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Analysis of the oocyte activating capacity and chromosomal complement of round-headed human spermatozoa by their injection into mouse oocytes

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Intracytoplasmic sperm injection (ICSI) in the human is a very effective procedure which allows the fertilization of the majority of oocytes even in cases of extreme oligoasthenoteratozoospermia. Round-headed acrosomeless human spermatozoa, however, form an exception to this rule, because in about half of the couples with globozoospermia all oocytes remain unfertilized after injection. The incapacity of the spermatozoon to activate the oocyte following injection of round-headed spermatozoa could be the underlying mechanism. To investigate this hypothesis, activation rates of mouse oocytes injected with spermatozoa from a patient with globozoospermia were compared with those obtained after injection with normal spermatozoa. Of mouse oocytes surviving the injection with donor spermatozoa, 95% underwent activation, compared to none of the 88 mouse oocytes surviving the injection with round-headed spermatozoa. After fixation, prematurely condensed sperm chromosomes were found in these oocytes. Parthenogenetic activation of mouse oocytes (8% ethanol at 40 min after injection) injected with round-headed spermatozoa led to the activation of 96% of oocytes. These oocytes developed normally to the first mitosis and were fixed for analysis of the sperm karyotypes. The incidence of chromosomal abnormalities of round-headed spermatozoa (6%) was similar to that in spermatozoa from a fertile donor (9%). These data provide further information on the basic defect in cases of globozoospermia and demonstrate that globozoospermia is not associated with sperm karyotype abnormalities.

Key words: globozoospermia/heterospecific fertilization/oocyte activation/sperm karyotype

Introduction

Round-headed spermatozoa syndrome or globozoospermia is a rather rare congenital disorder observed in ~0.1% of all andrological patients (Schill, 1991). In the most severe cases, globozoospermia is characterized morphologically by the spherical appearance of the sperm heads and by the absence of the acrosome and postacrosomal sheath in all spermatozoa. These spermatozoa can also have abnormal mitochondria and midpieces as well as abnormal nuclear membranes (Schirren *et al.*, 1971; Pedersen and Rebbe, 1974). Biochemically, they are characterized by the absence or reduced activity of acrosin, which is an acrosome protease, and of calicin, a cytoskeletal protein (Florke-Gerloff *et al.*, 1984; Longo *et al.*, 1987; Lalonde *et al.*, 1988; Escalier, 1990; Courtot, 1991). Round-headed acrosomeless spermatozoa are incapable of binding to and penetrating zona pellucida-free hamster oocytes, but they are able to undergo nuclear decondensation if incubated in a lysate of hamster oocytes or able to form male pronuclei if injected into hamster oocytes (Weissenberg *et al.*, 1982; Syms *et al.*, 1984; Lanzendorf *et al.*, 1988). In experiments with human oocytes it was reported that round-headed sperm cells are unable to bind to human zona pellucida (Aitken *et al.*, 1990), as well as unable to bind to or fuse with human oocyte membrane when injected subzonally (Dale *et al.*, 1994). The fertilization rate after intracytoplasmic sperm injection (ICSI) is reduced or zero (Liu *et al.*, 1995), but a few pregnancies with ICSI have been reported (Lundin *et al.*, 1994; Bourne *et al.*, 1995; Liu *et al.*, 1995; Trokoudes *et al.*, 1995). These data suggest that round-headed spermatozoa lack the capacity to penetrate oocytes and may also be deficient in their oocyte-activating capacity. The subject is complicated, however, by the fact that some patients can have a heterogeneous population of spermatozoa, in which round-headed acrosomeless spermatozoa are mixed with relatively normal ones (Florke-Gerloff *et al.*, 1984; Lanzendorf *et al.*, 1988). On the other hand, the current knowledge about the chromosomal complement of spermatozoa of male infertility patients is limited. Fluorescent in-situ hybridization (FISH) provides information on numerical but not structural chromosomal abnormalities in these spermatozoa. The use of the hamster oocyte fertilization test is limited to spermatozoa that are able to fuse with these oocytes (Moosani *et al.*, 1995). Recently we have demonstrated that mouse oocytes can be used for analysis of the oocyte-activating capacity and karyotyping of human spermatozoa (Rybouchkin *et al.*, 1995a). In the present study we used this model to analyse the reproductive characteristics of round-headed human spermatozoa.

Materials and methods

Semen characteristics and preparation for injection

The semen sample from a 36 year old patient had the following characteristics: volume, 4.2 ml; concentration, 18×10^6/ml; round-headed spermatozoa, 91% (72% 'small-headed' and 18% 'big-headed' with diameters of 3.6 and 4.3 μm respectively); spermatozoa with slightly pear-shaped heads, 9%; total motile spermatozoa, 39%. The

gelatin halo test showed complete absence of acrosin, and triple staining proved the absence of an acrosome in all spermatozoa The spermatozoa that were injected into mouse oocytes were recovered either from the fresh semen sample or after thawing of frozen samples Cryopreservation of the spermatozoa was performed according to Prins and Weidel (1986) using a TEST–yolk buffer (Irvine Scientific, Santa Ana, CA, USA) as a cryoprotectant. After washing twice in M2 medium (Fulton and Whittingham, 1978), fresh or thawed semen aliquots were resuspended in calcium-free M2 medium (M2CF) and motile sperm cells were recovered by a 'side-migration' procedure (Dozortsev *et al.*, 1996). Motile, debris-free spermatozoa were transferred to a drop of M2CF medium under mineral oil and an equal volume of injection medium [8% polyvinylpyrrolidone (PVP), 360 kDa in M2CF] was added to a final concentration of 3–4% PVP

Semen samples from a fertile healthy donor were used as a positive control and were treated in the same way. Negative controls to assess the occurrence of oocyte activation by the injection procedure itself were prepared from aliquots of washed donor spermatozoa which were treated twice with unprotected freezing in liquid nitrogen and thawing at 37°C, followed by washing in calcium-free phosphate buffer and incubation at 90°C for 30 min. They were finally washed in M2CF medium and resuspended in a 4% PVP solution in M2CF

Oocyte collection, injection and treatment

Mouse oocytes were recovered from superovulated hybrid female F1 B6/D2 mice (IFFA CREDO, Les Oncins, France). Superovulation was induced by s.c. injection of 8 IU of pregnant mares' serum gonadotrophin followed by 1 p. administration of 8 IU human chorionic gonadotrophin (HCG) 48 h later Animals were killed by cervical dislocation 13–13.5 h after HCG administration Oocytes were cleaned from cumulus cells by 6 min incubation in M2 medium with 100 IU/ml hyaluronidase (Sigma, Borneu, Belgium) and washed in three drops of plain M2 medium. They were further incubated before and after sperm injection in M2 medium in a humidified thermostat at 37°C.

The injection of mouse oocytes with human spermatozoa was done at 17–23 h post-HCG administration, since, according to our preliminary results, at this time the oocytes are more resistant to the injection procedure. Immediately before injection, groups of 15 oocytes were transferred into a drop of M2 medium at 17°C for a 15 min preincubation (Kimura and Yanagimachi, 1995), and the injection, which never took longer than 30 min, was also performed at this temperature. The injection needle had inside and outside diameters of 6 and 8 μm respectively, and had a sharp spike, which was prepared under an inverted microscope with ×400 magnification, using a heating filament under the control of an MF-9 microforge (Narishige, Tokyo, Japan). The holding pipette had inside and outside diameters of 5 and 90 μm respectively. The small opening of the holding pipette was important to ensure a high survival rate of the mouse oocytes during sperm injection. The patient's motile round-headed spermatozoa as well as those of the positive control were immobilized by touching their tails with the injection needle and were injected into the oocytes with the smallest amount of injection medium possible After injection, the oocytes were kept in M2 medium for 10 min at 17°C and 20 min at 25°C, after which they were transferred for further incubation in M2 at 37°C After 15 min incubation at 37°C, some of the groups of oocytes were exposed to 8% ethanol solution in M2 medium for 4 min at 25°C, washed in several drops of M2 medium and returned to the incubator In one experiment the mouse oocytes were injected with donor and round-headed spermatozoa, resuspended in the injection medium containing 1.7 mM of calcium at 37°C, to analyse the influence of these conditions (which are close to the conditions of ICSI in our clinical laboratory) on the activation rate of the injected oocytes

Cytological and cytogenetic analysis

Mouse oocytes injected with patient's or donor spermatozoa were scored for pronuclei formation between 10 and 16 h after injection and those with clearly visible, well developed pronuclei were considered to be activated The oocytes without pronuclei were fixed and analysed to assess the presence of sperm chromosomes The zona pellucida was removed from the oocytes by incubation in a 0.5% pronase solution for 6 min immediately before fixation. Zona pellucida-free oocytes were transferred in groups of 4–6 into a 75 mM KCl solution for an 8–10 min hypotonic treatment. Precooled (–25°C) fixative (1–2 ml; 3 1, methanol:acetic acid) was transferred from a stock solution to a watchglass (precooled at the same temperature) at the end of the hypotonic treatment and kept at room temperature for ~2 min Oocytes were fixed one by one by transferring them into the watchglass with fixative with a small quantity of hypotonic solution This was done under direct visual control using a dissecting microscope After 5–20 s fixation, the swollen oocytes were transferred with a small amount of the fixative to a slide, close to a mark made on the glass by a diamond pencil. After the fixative started to dry on the slide, attention was paid to the signs of oocyte spreading. If no such signs were seen, an additional quantity of fixative was added to the slide close to the oocyte This addition of fixative, however, had to be gentle and not excessive, to prevent oocyte detachment and movement out of the field or tearing of the oocyte cytoplasm, with breaking of the metaphases. Three to five additional drops of fixative were usually enough to obtain a satisfactory final spreading of the oocyte cytoplasm

The oocytes that had displayed two pronuclei after injection with control or patient's spermatozoa and activation by ethanol were also fixed in the same way, the only difference being that they were incubated several hours before pronuclear disappearance in medium with 0.2 μg/ml nocodazole to prevent mouse and human pronuclear joining and the formation of a common metaphase plate Preliminary experiments revealed that human chromosomes undergo condensation quickly in prometaphase-arrested mouse zygotes, forming tight clumps which are difficult to spread even after long incubation in a hypotonic solution Hence, starting from 14 h after ethanol treatment, zygotes incubated in nocodazole were checked every 30–40 min for the disappearance of pronuclei, and those without pronuclei were fixed 1–1 5 h afterwards.

G-banding of chromosomes and inclusion criteria

Fresh metaphase spreads were treated for 10 min at 100°C followed by incubation in 1× sodium chloride/sodium citrate (SSC) at 50°C for 2–8 min, depending on the age of the spreads (from 1 to 8 days) Afterwards the slides were air-dried and treated with a 0 1% trypsin solution (Difco, Detroit, MI, USA, 1:250) in a phosphate buffer with pH 6 8 for 1–2 s at 37°C Finally, the slides were washed with distilled water, air-dried and stained (under an inverted microscope at ×400 magnification) with a drop of fresh Wright stain solution (Sigma, W3000)

The ISCN (1985) criteria were used for the assessment of chromosome aberrations, but centromere gaps were not recorded as aberrations, since they are considered to be normal for pronuclear chromosomes (Chernos *et al*, 1986; Navarro *et al.*, 1987) If hypoploidy was found in both the mouse and human chromosomal sets in the same oocyte, this was considered to be an artefact and the oocyte excluded from the results.

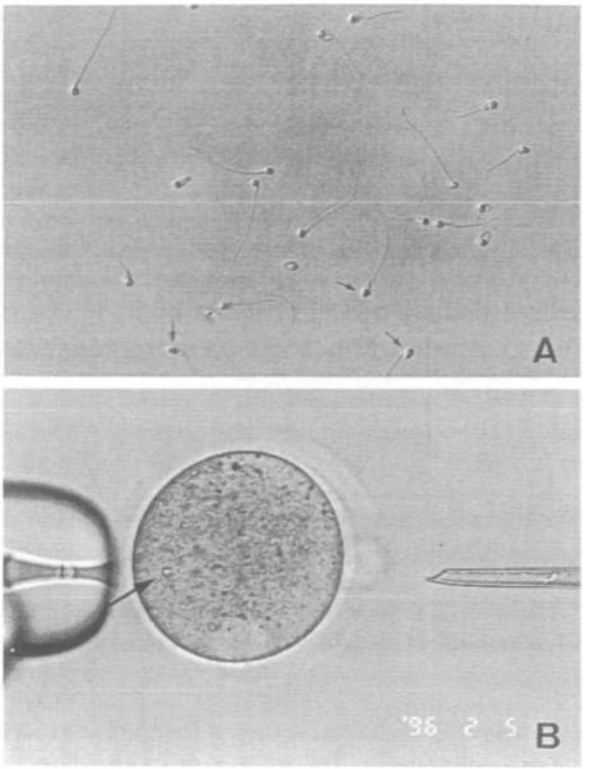

Figure 1. (**A**) Round-headed human spermatozoa observed under Nomarski optics. Note the pear-shaped head morphology in some of the spermatozoa (marked by arrows). (**B**) Mouse oocyte with injected sperm cell (marked by arrow). Original magnification ×400 for both pictures.

Results

The different forms of the globozoospermic patient's spermatozoa were injected into 118 mouse oocytes (Figure 1A,B). None of the 88 mouse oocytes that survived ICSI with spermatozoa from the globozoospermic patient underwent activation during the next 16 h of culture. In contrast, 61 out of 75 mouse oocytes injected with donor spermatozoa survived, and 58 (95%) of these underwent activation with formation of one or two pronuclei and extrusion of the second polar bodies. Of 50 oocytes injected with inactivated spermatozoa (negative control), 44 survived and three formed pronuclei at 10 h post-injection (Table I). To reproduce more closely the conditions of our clinical ICSI procedure, some round-headed spermatozoa were injected into mouse oocytes at 37°C, after resuspension in injection medium containing calcium. The survival rate in this experiment was 50% and three of 18 surviving oocytes underwent presumably parthenogenetic activation (data not shown).

Ethanol stimulation of mouse oocytes surviving the injection with human spermatozoa induced activation in the large majority of oocytes, independently of the origin of the injected spermatozoa (Table II). The size of developed pronuclei or the timing of progression of the zygotes to the first mitosis was also not different between oocytes injected with round-headed or normal spermatozoa.

Cytogenetic analysis of mouse oocytes which were not activated after injection of either patient or donor spermatozoa revealed prematurely condensed G_1 sperm chromosomes (chromatids) along with metaphase II mouse chromosomes in all cases (Figure 2A). Interestingly, in some oocytes the sperm chromatids were scattered so well that it was possible to count their number after fixation and staining. Nevertheless, the number of such spreads was not large enough for quantitative analysis of sperm karyotype abnormalities at this stage. From the 220 mouse oocytes injected with round-headed and normal spermatozoa followed by ethanol stimulation, 83 karyotypes were obtained. Analysis of G-banded sperm karyotypes obtained after fixation of the oocytes at the first cleavage division (Figure 2B) revealed that most of the round-headed human spermatozoa had a balanced karyotype, the number of structural chromosomal aberrations not being higher than in the positive control (Table III).

Of 50 sperm karyotypes obtained after injection of round-headed human spermatozoa into mouse oocytes, 11 (22%) showed centromeric 'gaps' in one or more chromosomes. The 'gaps' were most often found in chromosome 16 (nine karyotypes; Figure 2b), chromosome 7 (five karyotypes) and occasionally in chromosomes 10 and 8 (two and one karyotypes, respectively). The 'gaps' varied in size from about one to five chromatid widths and were recognized under phase-contrast observation of air-dried SSC-treated spreads as dark spots. They also stained dark by Wright stain if observed under air-dry objectives, but appeared as poorly visible bluish threads or spots if observed under oil-immersion objectives. The same kind of 'gaps' were found in karyotypes of human spermatozoa retrieved from the healthy donor, albeit at a smaller rate (9%) (Table III). Analysis of mouse oocyte chromosome complements after injection of round-headed spermatozoa and ethanol stimulation revealed that only two out of 51 complements were aneuploid with 19 chromosomes in both.

Note added

After the manuscript had been submitted, a clinical ICSI trial was performed with the patient described and his partner, since no relationship between the reactions of mouse and human oocytes to ICSI with the same spermatozoa have yet been established. None of the four human oocytes injected with round-headed spermatozoa was fertilized, demonstrating the good association between results from mouse and human oocytes in response to injection with round-headed human spermatozoa. Three other oocytes were injected with round-headed spermatozoa and then twice with 0.1 M calcium to induce activation. Two of them survived the injection and underwent activation, but formed zygotes with one and three pronuclei.

Discussion

The aetiology of globozoospermia is unknown, but is probably of a polygenic and polymorphic origin (Florke-Gerloff *et al.*, 1984). Further investigation of this phenomenon could give valuable information on general mechanisms of spermatogenesis as well as on different aspects of sperm–egg interaction. Analysis of the oocyte-activating capacity and karyotype of these spermatozoa is also of important therapeutic relevance. Estimation

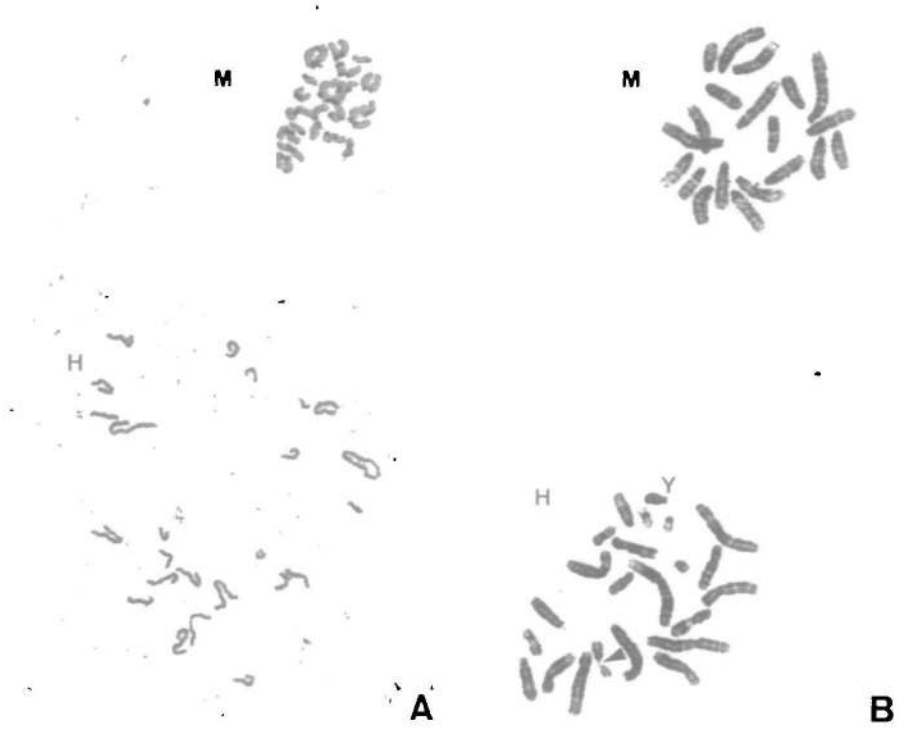

Figure 2. (A) Well scattered prematurely condensed human sperm chromosomes (H) along with mouse oocyte chromosomes (M) obtained after fixation of an unactivated mouse oocyte injected with a round-headed spermatozoon (24 chromatids can be counted on the picture, presuming either disomy or a chromatid strand break in this spermatozoon). Original magnification ×400. (B) Human (23,Y) (H) and mouse (20) (M) metaphase chromosomes obtained by fixation of a mouse oocyte at the first cleavage division after its injection with a round-headed human spermatozoon followed by ethanol stimulation (The arrowhead indicates the typical small centromere gap in human chromosome 16) Original magnification ×1000

Table I. Mouse oocyte activation after intracytoplasmic injection of human spermatozoa from a patient with globozoospermia and from a healthy donor

Sperm source[a]	Head morphology	No of oocytes injected	No of oocytes survived	No of oocytes activated			
				Total (%)	1PN	2PN	3PN
Patient	round, small	74	55	0 (0)	–	–	–
	round, big	23	16	0 (0)	–	–	–
	pear-shaped	21	17	0 (0)	–	–	–
Donor (+)	normal	75	61	58 (95)	6	52	0
Donor (–)	normal	50	44	3 (7)[b]	1	2	0

[a](+) = positive control initially motile spermatozoa were injected, (–) = negative control frozen–thawed and heat-treated spermatozoa were injected
[b]The difference in oocyte activation rates between negative controls and round-headed spermatozoa with small heads was significant at $P < 0.05$
PN = pronuclear

Table II. Mouse oocyte activation after intracytoplasmic injection of round-headed and normal human sperm followed by ethanol exposure

Sperm source[a]	No of oocytes injected	No of oocytes survived	No of oocytes activated			
			Total (%)	1PN	2PN	3PN
Patient	130	97	93 (96)	5	84	4
Donor (+)	90	82	82 (100)	18	62	2
Donor (–)	74	50	42 (84)	4	34	4

[a](+) = positive control initially motile spermatozoa were injected, (–) = negative control· frozen–thawed and heat-treated spermatozoa were injected
PN = pronuclear

of the oocyte-activating capacity of the round-headed human spermatozoa can hardly be done by injection of these sperm cells into hamster oocytes (Lanzendorf *et al.*, 1988), since activation of hamster oocytes can be rather easily induced by the injection procedure itself (Uehara and Yanagimachi, 1977). Hamster oocytes are also probably not useful for karyotyping of human spermatozoa after the intracytoplasmic injection, since only a low number of human sperm karyotypes per 100 hamster oocytes injected has been reported so far (Martin *et al*, 1988). We recently developed the mouse oocyte activation test for evaluation of the oocyte-activating capacity and karyotyping of human spermatozoa (Rybouchkin *et al.*, 1995a). In the present study we used this test to analyse the reproductive characteristics of round-headed spermatozoa obtained from a patient with globozoospermia.

Our results revealed that round-headed spermatozoa are deficient in oocyte-activation capacity and that this deficiency is independent of the variations in morphology of the sperm heads. The most probable reason for this deficiency is the absence or down-regulation of the sperm-associated oocyte-activating

Table III. Cytogenetic findings in mouse oocytes injected with human spermatozoa, displaying two pronuclei after ethanol stimulation and fixed at the first cleavage division

Complements	Patient	Donor
Normal	36	27
One or more centromeric 'gaps'	11	3
Total (%)	47 (94)	30 (91)
Abnormal		
Numerical	46,YY	0
Structural	23,Y,ctb(4)(p)chte(2;4)(p;q)tr, sym,incompl	23,Y,csb(1)(32p)+ace
	23,Y,+min	23,X,csb(9)(11q) 23,Y,+ace
Total (%)	3 (6)	3 (9)
Total analysed (X/Y)	50 (28/22)	33 (18/15)

ctb = chromatid break, csb = chromosome break, chte = chromatid exchange, tr = triradial, sym = symmetrical, min = minute fragment, ace = acentric fragment; p = short arm, q = long arm.

factor (SAOAF) in these spermatozoa. The presence of SAOAF in human spermatozoa, which is capable of inducing cytosolic free calcium oscillations and oocyte activation after ICSI, was recently demonstrated (Homa and Swann 1994; Dozortsev *et al.*, 1995). An oligomeric protein 'oscillin', which is capable of inducing calcium oscillations after injection into mouse oocytes, was recently isolated from cytosolic extracts of hamster sperm cells. It has a monomer molecular mass of ~33 kDa and was proposed as a hamster homologue to the human SAOAF (Parrington *et al.*, 1996). It would be very interesting to analyse the presence of oscillin in round-headed human spermatozoa. An association between the absence of this protein and the absence of acrosin and calicin in these spermatozoa (Longo *et al.*, 1987; Lalonde *et al.*, 1988; Escalier, 1990; Courtot, 1991) would suggest an abnormality in the factor(s) regulating the expression of a whole number of genes during the very late stages of spermatogenesis in patients with globozoospermia. Indeed, our finding of mostly normal karyotypes in round-headed spermatozoa implies that the processes of meiotic chromosome segregation develop normally and suggests the absence of any pathological effects on the spermatocyte stage of spermatogenesis. This is in agreement with morphological findings on testicular biopsy specimens of globozoospermia patients (Kullander and Rausing, 1975).

Another possible explanation for the total absence of mouse oocyte activation after injection of round-headed human spermatozoa could be the presence of an oocyte activation inhibitor(s) in the patient's spermatozoa (or seminal fluid). The careful washing of the patient's spermatozoa and the high rate of mouse oocyte activation with ethanol after injection of the round-headed human spermatozoa, however, makes this explanation very unlikely. The fact that some of the negative controls were activated could indicate that at least part of SAOAF is anchored on the sperm cell nucleus, so that repeated unprotected freezing–thawing and heat treatment do not remove it completely. Further biochemical characterization of human SAOAF and hamster oscillin will clarify this matter.

Whether or not globozoospermia is invariably associated with complete SAOAF deficiency is difficult to assess yet. Fertilization rates of up to 50% after ICSI of human oocytes with round-headed spermatozoa have been reported (Lundin *et al.*, 1994; Liu *et al.*, 1995; Trokoudes *et al.*, 1995). This could be compatible with the fact that different types of globozoospermia have been described (Florke-Gerloff *et al.*, 1984; Lanzendorf *et al.*, 1988; Singh, 1992). On the other hand, it is also possible that oocytes of some patients are more prone to parthenogenetic activation induced by the injection procedure itself. Indeed, in the mouse there are strain-specific differences in the susceptibility of oocytes to undergo parthenogenetic activation, as described by Marcus (1990) and confirmed by Rybouchkin *et al* (1996). Our results on the increased activation rates of mouse oocytes after injection under conditions close to those of our clinical ICSI procedure also support the idea that these conditions are favourable for the induction of parthenogenetic activation. ICSI of spermatozoa obtained from different globozoospermic patients into a homogeneous population of mouse oocytes could help to clarify this question.

Many reports have recently been published on the analysis of human sperm karyotypes using the hamster zona-free oocyte system (Martin *et al.*, 1995; Moosani *et al.*, 1995; Tusell *et al.*, 1995), but much less is known about sperm karyotypes after ICSI into oocytes. This topic is of great clinical relevance because of the increasing use of this procedure in assisted reproduction in humans. To our knowledge, no other work has been done since that of Martin *et al.* (1988) on human sperm karyotypes after ICSI. Hamster oocytes, however, are vulnerable, resulting in a low number of the injected oocytes developing to metaphase (Lanzendorf *et al.*, 1988; Martin *et al.*, 1988; our unpublished observations). With mouse oocytes, an average of 35 karyotypes per 100 injected oocytes can be expected. This model is therefore useful for the analysis of human sperm karyotypes, especially in those patients whose spermatozoa are incapable of fusing with hamster oocytes. The incidence of chromosomal abnormalities in the spermatozoa of our patient with globozoospermia was low (6%). According to the data from hamster oocytes, the incidence of all chromosomal abnormalities in human spermatozoa of fertile men is 12–14% (range 0–22%; Martin *et al.*, 1991; Estop *et al.*, 1995; Moosani *et al*, 1995). Whether the incidence we found in this single patient is representative for all cases of globozoospermia remains to be determined, but it appears that globozoospermia is not associated with an increase in numerical or structural chromosomal abnormalities. Our findings in both normal and globozoospermic sperm cells are in contrast to the high incidence of chromosomal abnormalities (42%) after ICSI of human spermatozoa reported by Martin *et al* (1988). One of the possible explanations for this discrepancy could be the prolonged capacitation (1–3 days) of human spermatozoa before injection used by these authors. Indeed, prolonged incubation of human or mouse spermatozoa *in vitro* can lead to a significant increase in structural chromosomal abnormalities (Munné and Estop, 1991, 1993).

In conclusion, the development of a new animal model for the analysis of the different aspects of sperm–egg interaction after gamete fusion (Rybouchkin *et al.*, 1995a) has made it possible to estimate the oocyte-activating capacity of human spermatozoa after ICSI and their karyotype. It was found that some morpholo-

gically 'normal' human spermatozoa of male infertility patients, who had very low or no fertilization after ICSI, have a low or absent oocyte-activating capacity after injection into mouse oocytes (Rybouchkin *et al.*, 1995b). The use of this model has demonstrated that globozoospermia can be associated with the total absence of activating capacity in human spermatozoa but that this is not associated with particular chromosomal abnormalities. These findings point to the existence of an unsuspected type of human sperm pathology, which consists of the inability of the spermatozoon to activate the oocyte. It will be possible to circumvent the absence of oocyte activation in the future by parthenogenetic or oscillin-induced activation of injected human oocytes. Finally, the results obtained with intracytoplasmic injection of human spermatozoa into mouse oocytes also indicate that no increase of sperm or oocyte chromosome abnormalities is induced by the injection procedure itself.

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