ORIGINAL ARTICLE

TESTICULAR VERSUS EPIDIDYMAL SPERMATOZOA IN INTRACYTOPLASMIC SPERM INJECTION TREATMENT CYCLES

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Background: Normal fertilization and ongoing pregnancy can be achieved using intracytoplasmic sperm injection (ICSI), even with severely immature spermatozoa. However, the published literature documents conflicting results as to the outcome of ICSI. **Methods:** Surgical extraction of spermatozoa in 111 ICSI treatment cycles performed over five years at the Assisted Conception Unit (ACU), University College Hospital (UCH), was retrospectively evaluated to compare the outcome of ICSI treatment using either testicular or epididymal spermatozoa. **Results:** A higher normal fertilization rate and lower abnormal fertilization rate was observed in the epididymal spermatozoa group than in the testicular spermatozoa group. Embryo development on day 3 after fertilization and implantation was significantly better in the epididymal spermatozoa group. Clinical and ongoing pregnancy rates were higher and the spontaneous miscarriage rate lower in the epididymal spermatozoa group, but only the clinical pregnancy rate reached statistical significance. **Conclusions:** The origin of surgically extracted spermatozoa has an effect on the success of assisted reproduction using ICSI, and the immaturity of testicular spermatozoa may affect fertilization, embryo development, implantation and pregnancy.

Keywords: IVF, male factor, azoospermia, surgical extraction of spermatozoa, outcome

INTRODUCTION

The ICSI with surgical extraction of spermatozoa has added a new dimension to the treatment of severe male factor infertility. ICSI bypasses the natural mechanisms critical for oocyte penetration and hence allows the use of surgically extracted immature spermatozoa for fertilization.

In patients with obstructive azoospermia (OA), spermatozoa can be extracted from the testes or epididymis. In patients with non-obstructive azoospermia (NOA) testicular biopsy is generally required[1], although successful epididymal recovery is also reported.[2]

Some authors report that the source of surgically extracted spermatozoa in OA does not have any impact on fertilization and pregnancy rates[3] and that testicular spermatozoa recovered from patients with OA and NOA are as effective as ejaculated spermatozoa.[4] It is also reported that once fertilization is achieved, embryonic development, pregnancy and miscarriage rates are similar regardless of the aetiology of azoospermia and the source of the spermatozoa.[5,6] To the contrary, the European Society for Human Reproduction and Embryology (ESHRE) ICSI Task Force reports lower fertilization, but comparable pregnancy rates and perinatal outcome, in patients with NOA when compared to patients with OA.[7] Lower fertilization rates, but also lower implantation rates, are reported for testicular spermatozoa in patients with NOA when compared to patients with OA.[8] Similar fertilization and pregnancy rates are also reported for NOA and OA[9,10] but with higher miscarriage rates in NOA.[11]

In this review, we retrospectively evaluated ICSI treatment cycles using surgically extracted spermatozoa performed over five years at the ACU, UCH to compare the outcome of treatment cycles using either testicular or epididymal spermatozoa.

MATERIALS AND METHOD

A retrospective data analysis was conducted on 111 consecutive, unselected ICSI treatment cycles in which surgically extracted spermatozoa was used at the ACU, UCH, between July 2001 and September 2006. No exclusion was applied on the basis of the type (primary or secondary), duration, or aetiology of infertility of the female partner, or on the number and outcome of previous fertility treatments.

Demographic characteristics of the couples and clinical and embryological variables of the ICSI cycles were recorded. Clinical pregnancy, diagnosed by serum beta-hCG levels on day 15 after embryo transfer and confirmed by ultrasonography at week 7 of pregnancy, was documented as the primary outcome measure. Ongoing pregnancy was defined as pregnancy beyond the first trimester. The implantation rate was defined as gestational sac per embryo transferred.

Routine andrological work-up of the male partner included conventional semen analysis, endocrine profile, karyotype analysis and cystic fibrosis screening. Routine gynaecological work-up of the female partner included a dynamic ovarian reserve test[12,13] to evaluate the antral follicle count, basal FSH and oestradiol (E_2) levels and E_2 response to 300 IU of recombinant FSH stimulation (delta E_2). Saline contrast hydrosonography was also performed for assessment of the uterine cavity.

All female partners underwent a long GnRH agonist stimulation protocol started on day 21 of the menstrual cycle as detailed in Ranieri *et al.*[12,13] A maximum of three cleavage stage embryos (25% of all

cycles) were transferred. Three cycles had blastocyst embryo transfer on day 5 of fertilization. For male partners with irreparable OA, percutaneous epididymal sperm aspiration (PESA) was performed on the day of oocyte collection. If PESA failed to extract any spermatozoa, testicular samples were extracted mostly by fine needle aspiration (TEFNA) and used for fertilization. Epididymal samples obtained during vasectomy reversal procedure by PESA or microepididymal sperm aspiration (MESA) were cryopreserved for future use. For male partners with NOA, samples were extracted directly from the testes using testicular sperm extraction (TESE) and samples were cryopreserved for future use. Since fresh testicular and frozen epididymal samples were few, these cases were excluded from the main data analysis.

The statistical analysis was performed using SPSS-10. The χ^2 test was used to analyse nominal variables. Fisher's exact test was computed when a table had a cell with an expected frequency <5. The Yates corrected chi-square was computed for all other 2 by 2 tables. Normally distributed metric variables were tested with the *t*-test. Ordinal variables or not-normally distributed metric variables were analysed with the Mann-Whitney U test. If more than two groups had to be analysed, normally distributed metric variables with equal variances (Levene test) were examined by means of the one-way ANOVA test. For not-normally distributed metric variables or for variables with unequal variances, the Kruskal-Wallis one-way ANOVA test was used. For correlation analysis, the Spearman rank correlation coefficient was used. All tests were two-tailed with a confidence level of 95% ($p<0.05$).

RESULTS

A total of 111 consecutive, unselected ICSI treatment cycles were reviewed. Epididymal extraction of spermatozoa was performed in 50 cycles: by PESA in 45 cycles and MESA in 5 cycles; with fresh spermatozoa used for ICSI in 44 cycles (37 OA, 7 NOA) and frozen-thawed used for ICSI in 6 cycles (6 OA). Testicular extraction of spermatozoa by TESE was performed in 61 cycles: with frozen-thawed spermatozoa used for ICSI in 53 cycles (24 OA, 29 NOA) and fresh used for ICSI in 8 cycles (2 OA, 6 NOA). Only frozen-thawed TESE (53 cycles) and fresh PESA (44 cycles) ICSI cycles were included in the comparison.

There were no significant differences in the distribution between PESA and TESE cycles when demographic characteristics of the couples were evaluated for female age, duration of infertility, number of previous pregnancies, and number of previous IVF attempts (Table- 1). Also similar in both groups were the antral follicle count, basal FSH and E_2 levels, dynamic response of E_2 to FSH stimulation, total dose of gonadotrophin used, and number of mature oocytes retrieved. However, In the PESA group the number of 2-PN embryos created and cryopreserved was significantly higher and the number of 3-PN embryos created lower; the latter was short of statistical significance (Table-1).

Table-1: Demographic characteristics, clinical and embryological variables in TESE and PESA groups

variable	Test	N	Mean±SD	*p*
Female Age	Frozen TESE	53	33.4±5.0	0.25
	Fresh PESA	44	34.5±3.9	
Duration of Infertility	Frozen TESE	49	45.2±32.0	0.41
	Fresh PESA	40	45.0±36.4	
Gravidity	Frozen TESE	50	0.4±0.9	0.12
	Fresh PESA	44	0.5±0.7	
Previous IVF Treatments	Frozen TESE	50	1.5±0.9	0.89
	Fresh PESA	44	1.5±0.8	
Antral Follicle Count	Frozen TESE	43	12.1±3.9	0.07
	Fresh PESA	30	10.5±5.0	
Baseline FSH	Frozen TESE	47	6.7±2.1	0.84
	Fresh PESA	43	6.6±1.6	
Baseline E_2	Frozen TESE	47	184.2±58.1	0.14
	Fresh PESA	43	203.9±67.9	
Delta E_2	Frozen TESE	47	343.0±161.2	0.91
	Fresh PESA	43	352.4±196.9	
Gonadotrophin Dose	Frozen TESE	50	47.1±15.9	0.97
	Frozen PESA	44	47.5±14.8	
Oocytes Used	Frozen TESE	53	9.8±4.7	0.47
	Frozen PESA	44	10.6±5.2	
2-PN Embryos	Frozen TESE	53	5.4±2.9	<0.01
	Frozen PESA	44	7.4±3.9	
3-PN Embryos	Frozen TESE	53	0.07±0.2	0.06
	Frozen PESA	44	0.0±0.0	
Cleaved Embryos	Frozen TESE	53	4.9±3.0	<0.01
	Frozen PESA	44	7.2±3.9	
Embryos Frozen	Frozen TESE	52	1.0±1.9	0.05
	Frozen PESA	44	2.0±2.7	

In the PESA group, the stage of the best two embryos selected for transfer was significantly higher on day 3 after fertilization than in the TESE group. However, the differences in grading of these embryos were not statistically significant (Table-2).

In the PESA group implantation of the embryos, in terms of number of gestational sacs and foetal cardiac activity observed per embryo transfer, was significantly higher than in the TESE group, as was the clinical pregnancy rate (Table-3). However, differences in the ongoing pregnancy rate fell short of statistical significance (Table-4). A higher spontaneous miscarriage rate in the TESE group was not statistically significant (31.8% vs 28.5%). Clinical pregnancy rates were not affected by the aetiology of the azoospermia (Table-5).

Frozen-thawed testicular spermatozoa had lower motility, lower normal morphology and lower concentration than fresh epididymal spermatozoa, but only the difference in motility was statistically significant (Table-6). Neither motility nor morphology of spermatozoa affected the treatment outcome, and no association was noted between the sperm concentration

and morphology, and the number of 2-PN and cleavage stage embryos that developed.

Table-2: Embryo development in TESE and PESA groups

Stage of		N	Mean±SD	*p*
1st Embryo Transferred	Frozen TESE	52	6.07±1.93	0.03
	Fresh PESA	44	6.90±1.56	
2nd Embryo Transferred	Frozen TESE	51	6.02±1.93	0.03
	Fresh PESA	44	6.77±1.43	
3rd Embryo Transferred	Frozen TESE	15	5.33±1.45	0.58
	Fresh PESA	10	5.60±1.84	

Table-3: Implantation variables in TESE and PESA groups

		N	Mean±SD	*p*
Gestational Sac per Embryo transfer	Frozen TESE	53	0.43±0.60	0.02
	Fresh PESA	44	0.79±0.82	
Foetal Heart Activity per Embryo transfer	Frozen TESE	53	0.41±0.60	0.02
	Fresh PESA	44	0.77±0.83	

Table-4: Pregnancy outcome of ICSI treatment in TESE and PESA groups

	Clinical Pregnancy		Ongoing Pregnancy	
	No	Yes	No	Yes
Frozen TESE	58.5%	41.5%	71.7%	28.3%
Fresh PESA	36.4%	63.3%	53.4%	46.6%
p	0.03		0.10	

Table-5: Pregnancy outcome of ICSI treatment in TESE and PESA groups in reference to the type of azoospermia

		Clinical Pregnancy		*p*
		No	Yes	
Frozen TESE	NOA	58.6%	41.4%	0.98
	OA	58.3%	41.7%	
Fresh PESA	NOA	14.3%	85.7%	0.18
	OA	40.5%	59.5%	

Table-6: Sperm parameters in TESE and PESA groups

	Frozen TESE	Fresh PESA	*p*
Motile	21.2%	69%	<0.01
Non-motile	23.4%	9.5%	
Twitching	55.4%	21.5%	
Abnormal Morphology	8.8%	4.5%	0.34
Normal Morphology	67.6%	81.8%	
Very Abnormal Morphology	23.6%%	13.7%	
Concentration per high power filed (Mean ± SD)	3.84±2.41	5.09±4.50	0.15

DISCUSSION

The role of surgical extraction of spermatozoa in the treatment of severe male factor infertility is well established. Normal fertilization and ongoing pregnancy can be achieved using ICSI, even with severely immature spermatozoa.[1,14] However, the literature reports conflicting results as to the outcome of these techniques, due to different underlying aetiologies of azoospermia and the different sources of the spermatozoa used.

Female partners in both the TESE and PESA groups shared similar demographic characteristics with prognostic significance and similar ovarian reserve values. Furthermore, the total dose of hMG stimulation utilised and the ovarian response to stimulation were similar in both groups. Hence, there was no confounding factor that might be related to ovarian factor that could affect the comparison of the two groups. With two study populations having a comparable background prognosis for assisted conception, any variation observed in the outcome of their treatment should be primarily a function of male factor.

In the PESA group, a higher fertilization rate with more 2-PN embryos created and cryopreserved and a lower abnormal fertilization rate with less 3-PN embryos created was observed, than in the TESE group. Embryo development in terms of blastomere count on day 3 following fertilization was also better for the embryos transferred in the PESA group. Consequent to better fertilization and embryo quality, embryos in the PESA group showed higher implantation rates than in the TESE group. Clinical and ongoing pregnancy rates were higher and the spontaneous miscarriage rate lower in the PESA group, but only the former reached statistical significance.

The published data on fertilization, pregnancy and miscarriage rates after ICSI with testicular or epididymal spermatozoa are discordant. Comparison is difficult since the series are most often small, report mixed cases of OA and NOA, and the origin of the spermatozoa is not always specified. However, some general trends are identifiable. In male partners with OA, fertilization and pregnancy rates following ICSI using epididymal spermatozoa are generally higher than following ICSI using testicular spermatozoa, albeit at a statistically insignificant level. However, when ICSI using testicular spermatozoa from men with NOA or OA is compared, the OA testicular spermatozoa provide higher fertilization and pregnancy rates (Table-7).

A possible reason for lower fertilization rates following ICSI with testicular spermatozoa may be the lower concentration of normal mature spermatozoa.[15] Furthermore, subsequent embryo development and pregnancy rates may be influenced by the immaturity of the testicular spermatozoa used for ICSI and these appear to be more prominent in cases with NOA.[16,17]

Miscarriage rates following ICSI with surgically extracted testicular spermatozoa are reported to be higher[18,19] or similar[6,20–23] than those following ICSI with ejaculated or surgically extracted epididymal spermatozoa. The differences between testicular and epididymal spermatozoa may reflect the respective influence of spermatogenetic defects and

spermatozoa immaturity in miscarriage. Spermatozoa produced by defective spermatogenesis may be associated with a higher aneuploidy rate[24] or other genetic alterations and may be responsible for embryos being less able to develop to the blastocyst stage[25] and to implant[9].

In our study, testicular spermatozoa samples were characterised by lower motility, poorer morphology and lower concentrations in comparison with epididymal samples. Although the numbers were too low to draw any firm conclusions, the freezing process did not appear to be the underlying reason for the observed differences. We observed no demonstrable difference in motility, morphology and numbers between frozen-thawed and fresh testicular spermatozoa samples. Nevertheless, none of the sperm parameters had an effect over the treatment outcome report. Similar fertilization and cleavage rates in patients with severe spermatogenetic defects and in patients with normal spermatogenesis in cases of OA have been reported elsewhere.[26] Hence, the difference must lie in aspects of spermatozoa function not captured by routine analysis.

Some authors verify comparable fertilization, embryo cleavage, and pregnancy and miscarriage rates with fresh compared to frozen testicular spermatozoa in ICSI cycles[21,27–29] and similar conclusions are reported for epididymal spermatozoa.[28,30,31] To the contrary, Friedler *et al* report higher fertilization but similar pregnancy rates with fresh epididymal sperm[21], while others report similar fertilization but lower pregnancy rates with frozen epididymal sperm[6]. Others report a significant improvement in clinical pregnancy rates with the use of frozen epididymal or testicular spermatozoa compared to fresh.[29,32] The differences are likely to be multi-factorial and should be reviewed within the context of the overall success of the IVF programmes. The pregnancy rates observed in our study were generally higher when compared with previously published reports (Table-7).

CONCLUSION

The origin of surgically extracted spermatozoa has an effect on success of assisted reproduction. The immaturity of testicular spermatozoa may affect fertilization, embryo development, implantation and pregnancy.

Table-7: Published studies on surgical extraction of spermatozoa and treatment outcome

	Fertilization rate	Pregnancy rate	Miscarriage
Aboulghar 1997	Epididymal OA 55% Testicular OA 52%* Testicular NOA 38%*	Epididymal OA 36% Testicular OA 34%* Testicular NOA 14%*	No difference Miscarriage
Mansour 1997	Epididymal OA 60% Testicular OA 54%* Testicular NOA 39%*	Epididymal OA 27% Testicular OA 32%* Testicular NOA 11%*	
Silber 1997	Epididymal OA 58% Testicular OA 52% (NS)	Epididymal OA 48% Testicular OA 34% (NS)	
	No difference OA, NOA	No difference OA, NOA	
Tarlatzis 1998	OA 55% NOA 47%	No difference	Similar Perinatal outcome
	Epididymal 62% Testicular 52%	Epididymal 22% Testicular 19%	
Ghazzawi 1998	Epididymal OA 75% Testicular NOA 69% (NS)	Epididymal OA 28% Testicular of NOA 21% (NS)	High Miscarriage Testicular NOA
Palermo 1999	Epididymal OA 73% Testicular OA 80%* Testicular NOA 57%*	Epididymal OA 61% Testicular OA 57%* Testicular NOA 49%*	No difference Miscarriage Congenital anomaly
Ubaldi 1999	Testicular OA Testicular NOA Ejaculated (NS)	Testicular OA Testicular NOA Ejaculated (NS)	
Croo 2000	Testicular OA 75%* Testicular NOA 68%* Maturation arrest 47%*	No difference Embryo quality Testicular OA 37% Testicular NOA 37%	
Vicari 2001	No difference Epididymal OA Testicular NOA	No difference Epididymal OA Testicular NOA	Higher in NOA
Friedler 2002	Epididymal OA 56% Testicular NOA 51% (NS)	Epididymal OA 35% Testicular NOA 32% (NS)	High Miscarriage Testicular
Junior[33] 2002		No difference Epididymal Testicular	High Miscarriage Testicular
Buffat 2006	Epididymal OA 59% Testicular OA 52% (NS)	Epididymal OA 22% Testicular OA 24% (NS)	High Miscarriage Testicular

*Significant, (NS) =Non-significant

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