Severe oligoasthenoteratozoospermias, secretory and obstructive azoospermias: Motility as a criterion of sperm viability

I. Molina · J. Alfonso · C. C. Duque · L. García-Reboll ·
M. Pérez-Camps · A. Romeu

Received: 15 May 2006 / Accepted: 9 October 2006 / Published online: 16 February 2007
© Springer Science+Business Media, LLC 2007

Abstract *Purpose:* Comparison of pregnancy rates in cases of Secretory Azoospermias (SA), Obstructive Azoospermias (OA) and severe Oligoasthenoteratozoospermias (OATZ). Evaluation of sperm motility as a quality criterion.

Methods: In SA cases ($n = 35$), 9 samples were cryopreserved. In OA cases (epididymal aspiration: $n = 91$; testicular biopsy: $n = 206$), all samples were cryopreserved. 596 OATZ ejaculates were included.

Results: In SA cases, 2 pregnancies were achieved from 9 ICSI cycles. In OA, motile sperm rates were higher in testicular biopsies. After thawing sperm motility was not different between testicular and epididymal origin. 2 pregnancies were achieved with immotile testicular sperm after thawing, but none with immotile epididymal sperm. In OATZ cases, one pregnancy was obtained from the 9 cryopreserved ejaculates and 35.3% with fresh motile sperm.

Conclusions: In SA cases, the use of donor sperm is recommended due to the lower pregnancy rate achieved. Moti-

lity, before and after cryopreservation, as a criterion of sperm viability is discussed and its use should be reconsidered in some cases.

Keywords Epididymal aspiration · Frozen-thawed sperm · Oligoasthenoteratozoospermia · Sperm motility · Testicular biopsy

Introduction

At present, ICSI is the technique used in severe male factor cases, both severe Oligoasthenoteratozoospermias (OATZ) and Azoospermias. However, fertilization and pregnancy rates are lower in Secretory Azoospermias (SA), where severe testicular failure patients are included [1, 2], than in Obstructive Azoospermias (OA).

In spite of different gametic maturation levels and other sperm characteristics, according to pathology and reproductive tract level where spermatozoa are obtained, the criterion used to evaluate their normality in cryopreservation and ICSI is usually sperm motility [3, 4]. Although this criterion was first established for semen samples which were used in conventional IVF [5, 6] or in Artificial Insemination (AI) [7, 8], techniques in which sperm motility is essential. However, motility does not necessary involve sperm normality [9]. In fact, certain immotility cases do not necessarily involve fertilization failure, at least by ICSI [10–12].

So, the aim of this study is to compare the results obtained from the different male pathologies evaluated by pregnancy rates and to analyse and validate the motility criteria in sperm samples depending on pathology, condition (fresh/frozen) and the tract level where they are obtained.

I. Molina (✉) · C. C. Duque · L. García-Reboll · A. Romeu
Human Assisted Reproduction Unit,
La Fe University Hospital of Valencia
46009, Valencia, Spain
e-mail: molina_inmbot@gva.es

J. Alfonso
Reproductive Medicine Institute (IMER)
46009, Valencia, Spain

M. Pérez-Camps
Laboratory of Animal Reproduction and Biotechnology,
Polytechnic University of Valencia,
Spain

Materials and methods

Data were analysed from patients included in the Human Assisted Reproduction Programme of La Fe University Hospital of Valencia from January 2000 to December 2003. The OATZ and azoospermia diagnoses were the inclusion criteria in this study. 35 of the 332 diagnosed azoospermias were defined as Secretory Azoospermias (SA). This group includes severe secretory azoospermias (FSH ≥ 30 mIU/ml), sperm maturation arrest, Sertoli cell-only syndrome, sclerosis, and tubular atrophy cases. Patients with Klinefelter syndrome were not included.

The other 297 were Obstructive Azoospermias (OA). Patients with obstruction due to agenesis, aplasia, infection, trauma and vasectomy were included in this group. Sperm samples were obtained by testicular biopsy in SA cases (for details see below). 9 of the 35 SA which presented at least 1 motile spermatozoon per field were frozen (see below). All of them were thawed and used for intracytoplasmic sperm injection during the studied period.

Percutaneous puncture and epididymal aspiration were performed in OA (91 cases). If it was not possible or there were no motile spermatozoa, testicular biopsy was performed (206 cases). Testicular or epididymal samples with at least 1 motile spermatozoon per field were frozen for their ulterior microinjection.

In the OATZ group, 596 semen samples were included. Of these, 587 were fresh ejaculates and only 9 were ejaculates previously cryopreserved.

When motile sperm were not observed in the ejaculate, or after thawing of samples for subsequent microinjection, cycle cancellation or the use of donor sperm were advised. Obviously, these cases were not included in the present study. However, in the case of patients disagreement, immotile sperm from ejaculate, epididymis or testicle were microinjected.

Treatment testicular biopsy

Local anaesthesia of spermatic cord was achieved with 10 ml of 2% lidocaine clorhidrato solution (Lidocaina Inyectable Braun 2%, B. Braun Medical SA). After a few minutes, a skin weal was raised in the scrotum adjacent to the middle region of the testis, a 1 cm transverse incision was made and the tunica vaginalis space entered. Three incisions of 0.5 cm each then enabled excision of 9 small pieces (3 each) of the seminiferous tubules. A preliminary sample microscopic check at the end of each biopsy avoided unnecessary tissue sampling. After careful cleaning and haemostasis, the tunica albuginea, the vaginal, the scrotum layers and the skin were closed. The procedure took about 20–30 min and was

performed entirely on an outpatient basis, enabling a rapid recovery with minimal complaints and total absence of surgical complications.

Preparation of samples

In TB cases, testicular tissue fragments removed were dislacerated using two scalpel blades in buffered medium (FM, Medicult, Jyllinge, Denmark, N° Cat. 10840500). Presence and sperm motility were evaluated using inverted phase-contrast microscope. In AE cases, presence and sperm motility were evaluated after their removal using inverted phase-contrast microscope.

Finally, in the case of fresh ejaculates, samples were allowed to liquefy for 30–40 min. at room temperature prior to analysis. Sperm count and sperm motility were evaluated. Semen samples were concentrated by centrifugation at 1500–3000 g for 5 min. The supernatant was removed and the pellet resuspended in 50–100 μ l of IVF (Medicult, Jyllinge, Denmark, N° Cat. 10310125). Observation of at least 1 motile spermatozoon was evaluated.

Freezing/Thawing

Sperm samples (TB, EA and ejaculates) were diluted 1:1 in Test Yolk Buffer (Irvine Scientific, Santa Ana, CA, USA, N° Cat. 90128) and stored in 2 mL cryotubes (Irvine Scientific, Santa Ana, CA, USA, N° Cat. 368632–1). Samples were kept at room temperature for 20 min. at 4°C for 20 min. then in nitrogen vapour for 20 min. and were finally immersed and stored in liquid nitrogen.

Samples were thawed, keeping (them) at room temperature. The cryoprotectant was removed by several centrifugations and the final pellet was resuspended in 50–100 μ l of IVF.

Intracytoplasmic sperm injection, embryo culture and embryo transfer were carried out according to standard clinical procedures.

Pregnancy test was carried out by seric β -hCG determination after 12 days of embryo transfer. If the test was positive, pregnancy was confirmed by transvaginal ultrasound after 2 weeks.

Statistical analysis

Sperm recovery rates, sperm motility before and after thawing, and pregnancy rates were analysed in different seminal pathologies (SA, OA, OATZ) and sperm samples (TB, EA, Ejaculate).

Results were analysed by Chi-Square test.

Table 1 Sperm parameters in cases of secretory azoospermias and pregnancy results

	<i>n</i>	%
N° Testicular Biopsies (TB)	35	
N° of TB with spermatozoa (spz)	15	42.8 (15/35)
N° with motile spz/TB with spz	9	60 (9/15)
N° with motile spz/TB (all frozen-thawed)	9	25.7 (9/35)
N° with post-thawing motile spz	6	66.6 (6/9)
N° with post-thawing immotile spz	3	33.3 (3/9)
% pregnancies using motile spz after thawing	1	16.6 (1/6)
% pregnancies using immotile spz after thawing	1	33.3 (1/3)
% pregnancies/TB	2	5.7 (2/35)

Results

A. Secretory azoospermias: Sperm parameters in secretory azoospermias and pregnancies obtained using post-thawing spermatozoa are presented in the Table 1. It may be highlighted that only in 15 of 35 testicular biopsies, spermatozoa were obtained. Motile sperm were found in 9 of the 15 cases and they were motile after thawing only in 6 cases. Both the 6 motile samples and the 3 immotile samples were used in ICSI, obtaining 1 pregnancy in each case (1/6 and 1/3). So, two pregnancies were obtained in 9 ICSI cycles (Table 1).

B. Obstructive Azoospermias: In these cases, sperm was attempted to be obtained firstly from epididymis and from testicle as a final option. This fact allows to evaluate possible differences according to sperm origin, with regard to sperm parameters and pregnancy rates, which are presented in Table 2.

Sperm recovery rate according to intervention (Testicular Biopsy: TB; Epididymal Aspiration: EA) did not present significant differences, although it was slightly higher in TB. However, there are significant differences in motile

sperm recovery rates between testicular biopsy (91.2%) and epididymal aspiration (76.9%) groups. Significant differences were not observed in sperm motility after thawing, depending on sperm origin, even though they seemed to be in favour of TB (TB: 96.7%; EA: 92%). Ongoing pregnancy rate obtained using motile sperm after thawing was lower in EA group, but these differences did not reach levels of significance (TB: 33.3%; EA: 26.1%). Two pregnancies were achieved using immotile testicular sperm after thawing (2/3), but no pregnancy was achieved with immotile epididymal sperm after thawing (0/2) (Table 2).

C. Severe oligoasthenoteratozoospermias (count $\leq 1 \times 10^6$ spz/mL): In OATZ cases, sperm cryopreservation is an usual practice, due to the possible absence of spermatozoa in the ejaculate the day of microinjection. However, as it is shown in Table 3, in the vast majority of cases fresh sperm samples can finally be used (98.5%, 578/587), with pregnancy rates of 35.3% (204/578). Motile fresh, immotile fresh and frozen-thawed sperm samples were used in OATZ cases, but only in 9 cases (9/587) immotile sperm were microinjected, but the only initiated pregnancy was miscarried. ICSI was performed using thawing motile sperm when fresh sperm were not available that day (9/250), but only one pregnancy was achieved (1/9). The low pregnancy rate obtained with thawed sperm and the low number of samples that finally were necessarily thawed, advise us, facing future, to be more restrictive in the storage of this type of samples (Table 3).

Discussion

It has to be outlined that 89% of azoospermias considered were obstructive against secretory azoospermias.

Table 2 Differences in sperm parameters and pregnancy results between testicular biopsy and epididymal punction procedures in obstructive azoospermias

	Obstructive azoospermias		Epididymis	
	Testicle <i>n</i>	%	<i>n</i>	%
Initial cases	206	–	91	–
N° of cases with spermatozoa (spz)	198	96.1 (198/206)	79	86.8 (79/91)
N° with motile spz/cases with spz	188	94.9 (188/198)	70	88.6 (70/79)
N° with motile spz/case	188	91.2 (188/206) ^a	70	76.9 (70/91) ^b
N° with thawing spz	90	47.8 (90/188)	25	35.7 (25/70)
N° with post-thawing motile spz	87	96.6 (87/90)	23	92 (23/25)
N° with post-thawing immotile spz	3	3.3 (3/90)	2	8 (2/25)
% pregnancies using motile spz after thawing	29	33.3 (29/87)	6	26.1 (6/23)
% pregnancies using immotile spz after thawing	2	66.6 (2/3)	0	0 (0/2)

Note. Columns with different superscripts are significantly different ($p < 0.05$).

Table 3 Sperm parameters in cases of severe oligoasthenozoospermias (count $\leq 1 \times 10^6$ spz/mL) and pregnancy results

	<i>n</i>	%
Initial samples	596 (9 + 587)	
N° Frozen-thawed samples	9	3.6 (9/250)
N° of fresh samples with motile spz	578	98.5 (578/587)
Pregnancy rate using motile fresh spz	204	35.3 (204/578)
Pregnancy rate using immotile fresh spz	1	11.1 (1/9)
% frozen-thawing pregnancies	1	11.1 (1/9)

Regarding SA, only two pregnancies were achieved of the 35 cases included in the study. One of them was obtained using post thawing immotile sperm. According to the standard protocol, only sperm that were immediately motile after testicular biopsy were frozen.

The use of motility as a criterion to evaluate sperm viability after thawing is not advised in SA cases, because pregnancies were obtained using immotile sperm samples after thawing. On the other hand, sperm motility criterion should not be used in fresh SA samples, where, in contrast with OA samples, the culture of immotile sperm does not involve further motility [13]. Fertilization and pregnancy rates using motile testicular sperm from SA males are very low, as were those obtained by Vernaev *et al.* (2003b) [14]. In SA males, sperm aneuploidy rates are higher [2, 14]. Furthermore, genomic imprinting could be altered [15], and abnormal male pronuclei decondensation can be shown [16]. Therefore, all these factors recommend the issue of genetic testing (including Y-chromosome deletions) and other predictive markers on outcomes, almost in such cases (SA). Unfortunately, the high cost of these probes prevents their routinely application, at least in public hospitals, like our case. Moreover extended in vitro culture could alter epigenetic control of imprinting during embryogenesis [17]. So, the low pregnancy rates obtained in SA cases in our work and the higher risk of newborns with malformations [2, 18] recommend the use of donor sperm in these cases. Moreover, the average number of ovarian stimulation cycles needed in women will be double that in IVF cases using donor sperm.

In OA cases, the higher frequency of motile sperm recovery at testicular level versus epididymal level (90% vs 76%) recommends the direct obtaining of sperm from testicle. Furthermore, there are other advantages, being fast, easy and cheap [19]. This low frequency of samples with motile sperm from epididymal level may be due to post-epididymal obstructions effects [20]. Under normal conditions, epididymal fluid protects sperm from degenerative changes. However, in chronic obstructions, this mechanism is modified and the motility pattern is inverted [21–23]. Testicular sperm quality in OA cases is similar to normal males. This suggests that damage is produced in the epididymis after sperm are produced in the testicle [24]. In fact, the use of immotile sperm from epididymis and from ejaculates leads to fertiliza-

tion and pregnancy rates lower than testicular sperm from the same patients, even if they are immotile [10, 25, 26]. Also, the slightly higher pregnancy rates obtained when spermatozoa from testicular biopsy, motile after thawing, are used, validate this recommendation, as it is also validated by the fact that pregnancies were achieved using post-thawing immotile spermatozoa from testicle, but not when post-thawing immotile spermatozoa from epididymis were used.

So, these latter results show that the motility criterion seems to be a more recommendable method to evaluate epididymal samples, but not testicular samples in this type of pathology. On the other hand, the differential freezing response may be indicative of a lower sperm quality of epididymal spermatozoa, even if they initially show motility.

In vitro culture of testicular sperm before ICSI makes it possible that, in most cases, they show motility after 3–5 h [27]. However, there is evidence in the literature that prolonged incubation to acquire motility has adverse effects on sperm viability, DNA ultrastructure and ROS production [28–30, 31]. It must be taken into account, when they were frozen, that the culture before and after cryopreservation aged spermatozoa [13]. In our case, unlike Park *et al.* (2003) [27], motile sperm were found in 95% of biopsies performed.

So, all these results support the proposal that, in OA cases, spermatozoa should be obtained directly from testicle.

In our Reproduction Unit, pregnancy rates obtained using sperm from either oligoasthenoteratozoospermic or normozoospermic males were similar, and were also similar to those obtained by other authors [32–35] as long as immotile sperm from fresh ejaculates were not microinjected. In that case, no successful pregnancies were achieved. So, the intracytoplasmic sperm injection with immotile sperm is inadvisable in OATZ cases.

In conclusion, the results obtained in OA cases point out that the application of motility criterion at testicular level before sperm cryopreservation should be reconsidered. In addition, application of this criterion should also be reconsidered after thawing in OA and SA cases.

Acknowledgements The authors are indebted to Professor García-Ximénez of the Laboratory of Animal Reproduction and Biotechnology, Polytechnic University of Valencia, Spain, for reviewing the original manuscript and giving his invaluable advice.

References

- Madgar I, Hourvitz A, Levron J, Seidman DS, Shulman A, Raviv GG, Levran D, Bider D, Mashiach S, Dor J. Outcome of in vitro fertilization and intracytoplasmic injection of epididymal and testicular sperm extracted from patients with obstructive and nonobstructive azoospermia. *Fertil. Steril* 1998;69:1080–84.
- Vernaev V, Bonduelle M, Tournaye H, Camus M, Van Steirteghem A, Devroey P. Pregnancy outcome and neonatal data of children born after ICSI using testicular sperm in obstructive and non-obstructive azoospermia. *Hum Reprod* 2003a;18:2087–93.
- Verheyen G, Joris H, Crits K, Nagy Z, Tournaye H, Van Steirteghem A. Comparison of different hypo-osmotic swelling solutions to select viable immotile spermatozoa for potential use in intracytoplasmic sperm injection. *Hum Reprod Update* 1997;3:195–203.
- Aktan TM, Montag M, Duman S, Gorkemli H, Rink K, Yurdakul T. Use of a laser to detect viable but immotile spermatozoa. *Andrologia* 2004;36:366–9.
- Verheyen G, Tournaye H, Staessen C. Controlled comparison of conventional in-vitro fertilization and intracytoplasmic sperm injection in patients with asthenozoospermia. *Hum Reprod* 1999;14:2313–9.
- Moilanen JM, Tulppala M, Reima I. Fertilization, embryo quality, and cryosurvival in in-vitro fertilization and intracytoplasmic sperm injection cycles. *J Assist Reprod Genet* 1999;16:17–23.
- Dickey RP, Pyrzak R, Lu PY. Comparison of the sperm quality necessary for successful intrauterine insemination with WHO threshold values for normal sperm. *Fertil Steril* 1999;71:684–9.
- Hauser R, Yogev L, Botchan A. Intrauterine insemination in male factor subfertility: significance of sperm motility and morphology assessed by strict criteria. *Andrologia* 2001;33:13–17.
- Zeyneloglu HB, Baltaci V, Ege S, Haberal A, Batioglu S. Detection of chromosomal abnormalities by fluorescent in-situ hybridization in immotile viable spermatozoa determined by hypo-osmotic sperm swelling test. *Hum Reprod* 2000;15:853–6.
- Nagy ZP, Liu J, Joris H. The result of intracytoplasmic sperm injection is not related to any of the three basic parameters. *Hum Reprod* 1995a;10:1123–9.
- Nagy ZP, Joris H, Verheyen G. Correlation between motility of testicular spermatozoa, testicular histology and the outcome of intracytoplasmic sperm injection. *Hum Reprod* 1998b;13:890–5.
- Shulman A, Feldman B, Madgar I. In vitro fertilization treatment for severe male factor: the fertilization potential of immotile spermatozoa obtained by testicular extraction. *Hum Reprod* 1999;14:749–52.
- Liu J, Tsai Y, Katz E, Compton G, Garcia JE, Baramki TA. Outcome of in-vitro culture of fresh and frozen-thawed human testicular spermatozoa. *Hum Reprod* 1997;12:1667–72.
- Vernaev V, Tournaye H, Osmanagaoglu K, Verheyen G, Van Steirteghem A, Devroey P. Intracytoplasmic sperm injection with testicular spermatozoa is less successful in men with nonobstructive azoospermia than in men with obstructive azoospermia. *Fertil Steril* 2003b;79:529–33.
- Tesarik J, Mendoza C. Genomic imprinting abnormalities: a new potential risk of assisted reproduction. *Mol Hum Reprod* 1996;2:295–8.
- Hewitson L, Simerly C, Schaten G. Fate of sperm components during assisted reproduction: implication for infertility. *Hum Fertil (Camb)* 2002;5:111–6.
- Gosden R, Trasler J, Lucifero D, Faddy M. Rare congenital disorders, imprinted genes, and assisted reproductive technology. *Lancet* 2003;361:1975–7.
- Silber S, Escudero T, Lenahan K, Abdelhadi I, Kilani Z, Munne S. Chromosomal abnormalities in embryos derived from testicular sperm extraction. *Fertil Steril* 2003;79:30–38.
- De Croo I, Van Der Elst J, Everaert K, De Sutter P, Dhont M. Fertilization, pregnancy and embryo implantation rates after ICSI in cases of obstructive and non-obstructive azoospermia. *Hum Reprod* 2000;15:1383–8.
- Silber SJ. Results of microsurgical vasoepididymostomy: role of epididymis in sperm maturation. *Hum Reprod* 1989;4:298–03.
- Silber SJ, Devroey P, Tournaye H. Fertilizing capacity of epididymal and testicular sperm using intracytoplasmic sperm injection (ICSI). *Reprod Fertil Dev* 1995;7:281–93.
- Moore H. Contribution of epididymal factors to sperm maturation and storage. *Andrologia* 1998;30:233–9.
- Nicopoulos JD, Ramsay JW, Almeida PA, Gilling-Smith C. Assisted reproduction in the azoospermic couple. *Br J Obstet Gynaecol* 2004;111:190–203.
- Steele EK, McClure N, Maxwell RJ. A comparison of DNA damage in testicular and proximal epididymal spermatozoa in obstructive azoospermia. *Mol Hum Reprod* 1999;5:831–5.
- Tournaye H, Liu J, Nagy PZ, Camus M, Goossens A, Silber S, Van Steirteghem A, Devroey P. Correlation between testicular histology and outcome after intracytoplasmic sperm injection using testicular spermatozoa. *Hum Reprod* 1996;11:127–32.
- Abdelmassih V, Balmaceda JP, Tesarik J, Abdelmassih R, Nagy ZP. Relationship between time period after vasectomy and the reproductive capacity of sperm obtained by epididymal aspiration. *Hum Reprod* 2002;17:736–40.
- Park YS, Lee SH, Song SJ, Jun JH, Koong MK, Seo JT. Influence of motility on the outcome of in vitro fertilization/intracytoplasmic sperm injection with fresh vs. frozen testicular sperm from men with obstructive azoospermia. *Fertil Steril* 2003;80:526–30.
- Nogueira D, Bourgain C, Verheyen G, Van Steirteghem AC. Light and electron microscopic analysis of human testicular spermatozoa and spermatids from frozen and thawed testicular biopsies. *Human Reprod* 1999;8:2041–49.
- Prins GS, Dolgina R, Studney P, Kaplan B, Ross L, Niederberger C. Quality of cryopreserved testicular sperm in patients with obstructive and non obstructive azoospermia. *J Urol* 1999;5:1504–8.
- Calamera JC, Fernández PJ, Buffone MG, Acosta AA, Doncel GF. Effects of long-term in vitro incubation of human spermatozoa: functional parameters and catalase effect. *Andrologia* 2001;2:79–86.
- Dalzell LH, Thompson-Cree ME, McClure N, Traub AI, Lewis SE. Effects of 24-hour incubation after freeze-thawing on DNA fragmentation of testicular sperm from infertile and fertile men. *Fertil Steril* 2003;79:1670–2.
- Nagy ZP, Liu J, Cecile J, Silber S, Devroey P, Van Steirteghem A. Using ejaculated, fresh, and frozen-thawed epididymal and testicular spermatozoa gives rise to comparable results after intracytoplasmic sperm injection. *Fertil Steril* 1995b;63:808–15.
- Vandervorst M, Tournaye H, Camus M, Nagy ZP, Van Steirteghem A, Devroey P. Patients with absolutely immotile spermatozoa and intracytoplasmic sperm injection. *Hum Reprod* 1997;12:2429–33.
- Nijs M, Vanderzwalmen P, Vandamme B, Segal-Bertin G, Lejeune B, Segal L, Van Roosendaal E, Schoysman R. Fertilizing ability of immotile spermatozoa after intracytoplasmic sperm injection. *Hum Reprod* 1996;11:2180–5.
- Van Steirteghem A, Nagy P, Joris H, Janssenswillem C, Staessen C, Verheyen G, Camus M, Tournaye H, Devroey P. Results of intracytoplasmic sperm injection with ejaculated, fresh and frozen-thawed epididymal and testicular spermatozoa. *Hum Reprod* 1998;13:134–42.