurrence of normal monospermic fertilization was low.

An efficient solution to this problem required a further substantial increase in the degree of assistance to fertilization, implying a direct selection of one spermatozoon and its deposition in the oocyte cytoplasm. The application of this technique, now known as intracytoplasmic sperm injection (ICSI), in human assisted reproduction was pioneered by Palermo et al. (1) and was soon demonstrated to be clearly superior to SUZI, which was the method of choice by that time (2). The direct transfer of the male gamete into the oocyte is the common denominator of all current techniques of micromanipulation-assisted fertilization, including those involving immature forms of male germ cells (see below).

2.3. Animal experimentation and human application

Animal experiments preceded the use in humans of all techniques of micromanipulation-assisted fertilization, although there is still some debate about the question of whether the knowledge resulting from these animal experiments was sufficient to guarantee the safety of human application or, at least, to reduce the potential risks to an acceptable level. The first successful fertilization of a mammalian oocyte by a direct intra-ooplasmic transfer of a spermatozoon was reported in hamsters more than 20 years ago (3). In rabbits and cattle, embryos obtained with the use of this method were transferred to recipient mothers, resulting in the birth of normal offspring (4). In general, animal experiments served to refine the methodology of gamete micromanipulation, to design the optimal media and instruments, and to exclude the existence of a high risk of major developmental abnormalities leading to somatic diseases or defects of behaviour. On the other hand, they did not totally exclude the eventual increased risk of minor abnormalities, of rare pathologies whose prevalence is extremely low in general population and of those human pathologies (including behavioural ones) for which there is currently no animal model.

3. CURRENT STATUS OF MICROMANIPULATION-ASSISTED FERTILIZATION

The human species appears to be unusually well-suited for micromanipulation-assisted fertilization, and particularly for ICSI, because of the relatively small size of spermatozoa as compared to oocytes. This favourable sperm-to-oocyte size relationship reduces the risk of oocyte damage during sperm injection and thus makes the procedure technically easier. Human ICSI is a clinically efficient method although it modifies some of the basic biological mechanisms underlying normal fertilization. Notwithstanding, these modifications must be taken into consideration because they can explain the increased vulnerability of embryos resulting from ICSI under certain special conditions (e.g. cryopreservation). The knowledge of these differences will also help control some of the potential risk factors associated with the clinical use of micromanipulation-assisted fertilization.

3.1. Modifications of basic biological mechanisms underlying fertilization

Since all current methods of micromanipulation-assisted fertilization imply a direct transfer of the male gamete (or of its precursor cell) to the oocyte cytoplasm, all natural preparatory steps, necessary for sperm penetration into the oocyte, are bypassed, whereas the conditions of oocyte activation and of pronuclear formation are modified. The possibility of using these modified conditions for deliberate manipulations of male germ cell ploidy (see below) is an interesting new finding, opening the way to methods using meiotically immature germ cells for fertilization.

3.1.1. Sperm capacitation

Sperm capacitation is a complex of different molecular and cellular events including modifications of the sperm surface, changes in the intracellular concentration of ions, the metabolism of cyclic nucleotides and protein phosphorylation/dephosphorylation reactions (5). Most of these events are required for a typical change in the pattern of sperm movement, referred to as hyperactivation, and for the acrosome reaction (see below) which is necessary for sperm penetration through the zona pellucida and fusion with the oocyte (6). Because spermatozoa need not be motile in order to fertilize in the case of ICSI, and their interaction with the zona pellucida as well as the fusion with the oocyte are bypassed by using the micromanipulation technique, there seems to be no need for sperm capacitation to achieve fertilization with the use of this technique.

3.1.2. Acrosome reaction

In normal fertilization, spermatozoa that have penetrated into oocytes have always undergone the acrosome reaction and, consequently, lack the acrosome. However, this is obviously not always the case when fertilization is assisted by ICSI. Because the role of the acrosome reaction is to prepare spermatozoa for actions necessary for active penetration into oocytes, it also

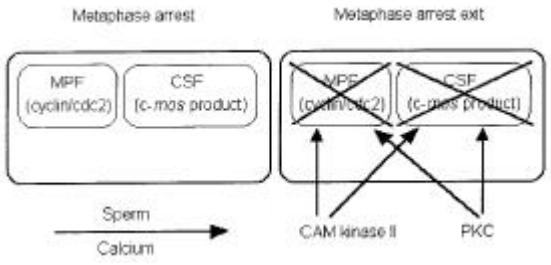

Figure 1. Schematic representation of the probable mechanism by which sperm-induced oocyte activation drives the oocyte exit from the metaphase II arrest. The metaphase II arrest is maintained by persisting high activities of metaphase-promoting factor (MPF; consisting of the regulatory component termed cyclin and the cyclin-dependent kinase cdc2) and cytostatic factor (CSF) that is a product of c-*mos* proto-oncogen. The calcium signal generated by the fertilizing spermatozoon activates protein kinases of which calmodulin-dependent protein kinase II (CAM kinase II) and PKC have been suggested as most likely candidates. The kinase activation is then postulated to lead to phosphorylation of MPF and CSF and their subsequent inactivation (reproduced from Ref. 18, with permission of the European Society for Human Reproduction and Embryology).

becomes unnecessary in the instance of ICSI. This has been demonstrated in sheep, where fertilization rates after ICSI were similar for acrosome-intact and acrosome-reacted spermatozoa (7). The only problem is related to the question of how an oocyte that has been injected with an acrosome-intact spermatozoon will cope with the sperm acrosome, which contains a battery of hydrolytic enzyme that, when activated in the oocyte cytoplasm, might produce harmful effects. These effects are more likely to occur in species whose spermatozoa possess large acrosomes. It is thus easy to understand that unlike species with relatively small sperm acrosomes, such as the mouse, previous induction of the acrosome reaction is important for the success of ICSI with hamster spermatozoa which possess much larger acrosomes, whereas the oocyte size in both species is about the same (8). The small size of the human sperm acrosome and the large size of the human oocyte represent an ideal combination for the success of ICSI with acrosome-intact spermatozoa, which may partly explain the relative ease with which human ICSI developed into a highly efficient method as compared with ICSI using gametes of other mammalian species. Electron microsopic studies performed on human oocytes freshly injected with human spermatozoa showed that the acrosome begins to be removed from the injected spermatozoa as early as 15 min after ICSI (9), and this process is achieved by multiple fusion and vesiculation of the sperm plasma and acrosomal membranes (10), in a similar way as observed during the physiological acrosome reaction (6).

3.1.3. Oocyte activation

Oocyte activation is a complex of molecular events that result in the exit of the oocyte from meiosis and activation of the mitotic cell cycle (figure 1). Because this cell cycle switch is obligatory for an oocyte to become an embryo, irrespective of the means whereby the male genome has been introduced into it, the importance of oocyte activation is the same in natural and micromanipulation-assisted fertilization. In fact, the failure of oocyte activation has been shown to be the main cause of fertilization failure after ICSI (11, 12). As in other mammalian species, human oocyte activation is mediated by a specific type of calcium signals, characterized by a series of sharp, transient increases of cytosolic free calcium concentration, termed calcium oscillations (13). Calcium oscillations also develop in human oocytes after ICSI (14). However, the temporal and spatial patterns of these oscillations are not exactly the same as after normal fertilization. When the pattern of sperm-induced calcium oscillations was compared in human oocytes fertilized by SUZI, a technique allowing the normal interaction between sperm and oocyte plasma membranes, and in those fertilized by ICSI, where all preliminary gamete contact is avoided, specific differences were found (15, 16). Calcium oscillations observed in oocytes fertilized by SUZI (15) were quite similar to those developing after non-micromanipulated IVF (13), whereas the use of ICSI was associated in typical changes concerning both the rhytm of the oscillations (15, 16) and the spatial propagation of the zone of increased calcium concentration (calcium wave) throughout the oocyte cytoplasm (17).

Experimental data about oocyte activation in human IVF and ICSI conform to a recent hypothesis which makes a strict distinction between two sperm functions called, respectively, trigger and oscillator (18). According to this hypothesis, the initial sperm-induced calcium increase during conventional (non-micromanipulated) IVF is triggered by a signal generated by the fertilizing spermatozoon at the oocyte plasma membrane (trigger function), whereas the ability of the oocyte to generate subsequent calcium oscillations is due to a sperm cytosolic factor modifying the characteristics of calcium release from the oocyte intracellular stores (oscillator function). In ICSI, the trigger function appears to be assumed by an alternative mechanism leading to a similar calcium increase - the influx of calcium from extracellular medium, which is known to be produced by the injection procedure itself (18). The efficiency of this pseudotrigger is potentiated by vigorous aspiration of oocyte cytoplasm just before expulsion of the spermatozoon from the microinjection needle or by movements of the needle within the oocyte during ICSI (16). One or both of these actions are used by most of laboratories in order to ensure the penetration of the oocyte plasma membrane before sperm injection (19, 20). The efficacy of the release of the oscillator, in its turn, can be increased by artificially destabilizing the sperm plasma membrane. This contention corresponds to empirical observations on increased fertilization rates after ICSI when the sperm flagellum is crushed mechanically with the microinjection needle just before the injection (21, 22, 23). Accordingly, oocytes fail to activate after ICSI either when the micromanipulation technique is not adequate to provoke sufficient calcium influx to substitute for the bypassed trigger function of the fertilizing spermatozoon, or when the

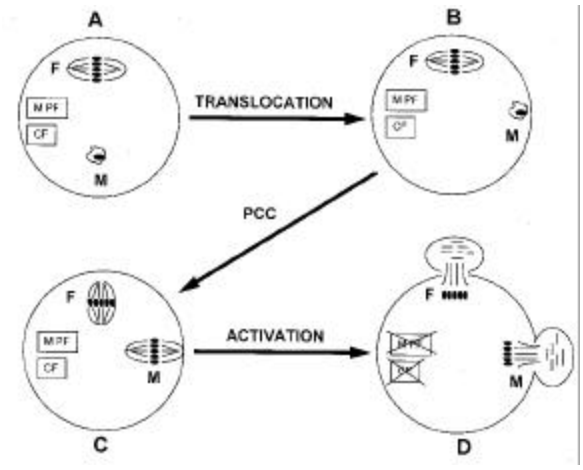

Figure 2. Schematic representation of the mechanism by which the oocyte can reduce the chromosomes of an injected male germ cell. (**A**) Mature oocyte with female chromosomes (F) maintained in metaphase configuration by the action of metaphase promoting factor (MPF) and cytostatic factor (CF), just after the injection of a male germ cell nucleus (M). The first polar body is not represented. (**B**) The injected male germ cell nucleus is translocated to the oocyte periphery. (**C**) In the absence of oocyte activation, the persisting MPF and CF have induced premature chromosome condensation (PCC) in the injected male nucleus (M) resulting in the formation of a second metaphase plate. (**D**) The delivery of an oocyte-activation signal leads to the elimination of the MPF and CF activities leading to the entry of both the female (F) and the male (M) chromosomes into anaphase followed by the extrusion of the oocyte polar body and one set of spermatocyte chromatids (adapted from Tesarik, Rev. Reprod., 1, 149-152, 1996).

injected spermatozoon is deficient in cytosolic factors responsible for the oscillator function.

Interestingly, normal oocyte activation can be achieved even with spermatozoa carrying subthreshold quantities of the oscillator, by enhancing the force of the artificial trigger substitute. This was shown both experimentally, by the demonstration that the onset of calcium oscillations after ICSI can be substantially accelerated by oocyte treatment with a calcium ionophore (24), and clinically, by the achievement of normal fertilization and pregnancy with ionophore-treated oocytes injected with spermatozoa deficient in the cytosolic oocyte-activating factors (25).

The nature of the sperm cytosolic factors, exerting the oscillator function, is still incertain. In spite of the identification of a highly conserved mammalian protein, named oscillin, that was claimed to induce calcium oscillations in oocytes (26), subsequent experiments questioned the role of this protein and suggested that the oscillator function is due to another, yet unknown molecule which is associated with the same fraction of sperm extracts from which the supposed oscillin has been isolated (27). Other recent studies suggested another candidate for the soluble oocyte-activating factor from mammalian spermatozoa - a truncated c-kit receptor (tr-kit) (28, 29) lacking the tyrosine kinase activity of the complete c-kit molecule and supposed to stimulate Ca^{2+} release from oocyte intracellular stores through the inositol 1,4,5-trisphosphate ($InsP_3$) receptor/Ca^{2+} channel by activating phospholipase C 1 which produces $InsP_3$ by hydrolysis of phosphatidylinositol-4,5-bisphosphate (30,31). However, a direct relationship between tr-kit and calcium oscillations remains to be demonstrated.

In addition to causing fertilization failure, abnormalities of oocyte activation can have developmental consequences reaching far beyond fertilization. By analogy with experimental data obtained in animal models (32-34), truncated calcium signals in human oocytes at fertilization are suspected to compromise the completion of meiotic division (18) and even to cause chromosome non-disjunction leading to aneuploidy (35). In fact, the failure of the second meiotic anaphase, leading to the retention of the second polar body and triploidy, is a relatively frequent anomaly in human ICSI (36), and some of the *de novo* arising numerical chromosomal abnormalities described in babies resulting from ICSI (37) may be due to abnormalities of the oocyte activation signal (35).

3.1.4. Manipulation of male germ cell ploidy

The finding that spermatozoa, as well as less mature germ cells, can be introduced into oocytes by a micromanipulation technique without triggering oocyte activation (38, 39) was at the origin of experimental studies in which ploidy of immature male germ cells was deliberately reduced within injected oocytes (figure 2). This was first achieved by injecting mouse secondary spermatocyte nuclei (2N haploid) into mouse oocytes (39). In the absence of oocyte activation, the injected oocytes maintained high levels of metaphase-promoting factor (MPF) which drove the injected spermatocyte nuclei to metaphase. The oocytes thus possessed two distinct metaphase plates, one with the oocyte's own chromosomes and the other with chromosomes originating from the injected spermatocyte nucleus. When the oocytes were subsequently activated by an electric discharge, the resulting drop of MPF activity led to the entry of both sets of chromosomes to anaphase. One set of spermatocyte chromatids was thus extruded from the, and the resulting zygotes were diploid, some of them developing into normal progeny (39).

More recently, a similar strategy was used in the mouse model to obtain normal diploid zygotes by using even less mature male germ cells, primary spermatocytes (4N diploid), that need to undergo two rounds of meiotic reduction before participating in syngamy with the oocyte. This was done either by injecting primary spermatocyte nuclei into immature (4N diploid) oocytes, waiting for the two spontaneous meiotic divisions of both the oocyte and the spermatocyte chromosomes (40) or by carrying out the first round of spermatocyte chromosome reduction in helper mature (2N haploid) oocytes with the subsequent removal of the reduced set of spermatocyte chromosomes and its

reinjection into another mature oocyte which was then treated as in the case of the injection of secondary spermatocyte nuclei (41). In both studies, fertilization and early embryonic development were achieved. However, none of the 61 transferred embryos implanted in the former study (40), whereas the latter study resulted in only two live births out of 258 transferred embryos (41). These low birth rates appear to be due to other factors (mainly of epigenetic nature) than errors of chromosome and chromatid partitioning during the artificial reduction of germ cell ploidy. The application of these techniques in human reproductive medicine, in the treatment of cases of meiotic and pre-meiotic spermatogenesis arrest awaits further evaluations concerning efficacy and safety.

3.2. Clinical efficacy

3.2.1. Ejaculated spermatozoa

Unlike previous methods of micromanipulation-assisted fertilization, studies comparing fertilization and pregnancy outcomes of ICSI performed with ejaculated spermatozoa for patients with different types of sperm pathology did not reveal any significant differences between patients with oligozoospermia, asthenozoospermia, teratozoospermia or their combinations, nor was there any relationship with the severity of each of these pathologies (42, 43). This does not appear suprising because, with the exception of oligozoospermia, the definitions of sperm pathology currently in use and the relating of their severity are based on the evaluation of the percentage of abnormal spermatozoa in the overall sperm population. Because every effort is usually done in ICSI to select the best-appearing spermatozoa for injection into oocytes, it is little important whether the overall sperm sample has as many as 99% abnormal spermatozoa; in fact it will always be the remaining 1% of normal-appearing spermatozoa from which those to be injected will be recruited.

The situation may be different when no normal-appearing spermatozoa can be found (44). This is especially true in cases in which 100% of spermatozoa are immotile because a living immotile spermatozoon cannot be distinguished easily from a dead spermatozoon. However, a relatively simple test of sperm viability, based on a specific deformation of living spermatozoa in hypo-osmotic solutions, can be applied with success in these cases (45, 46).

3.2.2. Epididymal spermatozoa

Epididymal spermatozoa, obtained by microsurgical (47) or percutaneous (48) epididymal sperm aspiration from patients with obstructive azoospermia can be used for ICSI, leading to fertilization and pregnancy outcomes identical to those achieved with ejaculated spermatozoa (49).

3.2.3. Testicular spermatozoa

In cases of obstructive azoospermia, and in many cases of nonobstructive azoospermia, spermatozoa for ICSI can be recovered directly from the testis, either surgically (50) or percutaneously, by fine-needle aspiration (51). Globally, ICSI results in these cases are similar to those with ejaculated spermatozoa (49). However, in cases of nonobstructive azoospermia with severe impairment of spermatogenesis, some authors reported lower success rates as compared to other ICSI indications. This is especially due to higher spontaneous abortion rates (52), whereas fertilization and pregnancy rates tend to be similar. Because nonobstructive azoospermia is merely a symptom, not a disease, and can be caused by a number of different aetiologies, the establishment of a relationship between each of these aetiologies, the gravity of testicular damage, and sperm performance in ICSI is a challenge for future clinical studies.

4. REAL AND POTENTIAL HEALTH HAZARDS

4.1. Real hazards

An increased risk of some health problems in babies resulting from the clinical application of ICSI, as compared to general population, has now been documented empirically. Most of these risks appear to be related to the dramatic enhancement of fertility in spontaneously infertile or subfertile men rather than to the manipulation itself.

4.1.1. Transmission of gene microdeletions/mutations causing infertility

Problems of spermatogenesis are often associated with microdeletions in the long arm of the Y chromosome, where several genes supposed to be involved in spermatogenesis have been identified. These include members of the *RBM* (RNA-binding motif) gene family (53) and another gene, also possessing an RNA-recognition motif, which has been termed *DAZ* (deleted in azoospermia) (54). If a spontaneous microdeletion or mutation occurs in some of these genes, leading to a substantial impairment of natural reproductive capacity of the given individual, ICSI can serve a vector of propagation of these modified genes to the male (but not the female) progeny. The failure of a clear relationship between the size and chromosomal location of Y-chromosome microdeletions, on the one hand, and the severity of spermatogenic impairment, on the other hand (55), can be partly explained by the recent discovery of an autosomal spermatogenesis-controlling gene, called *DAZLA* (DAZ-like autosomal), on the human chromosome 3 (56, 57). Unlike the Y-chromosome-associated genes, *DAZLA* is also intimately implicated in the development of the female gonad. Thus, trasmission of *DAZLA* anomalies by means of ICSI may compromise fertility of not only the male but also the female progeny. Further research into the potential association between *DAZLA* and human infertility is warranted.

4.1.2. Chromosomal abnormalities

A slight but significant increase in the incidence of numerical and structural anomalies concerning sex chromosomes has been documented in children resulting from ICSI (58). In many cases, this is apparently due to transmission of a chromosomal abnormality from the father or the mother. However, *de novo* sex chromosome abnormalities have also been described after ICSI (37). The possibility that these *de novo* chromosomal abnormalities can be due to some attributes of the ICSI technique, such as

the modified pattern of oocyte activation (15, 17) or an abnormal behaviour of the sperm centrosome (59-61), has been pointed out (35) and is now under investigation.

4.2. Potential hazards

Some concern has been raised as to the quality of developmental processes in embryos resulting from micromanipulation-assisted fertilization. However, these concerns are merely based on theoretical speculation and are not substantiated by any empirical data. However, they certainly merit attention and should orient our research effort in appropriate directions. Most of these concerns relate to the hypothetical perturbation of genomic imprinting mechanisms (processes occurring during gametogenesis and conditioning certain genes for exclusive or preferential expression from the paternal or from the maternal allele in some or all tissues). This is particularly relevant when incompletely mature male gametes (epididymal and testicular spermatozoa, spermatids, spermatocytes) or female gametes (germinal vesicle or metaphase I oocytes) are used (62). Even though animal experiments did not reveal any pathologies resembling those caused by imprinting abnormalities in either of these situations, this issue deserves further attention.

Developmental abnormalities during early stages of embryogenesis can also result from abnormal oocyte activation (34), which is not an uncommon situation when defective or immature gametes are used for human reproduction (18). However, such abnormalities are likely to lead to implantation failure or to an early pregnancy loss rather than to the development of an abnormal child.

5. NEW DEVELOPMENTS AND FUTURE DIRECTIONS

Some men with nonobstructive azoospermia due to defective spermatogenesis do produce early stages of germ cells, but these cells fail to mature and remain arrested at various stages of spermatogenesis. Most current efforts in the treatment of severe male infertility are aimed at the determination of the optimal strategy for assisted reproduction in these cases. The use of postmeiotic germ cells that have not yet entered the phase of spermiogenesis (round spermatids) for assisted reproduction has been predicted by Edwards et al. (63), based on successful animal experiments performed in the mouse (64). The completion of meiotic reduction in round spermatids avoids the need for an artificial manipulation of ploidy (see above), which is a prerequisite for successful conception with premeiotic and meiotic cells. The absence of sperm devices that serve for sperm penetration into the oocyte and that develop later in spermiogenesis (flagellum, acrosomal cap) is not a major problem when immature germ cells are introduced into oocytes by micromanipulation. The birth of normal babies after round spermatid injection (ROSI) into human oocytes demonstrated the feasibility of this method (65-67), which has later been confirmed by other studies (68, 69). However, current success rates of human ROSI still remain low, mainly because of the poor viability of round spermatids that can be recovered from patients with complete spermiogenesis failure (68, 70). Apoptosis of germ cells from patients with spermatogenic arrest at the round spermatid stage appears to be the main problem of ROSI (71).

In the mouse model, successful fertilization and birth were also achieved with secondary spermatocytes (39) and even with primary spermatocytes (40, 41). The problem of ploidy, arising from the use of meiotic and premeiotic male germ cells can be resolved by an artificial manipulation of germ cell ploidy (see above). However, the application of these methods to the treatment of human male infertility will be burdened by the same problem of germ cell apoptosis which already represents the main obstacle to the successful use of round spermatids (71). In spite of an isolated report on the successful use of secondary spermatocytes for human reproduction (72), the question of repeatability and efficacy of this method in its human application still remains open.

On the other hand, *in vitro* culture of human testicular cells can lead to transmeiotic differentiation of premeiotic and meiotic cells (primary and secondary spermatocytes), resulting in the formation of round spermatids that can further undergo certain phases of elongation resembling *in vivo* spermiogenesis (73, 74). If confirmed for a larger group of patients with spermatogenic arrest, *in vitro* spermatogenesis followed by the use of meiotically mature cells (spermatids) for assisted reproduction may be a more promising approach than a direct use of immature germ cells because the selection of non-apoptotic cells will be facilitated by assessing their *in vitro* development.

Last but not least, interspecies transplantation of spermatogenic stem cells into testicular seminiferous tubules, leading to the resumption of *in vivo* differentiation of the transplanted cells in the host testis has been described (75). Whereas interspecies transfer of human spermatogenic cells may be questioned from the point of view of ethics, the use of similar techniques can be envisaged for reimplantation of patient's own spermatogenic cells following *ex vivo* therapy. Thus explanted germ cells can be treated *in vitro* to repair a genetic or another defect, followed by reimplantation to the patient's testis. Even though this would obviate the need for the use of a host animal, germline gene therapy is currently banned in virtually all European countries. If ethical and technical issues questions associated with these techniques are resolved, their application would be very interesting because it would aim at the restoration of natural male fertility. This would be a step in the opposite direction to the tendency of distancing the procreative act from its natural framework, which currently prevails in the world of human assisted reproduction.

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