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Outcomes of patients undergoing conventional testicular sperm extraction for intracytoplasmic sperm injection in a South African fertility centre

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Research report submitted to the University of Witwatersrand Faculty of Health Sciences for the degree of Masters of Medicine (Urology) in June 2022

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Masters of Medicine

Research report in submissable format

This report has been written according to guidelines of *Current Urology* journal and has been submitted to this journal for publication.

Original article of 2500 – 6000 words

Calibri, size 11 font, double spacing, numbered pages

References in numerical order of appearance in American Medical Association (AMA) style

Declaration

I, Nicholas Brits, declare that this report is original work and no part has been copied from another source, unless clearly stated as such with appropriate referencing. This work has not been submitted to another University for a post graduate degree.



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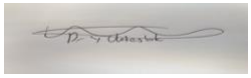
Signed 01 June 2022 in Johannesburg

Author's contribution **June 2022**

Title of manuscript: Outcomes of patients undergoing conventional testicular sperm extraction for intracytoplasmic sperm injection in a South African fertility centre

All authors were involved in research design, data analysis and contributed to the final manuscript. Dr N Brits, Dr Y Unterslak and M Marais were involved in data collection.





_____ _____

Dr N Brits

Urology Registrar

Dr Y Unterslak

Supervisor





_____ _____

M Marais

Supervisor

Dr M Nel

Supervisor

Dedication

I dedicate this to my wife, Iné, my daughter, Mila and my parents, Max and Miranda.

Thank you for your guidance, support and belief.

Presentations

24 November 2021 – Oral presentation at the Bert Myburgh Research Forum, University of Witwatersrand

September 2022 – Due for presentation at South African Urological Association (SAUA) Congress. Postponed since 2020 due to Covid-19.

Abstract

Background Surgical sperm retrieval for intracytoplasmic sperm injection (ICSI) has revolutionised the treatment of male factor infertility. This study aims to compare sperm retrieval rates (SRR) of conventional testicular sperm extraction (c-TESE) at our centre with SRR in the literature as well as describe the demographic and clinical features of our patients and the pregnancy outcomes following c-TESE and ICSI.

Materials and Methods A retrospective review of 163 men who underwent c-TESE for ICSI between January 2016 and September 2019 in a South African fertility centre.

Results Sperm retrieval rate was 58% (58 of 100) in the group with spermatogenic failure and 100% (63 of 63) in the group with post-testicular pathology. Preoperative follicle stimulating hormone (FSH) levels in those with spermatogenic failure demonstrated a statistically significant difference between the positive sperm retrieval group (6.50IU/l $\pm$4.74) and negative sperm retrieval group (15.4IU/l $\pm$7.67), $p<0.0001$. Intracytoplasmic sperm injection was performed for 115 of the 163 patients (70.6%), with an overall clinical pregnancy rate of 67.8% (78 of 115). A single ICSI cycle was performed in 61 couples and multiple cycles, defined as more than one cycle, were performed in 54 couples with a pregnancy rate of 62.3% and 27.6% per cycle, respectively.

Conclusion Conventional TESE in our setting has similar sperm retrieval rates to the international literature, however a significant proportion of our patients had a post-testicular cause of male infertility which may over-estimate sperm retrieval rates. Further studies are needed in South Africa to evaluate the causes and treatment options available and offered for male factor infertility. The information regarding sperm retrieval rates and pregnancy outcomes is valuable for counselling South African patients embarking on fertility treatment.

Acknowledgements

I would like to thank the patients and staff of Vitalab Centre of Assisted Conception for making this research report possible as well as my supervisors for their guidance and support.

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Author guidelines – *Current Urology*

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the footnotes below the tables to which they refer. Tables are cited in the text in numerical order. Each table should be able to be understood without consulting the text.

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List of abbreviations

ART	Assisted reproductive technology
CPR	Clinical pregnancy rates
c-TESE	Conventional testicular sperm extraction
FSH	Follicle stimulating hormone
ICSI	Intracytoplasmic sperm injection
m-TESE	Microdissection testicular sperm extraction
NOA	Non-obstructive azoospermia
OA	Obstructive azoospermia
SRR	Sperm retrieval rates

Introduction

Male factor infertility may account for 50% of infertility cases and has traditionally posed a significant challenge to both patients and reproductive specialists.[1,2] Previously, the only options left available to these couples were adoption or donor insemination. The advent of surgical sperm retrieval and advances in assisted reproductive technology (ART) with intracytoplasmic sperm injection (ICSI) have revolutionised the treatment options available to allow these patients to become biological fathers.[3–5]

In the azoospermic male, the surgical sperm retrieval technique depends on the underlying cause, i.e. obstructive or non-obstructive,[3] as well as the availability of surgical expertise to perform them. In South Africa there is limited funding and access to infertility treatment, and despite microdissection testicular sperm extraction (m-TESE) being described in 1999, the first m-TESE was performed in South Africa in 2014.[5–7] Conventional open testicular sperm extraction (c-TESE) is a widely used method, although sperm retrieval rates (SRR) are reported to be lower than m-TESE.[8–10]

This study aims to evaluate SRR and pregnancy rates of patients undergoing c-TESE and ICSI at our centre. The objectives of this study are to describe the patient demographics, risk factors and causes of infertility, evaluate preoperative follicle stimulating hormone (FSH) levels and histopathological findings and compare our SRR and pregnancy outcomes with the literature.

Materials and Methods

Study setting

Study done at a single, fee-for-service private fertility practice in Johannesburg, South Africa.

Patients

A retrospective review of all men who underwent c-TESE between January 2016 and September 2019.

Ethical approval was obtained by the University of the Witwatersrand Human Research Ethics Committee (Medical). All men who had undergone c-TESE for ICSI were included. Men without a partner or men who underwent c-TESE for sperm cryopreservation prior to chemotherapy were excluded. All patients and their partners were evaluated by a Reproductive Medicine Specialist with a detailed history, physical examination and appropriate investigations.

Background of procedures

FSH levels

The laboratory reference range for FSH is 1.0 – 12.0IU/L.

Conventional TESE and ICSI

At our centre, c-TESE is performed on an out-patient basis under conscious intravenous sedation and local anaesthesia. The procedure is performed by one of the six Reproductive Medicine Specialists who are all specialist gynaecologists. A midline scrotal incision is performed and dissection is carried out through the dartos muscle and tunica vaginalis until the tunica albuginea of the testis is exposed. A horizontal incision between 4mm and 10mm is made in the tunica albuginea and the extruded seminiferous tubules are excised with scissors. The tissue is examined immediately for the presence of spermatozoa. The seminiferous tubules are placed in a petri dish and examined by the embryologist under an inverted microscope to confirm the presence of motile or non-motile spermatozoa. The presence of spermatozoa, motile or non-motile, was deemed positive and the absence of spermatozoa was deemed a negative sperm retrieval. If no spermatozoa are found the incision is repeated at a second site on the same testis and subsequently on the contralateral testis if still no spermatozoa are found. The tunica albuginea, dartos muscle and skin are closed separately. If no spermatozoa are identified, the specimen is placed in Bouin's solution (Sigma Aldrich) and sent for histopathological assessment. Occasionally tissue is submitted for patients in whom spermatozoa are identified, especially if there are concerns regarding the quality of tissue harvested and the

cause of azoospermia. The retrieved sperm are then cryopreserved for later use or used immediately in a cycle of ICSI.

Pregnancy outcomes

The clinical outcomes following ICSI were classified as either a biochemical pregnancy, a clinical pregnancy or a live birth. After embryo transfer, a quantitative serum Beta-Human Chorionic Gonadotropin (β-hCG) is done on day 12. A positive result is regarded as a biochemical pregnancy. A transvaginal ultrasound is then performed at seven weeks following embryo transfer. The presence of a foetal heart is regarded as a clinical pregnancy. The number of foetal sacs is also documented. After confirmation of a clinical pregnancy, the patient is referred to an obstetrician for further antenatal care. While requests are made for feedback regarding birth details and outcomes, this is often not obtained.

Data analysis

Statistical analysis was performed using Stata 16 (StataCorp. 2019. *Stata Statistical Software: Release 16*. College Station, TX: StataCorp LLC). Data are presented as mean $\pm$ standard deviation for continuous variables and as counts and percentages for categorical variables. The Mann-Whitney U test was used for comparison between non-parametric quantitative values. A p-value <0.05 was considered significant. Univariate logistic regression, Receiver Operating Characteristics (ROC) curve and the area under a curve (AUC) was used in our predictive model. A value of 1.0 indicates a perfect test with a 100% sensitivity and specificity.

Results

Demographics of couples and male risk factors for infertility

In this study, 163 men were included with a mean age of 41.68 $\pm$ 8.61 years. The mean female partner age was 33.47 $\pm$ 5.09 years. The mean age in the men with spermatogenic failure (n=100)

was 39.09 (± 7.69) years and 45.49 (± 8.44) years in men with a post-testicular cause (n=63), p <0.0001. The demographics and male risk factors for infertility are shown in Table 1.

Table 1: Demographics of couples and male risk factors for infertility of men undergoing c-TESE

	Male	Female
Patients (n)	163	163
Age (years)	41.68 (±8.61)	33.47 (±5.09)
Primary infertility (n)	90 (55.2%)	111 (68.1%)
Secondary infertility (n)	73 (44.8%)	52 (31.9%)
Male risk factor (n) (%)		
Vasectomy	53 (32.5%)	
Years post vasectomy	9.5 (range 1 – 27)	
Undescended testes	17 (10.4%)	
Mumps	12 (7.4%)	
Chemotherapy	7 (4.3%)	
Varicocelectomy	4 (2.5%)	
Testicular torsion	3 (1.8%)	

(c-TESE = Conventional testicular sperm extraction)

The most common cause of infertility was a previous vasectomy, noted in 53 patients (32.5%) with a mean duration post-vasectomy of 9.5 ± 5.9 years. Of these 53 patients, 11 reported undergoing an unsuccessful vasectomy reversal. A post-testicular cause was identified in 63 patients. The group included two patients (genetically related) who were diagnosed with congenital bilateral absence of the vas deferens (CBAVD) with positive cystic fibrosis transmembrane conductance regulator (CFTR)

mutations, six patients had ejaculatory dysfunction and the cause of obstruction was undocumented in two patients.

Risk factors for spermatogenic failure included a history of undescended testes in 17 patients, however it was not documented whether this was unilateral or bilateral undescended testes. Other risk factors included post-pubertal mumps in 12 patients, chemotherapy in seven patients, a previous varicocelectomy in four patients and a history of testicular torsion in three patients. In 57 of our patients no cause could be identified.

Testicular histopathology following c-TESE

Testicular tissue was sent for histopathological assessment in 51 cases, 39 following negative sperm retrieval and 12 following positive sperm retrieval. The most common findings were Sertoli Cell Only syndrome in 16 patients (31.4%), maturation arrest in 11 patients (21.6%), hypospermatogenesis in six patients (11.8%) and mixed aetiology in eight patients (15.7%).

FSH levels of male patients

The FSH levels were recorded in 104 of 163 patients (63.8%) with an overall mean value of 9.12IU/l ± 7.36. FSH was recorded in 71 of the non-obstructive group (71%) with a mean of 11.14IU/l ±7.80 and mean FSH was found to be significantly higher in patients with negative sperm retrieval (15.4IU/l ±7.67) compared to positive sperm retrieval (6.50IU/l ±4.74), $p<0.0001$. FSH levels as a predictor of successful sperm retrieval in the spermatogenic failure group is demonstrated in a Receiver Operator Characteristics (ROC) curve in Figure 1. The area under the curve (AUC) was 0.8239. In the group with a post-testicular cause, FSH was recorded in 33 of 63 patients (52.4%) with a mean of 4.77IU/l ±3.54.

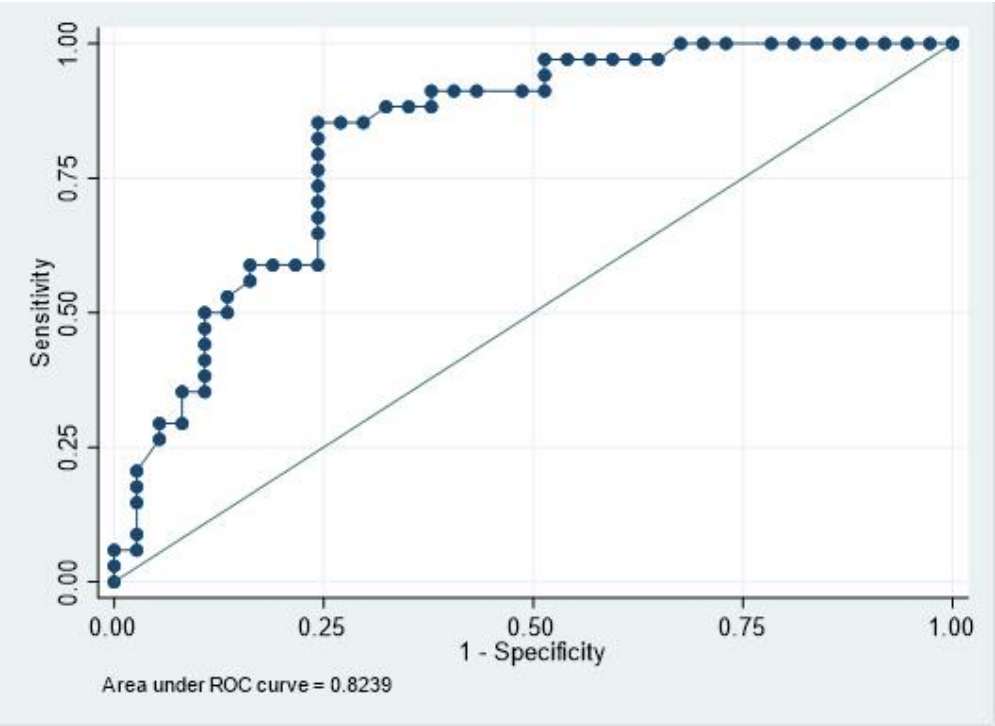


Figure 1: Receiver-operator characteristic (ROC) curve for FSH with area under ROC curve (AUC) of 0.8239

Sperm retrieval rates of c-TESE

Overall, 121 of the 163 patients (74.2%) had a positive sperm retrieval. In men with spermatogenic failure (n=100) SRR were 58% (58 of 100) and in men with a post-testicular aetiology the SRR were 100% (63 of 63). In the former group, the mean age of those with a positive sperm retrieval was 40.14 (±8.04) compared to 37.64 (±7.02) years in those with negative sperm retrieval, p = 0.1. The SRR according to age are shown in Figure 2. A repeat c-TESE was performed for 16 patients, with 12 positive sperm retrievals at the repeat procedure. Donor sperm was used for ICSI in 23 of the patients with spermatogenic failure and none of the patients with a post-testicular cause. Donor sperm was used in seven men with positive sperm retrieval and 16 men with negative sperm retrieval. The outcomes following c-TESE are shown in Table 2.

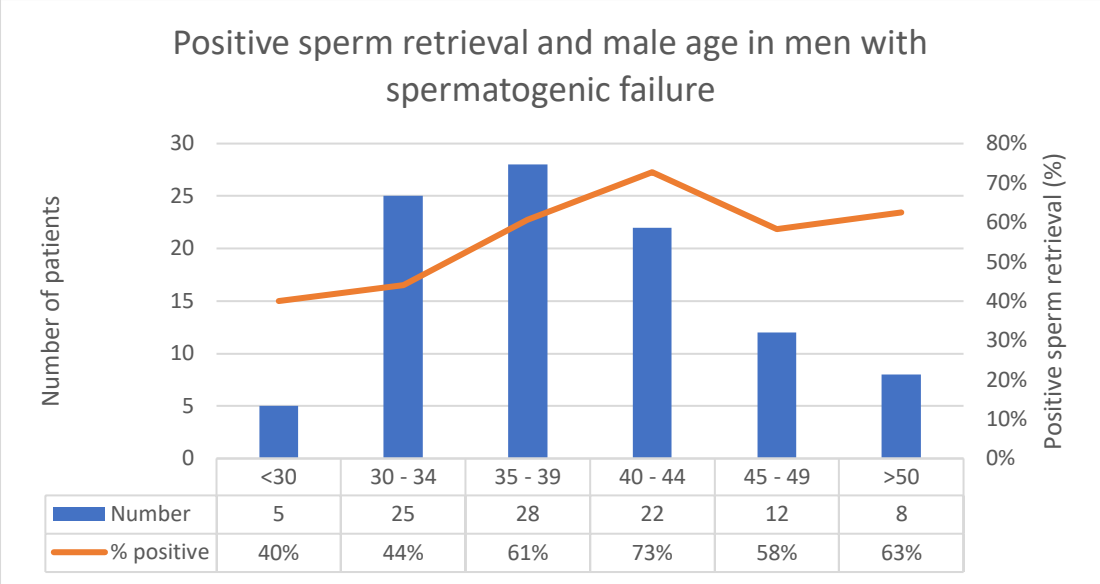


Figure 2: Graph showing sperm retrieval rates in spermatogenic failure group according to male age

Table 2: Sperm retrieval and pregnancy outcomes following c-TESE and ICSI

	Overall	Spermatogenic failure			Post-testicular		
		Total	(+) SR	(-) SR	Total	(+) SR	(-) SR
c-TESE (n)	163	100	58	42	63	63	0
ICSI performed (n)	115	67	51	16	48	48	0
Biochemical pregnancy (n) (Donor sperm) (n)	92 (23)	54 (23)	40 (7)	14 (16)	38 (0)	38 (0)	n/a (0)
Clinical pregnancy (n)	78	45	33	12	33	33	n/a

(cTESE = conventional testicular sperm extraction; (+) SR = positive sperm retrieval; (-) SR = negative sperm retrieval; ICSI = intracytoplasmic sperm injection; n/a = not applicable)

Pregnancy outcomes following ICSI

Intracytoplasmic sperm injection was performed in 115 of the 163 couples (70.6%) with a clinical pregnancy rate (CPR) following ICSI of 67.8% (78 out of 115). A total of 206 ICSI cycles were

performed with a single cycle in 61 couples (53% of couples) and multiple cycles, defined as more than one cycle, in 54 couples (47% of couples). Pregnancy rates were 62.3% (38 of 61) following a single cycle and 27.6% per cycle following multiple ICSI cycles. This resulted in 57 documented live births and a loss to follow up of 21 ongoing clinical pregnancies. The biochemical and clinical pregnancy rates are shown in Table 3.

Table 3: Pregnancy outcomes following c-TESE and ICSI

	Biochemical pregnancy (Pregnancy rate per cycle)	**Clinical pregnancy (Pregnancy rate per cycle)**
c-TESE (total = 163)	92 (56.4%)	78 (47.9%)
ICSI performed (total = 115)	92 (80.0%)	78 (67.8%)
Single ICSI cycle (n=61)	45 (73.8%)	38 (62.3%)
Multiple ICSI cycles (total = 145)	47 (32.4%)	40 (27.6%)
2 cycles (n=30)	26 (43.3%)	22 (36.7%)
3 cycles (n=14)	12 (28.6%)	11 (26.2%)
4 cycles (n=7)	6 (21.4%)	4 (14.3%)
5 cycles (n=3)	3 (20.0%)	3 (20.0%)

(c-TESE = conventional testicular sperm extraction; ICSI = intracytoplasmic sperm injection)

Discussion

The most common cause for male infertility was a previous vasectomy in 53 men (32.5%). Of these, only 11 (20.8%) had undergone an attempt at vasectomy reversal. Vasectomy is the commonest cause of acquired vasal obstructive azoospermia and the most common reason for a male to present to a fertility centre.[11,12] Vasectomy reversal has a reported probability of patency of 81% and was found to be the most cost effective treatment option for vasectomy-induced obstructive azoospermia and, in addition to this, it allows for multiple future spontaneous pregnancies.[13,14] The

low utilization of this treatment option in our study population may be attributable to perceived increased cost of vasectomy reversal compared to c-TESE, the obstructive interval post vasectomy, the desire for only a single future pregnancy or the lack of microsurgical expertise required to perform a vasovasostomy or vaso-epididymostomy.[15] Further studies are needed to explore these possibilities in our setting.

A history of cancer was identified in eight men, seven of whom received chemotherapy with or without surgery and one underwent surgery only. Of these eight men, four had undergone an orchidectomy for testicular cancer. Testicular cancer is associated with pre-treatment infertility, which may be further affected following surgery, chemotherapy or radiation. [16] Other acquired causes included two men with ejaculatory dysfunction due to spinal cord injury, two men with severe erectile dysfunction and two men with retrograde ejaculation. Both patients with spinal cord injury had unsuccessful penile vibratory stimulation and no viable sperm was obtained from the urine in both patients with retrograde ejaculation. In terms of congenital causes for infertility, 17 (10.4%) of patients gave a history of undescended testes, however it was not documented at what age corrective surgery took place or whether this was unilateral or bilateral. Undescended testes are the most common genital abnormality in male infants and its association with infertility is well described. [2] Factors which influence paternity and infertility rates include whether the undescended testes have been corrected, the age at which the correction took place and whether the undescended testes were unilateral or bilateral. After treatment of unilateral undescended testis the incidence of azoospermia is 13% compared to 80% in untreated, bilateral undescended testes. [2] Another congenital disorder identified was cystic fibrosis with CBAVD being found in two men. Congenital bilateral absence of the vas deferens is the most common congenital cause of vasal obstruction and occurs in approximately 1% of infertile men. [2,12]

Mean FSH levels were 11.14 ±7.80 in men with spermatogenic failure and 4.77IU/l ±3.54 in men identified to have a post-testicular pathology. In the former group, a significantly higher mean FSH was found in patients with negative sperm retrieval (15.4IU/l±7.67) compared to positive sperm retrieval (6.50IU/l±4.74), p<0.0001. In our study, FSH was shown to have an area under the ROC score of 0.8239. A test with a good fit and predictive value, i.e FSH level being able to predict positive sperm retrieval, will have an AUC approaching 1. An AUC of 0.5 would mean that the test has no predictive value.

Spermatogenesis is dependent on a functionally intact hypothalamic-pituitary-testicle axis and as a result, an elevated FSH level with small testes can be used to identify men with non-obstructive azoospermia. [17] However, in men with an identifiable obstructive or post-testicular cause, FSH is less important in the diagnostic evaluation as there is no interruption to this hypothalamic-pituitary-testicle axis. This may, in part, explain the low utilisation of FSH in our study in the evaluation of the group of men with a post-testicular cause of infertility. In men requiring surgical sperm retrieval, elevated serum FSH has been investigated as a predictor of sperm retrieval rates. Despite conflicting findings, FSH has been shown to have a poor predictive value for sperm retrieval in non-obstructive azoospermia with sperm retrieval occurring in cases with markedly raised FSH levels.[18–21] In our study, sperm retrieval rates were found to be highest in the age group of 40 – 44 years . Published data show conflicting results regarding male age as a predictor of sperm retrieval. [22,23] One study of m-TESE found higher SRR in men over the age of 50 (73%) compared to men under 50 (56%). [22]

Microdissection TESE for non-obstructive azoospermia is reported to have a higher sperm than c-TESE, with various studies reporting positive retrieval rates of 32.2% - 65.5% compared to 16.7% - 47%, respectively.[9,10,18] Our study showed a SRR of 58% (58 out of 100) in men with spermatogenic failure. SRR was 100% (63 out of 63) in men with a post-testicular cause of infertility. This is compared to the 90% SRR in obstructive azoospermia reported by Esteves et al. [3] However, due to

the heterogenous aetiology of male infertility in our cohort, direct comparison with other published SRR is not possible. Conventional TESE is performed under conscious sedation and generally takes 20 to 30 minutes, while m-TESE is performed under general anaesthesia and takes two to four hours. Microdissection TESE also requires the availability of microsurgical equipment and expertise.[5,9,10] In patients with vasectomy induced azoospermia, percutaneous epididymal sperm aspiration (PESA) could be considered as it is less invasive, does not require surgical exploration or microsurgical expertise or equipment and has equivalent SRR to m-TESE (90%) if used appropriately.[3]

The clinical pregnancy rate (CPR) for all patients undergoing c-TESE was 47.9% (78 out of 163), and 67.8% (78 out of 115) following ICSI. Not all patients that had positive sperm retrieval at c-TESE went on to have ICSI. The reasons for the 22 patients with positive sperm retrieval not undergoing ICSI were not identified. Donor sperm was used for ICSI in 23 patients with spermatogenic failure and none of the patients with a post-testicular aetiology. Of these 23 patients who used donor sperm, seven had positive sperm retrieval at c-TESE and 16 had negative sperm retrieval. The use of donor sperm allows men with negative sperm retrieval, or men who have positive sperm retrieval with either poor quality sperm or failed ICSI with their own sperm, the potential of achieving pregnancy and ultimately a live birth. A single ICSI cycle was done in 61 couples with pregnancy rates of 62.3%, while 54 couples had multiple ICSI cycles with a pregnancy rate of 27.6% per cycle. van Wely et al. reported CPR of 30% following m-TESE/ICSI in patients with OA, while Eken et al. reported CPR of 44.6% following m-TESE/ICSI in patients with NOA. [14,24] The CPR in our study may be affected by a number of factors, including patients with post-testicular pathology being incorrectly classified as spermatogenic failure, the fact that not all patients who had positive SRR went on to have ICSI, the use of donor sperm for ICSI as well as the high proportion of men with a post-testicular cause in this cohort.

Limitations of this study

The retrospective nature of this study meant that certain information, including testicular volume, FSH levels and live birth details, may be missing. Patients may, therefore, be misdiagnosed as non-obstructive resulting in over-estimated retrieval rates. Also, although the same procedure is followed, the sperm retrieval operation is performed by different Reproductive Medicine specialists which may result in variations in retrieval rates. Additionally, this study population is limited to patients who are able to afford fertility treatment and is therefore not representative of the entire South African population. The heterogenous aetiology of male infertility in this patient cohort makes comparison with other studies difficult.

Conclusions

In this South African review, sperm retrieval rates of c-TESE were similar to published results and clinical pregnancy rates of 67.8% were achieved for couples undergoing ICSI. This information is valuable for counselling patients who are embarking on their fertility journey. Further studies around the evaluation of male infertility and the appropriate use of sperm retrieval techniques are needed in our setting.

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Appendix A: Approved Research Protocol

Protocol not for examination purposes as per University guidelines

MMed Research Proposal

Outcomes of patients undergoing conventional testicular sperm extraction for intracytoplasmic sperm injection in a South African fertility centre

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List of abbreviations

ART	Assisted reproductive techniques
c-TESE	Conventional testicular sperm extraction
FSH	Follicle stimulating hormone
HS	Hypospermatogenesis
ICSI	Intra-cytoplasmic sperm injection
MA	Maturation arrest
m-TESE	Microdissection sperm extraction
NOA	Non-obstructive azoospermia
NP	Non-progressive
OA	Obstructive azoospermia
PR	Progressive
SCO	Sertoli cell only
SR	Sperm retrieval
SRR	Sperm retrieval rates
STD	Sexually transmitted disease
TESE	Testicular sperm extraction
WHO	World Health Organisation

1. Introduction

Infertility is the inability of a sexually active, non-contracepting couple to achieve a spontaneous pregnancy in one year and affects approximately 8 – 15% of reproductive-aged couples worldwide. [1,2] Female causes of infertility include ovulatory dysfunction and utero-tubal factors. [3] Female age over 40 is also associated with a significant reduction in fertility. [2,3] A male factor for infertility is present in 20 – 50% of couples seeking fertility treatment and, of those, azoospermia accounts for 10 – 15% of cases. [1,2,4]

Azoospermia is the absence of spermatozoa in the ejaculate and is classified as either pre-testicular, testicular (primary testicular failure) or post-testicular. [4-6] Post-testicular causes of azoospermia are due to obstruction or disorders of ejaculation. [6] Obstructive azoospermia (OA) is due to intra-testicular obstruction or conditions involving the epididymis, vas deferens or ejaculatory ducts. This may be congenital such as congenital bilateral absence of the vas deferens (CBAVD) associated with cystic fibrosis or acquired such as sexually transmitted diseases, vasectomy, or due to trauma or surgery. [1,6] Treatment for infertility associated with OA involves surgical correction of the obstruction, if possible, or sperm retrieval followed by assisted reproductive techniques (ART). [1,6,7]

Non-obstructive azoospermia (NOA) accounts for approximately 60% of patients with azoospermia and has traditionally posed a significant

challenge to both those seeking and providing fertility services. [4,8,9] NOA is caused by pre-testicular hypothalamic-pituitary disease, primary spermatogenic (testicular) failure or genetic disorders. [1,6] Conditions associated with NOA include acquired causes (pituitary tumours, testicular torsion, trauma, mumps orchitis and varicoceles) and congenital disorders (undescended testes or genetic abnormalities such as Kallmann syndrome, Klinefelter's syndrome and Y-chromosome microdeletions). [1,6,10] For men with NOA and primary spermatogenic failure, sperm retrieval and biopsy has become the standard of care in order to establish a histological diagnosis and retrieve sperm at the same setting for use in ART. [1,4,9,11]

1.1. Testicular sperm extraction (TESE)

Sperm retrieval via testicular sperm extraction (TESE) followed by intra-cytoplasmic sperm injection (ICSI) was first described in 1993 as treatment for infertility due to obstructive azoospermia. [12,13] Since then, TESE for ICSI has become widely accepted and implemented as the first line treatment for men with NOA. [4,7,9] Successful sperm retrieval with TESE can be achieved in men with NOA because of isolated foci of active spermatogenesis within the testes. [1,9,14]

Sperm retrieval rates (SRR) with conventional TESE (c-TESE) are between 16.7% and 47% [7,14-17] while Gnessi *et al.,* reported successful sperm retrieval with c-TESE of 63% in their study. [11] SRR with microdissection

TESE (m-TESE) are generally higher than c-TESE with success rates between 32.2 and 65.5%. [4,5,9,14,16,17]

1.2. Predicting successful sperm retrieval – FSH levels and histopathology

Fertility treatment is an emotionally and financially demanding journey. Being able to inform patients of success rates of TESE for ICSI is important in the counselling of couples. Several factors have been investigated for predicting successful sperm retrieval in men with azoospermia. These include, but are not limited to, patient age, testicular volume, Follicle Stimulating Hormone (FSH) level and histological diagnosis. [4,5,7,9,11] Of these, histological findings are generally the most accurate for predicting successful TESE.

Classically, because spermatogenesis is dependent on a functionally intact hypothalamic-pituitary-testicle axis, an elevated serum FSH level was thought to be a useful predictor of impaired spermatogenesis that could be used to select which patients would benefit from TESE. However, FSH concentration has been found to have a poor predictive value for successful TESE, with sperm retrieval even occurring in cases with markedly raised FSH levels. [5,7,14,18,19] Thus it is not recommended to exclude a patient based on the FSH level.

The histological pattern identified from testicular biopsy or at TESE has some predictive value of successful sperm retrieval. [4,7,9,18] The germ cell abnormalities are classified as hypospermatogenesis (HS) – all cell types up to spermatozoa are present but there is a reduction in the degree of normal spermatogenesis; maturation arrest (MA) – incomplete spermatogenesis, not beyond the spermatocyte stage; Sertoli cell only (SCO) – the absence of germ cells; and seminiferous tubule hyalinization – absence of seminiferous tubules. [7,20] The SRR for hypospermatogenesis is more favourable than maturation arrest which is in turn more favourable than SCO, with reported rates of 81%, 44% and 41% respectively. [7,9,18] This suggests that a testicular biopsy should be done prior to TESE. However, the issue of performing a pre-operative biopsy is controversial as it has a poor predictive value and may cause inflammatory changes, haematoma, parenchymal fibrosis or devascularization of the testis. [21,22]

1.3. Outcomes – pregnancy and live birth rates

There are a limited number of studies regarding the long term follow up and outcome of couples after undergoing TESE. Dafopoulos *et al.*, had a clinical pregnancy rate of 37.6% while Vloeberghs *et al.*, and Eken *et al.*, had clinical pregnancy rates of 49.4% and 44.6% and live birth rates of 36.7% and 28.3% respectively. [9,23,24] The clinical pregnancy rate per ICSI cycle is reported at 20.9 – 21.7%. [23,24] Some studies differentiate clinical pregnancy rates according to whether the underlying cause for

azoospermia was obstructive or non-obstructive. Palermo *et al.*, Adbel Raheem *et al.*, reported clinical pregnancy rates for men with OA of 57.1% and 37% respectively but only 49.1% and 32% for men with NOA while De Croo *et al.*, found no difference between OA and NOA. [25-27] The overall live birth rates for a couple that undergoes TESE is between 13.4 and 23%. [9,23,27]

1.4. Rationale for the study

This will be the first study in South Africa investigating FSH levels, histopathological findings at TESE and clinical outcomes from TESE and ICSI. Conventional TESE, rather than microdissection TESE, is performed at Vitalab and testicular tissue is only sent for histopathological assessment if no sperm are retrieved at TESE. Occasionally, donor eggs or donor sperm may be used for the ICSI cycle. Adequate counselling on clinical pregnancy outcomes is essential in order for a couple to make an informed decision. This study aims to fill the gaps in our knowledge about TESE and ICSI and equip patients and Reproductive Medicine Specialists with the necessary information to make informed decisions and provide appropriate care.

2. Study aim and objectives

2.1. Aim

The aim of this study is to evaluate the sperm retrieval rates, pregnancy rates and live birth rates of patients undergoing conventional TESE and ICSI at a South African fertility centre.

2.2. Objectives

2.2.1. To describe the demographic information of patients undergoing TESE and ICSI

2.2.2. To compare the FSH levels of patient who have positive sperm retrieval with those that have negative sperm retrieval

2.2.3. To determine if there is an FSH level which can predict unsuccessful sperm retrieval

2.2.4. To describe the histopathological pattern identified in patients with unsuccessful sperm retrieval

2.2.5. To compare the success rate of conventional TESE at Vitalab with the success rate of microdissection TESE in the literature

2.2.6. To determine the pregnancy rates and live birth rates of patients undergoing c-TESE and ICSI and compare this with literature

3. Methods

3.1. Study design

A retrospective record-review cohort study.

3.2. Study setting

The study will be done at Vitalab Centre for Assisted Conception, a private fertility clinic situated in Sandton, South Africa. There are six Reproductive Medicine Specialists that perform TESE at Vitalab. All patients that underwent TESE and ICSI will have their records reviewed to determine FSH levels, sperm analysis, sperm retrieval rates, histological patterns, whether ICSI was performed and the clinical outcomes.

3.3. Study population

All patients who underwent ICSI at Vitalab Sandton between 01 October 2016 and 30 September 2019. An estimated number between four and eight TESE and ICSI cycles are performed each month at Vitalab.

3.3.1. Inclusion Criteria:

All patients that underwent ICSI cycles with TESE sperm, with or without donor sperm and donor eggs

3.3.2. *Exclusion Criteria:*

Female patients over the age of 40 years

3.4. Data collection

All patients who underwent TESE during the study period will be identified. Patients eligible according to the inclusion and exclusion criteria will be included in this study. Their clinic records will be accessed in order to

capture the relevant data onto the data collection tool (Appendix A). The following data will be collected: patient age, race, medical and reproductive history, FSH level, semen analysis results, presence or absence of sperm, histopathological pattern identified, whether ICSI was performed and how many cycles, subsequent pregnancy and live birth outcomes. Data will be entered into the REDCAP data capture program.

4. Data analysis

Data collected will be analysed using STATA software with the help of a statistician from the University of the Witwatersrand. Descriptive data will be analysed to determine the prevalence of variables, medians and means. Analytical statistics and logistic regression will be used to determine any association between these variables and the outcomes. A p value of 0.05, odds ratio and 95% confidence intervals will be used. Normally distributed data will be analysed using parametric tests such as Student's T-test, ANOVA and Chi-square. Non-parametric tests will be used for non-normal data.

5. Ethics

All patients at Vitalab sign a consent form prior to starting their fertility treatment whereby they give consent for their data to be used on an anonymous basis for research purposes (Appendix B). Patient anonymity will be further ensured by only using file numbers and by the researcher

signing a non-disclosure agreement. Study information will be accessed via the Vitalab database with username and password-controlled computers. Ethics approval will be obtained from the University of the Witwatersrand Human Research Ethics Committee. Data collection will only commence once permission to conduct the study is obtained from Vitalab and an ethics clearance number is supplied.

6. Funding

Limited costs, such as printing and stationary, are anticipated in this study and all expenses will be funded by the researcher.

7. Limitations

This study is a retrospective review of patient records and data may be incomplete. There are six reproductive medicine specialists that have performed c-TESE at the clinic during the defined study period. The different clinicians may have different levels of expertise regarding TESE which may influence SRR. Most cases of TESE differentiate between obstructive azoospermia and non-obstructive azoospermia but in this study we will include all patients that have undergone TESE, regardless of the cause of azoospermia. Tissue is not routinely sent for histopathological assessment, only if no sperm are retrieved. Once a clinical pregnancy has been confirmed, patients are referred to their obstetrician for the remainder of their obstetric care. This may impact on follow up of live births.

8. Study timing

	Mar 20	Apr 20	May 20	Jun 20	Jul 20	Aug – Oct 20	Nov 20	Dec 20	Jan 21
Literature review									
Protocol									
Protocol deadline			13th						
Protocol assessment				10th					
Ethics									
Data Collection									
Data analysis									
Write up									
Submission / publication									

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Protocol appendix A: Data collection tool

1. Patient details and medical history

File number		
Study number		
Male age		
Race	Asian	Black
	Coloured	Indian
	White	Other
Height (cm)		
Weight (kg)		
BMI (kg/m^2)		
Co-morbidities, medication:		
Social habits	Smoking	Alcohol
	Recreational drugs	None
	Other:	
Erectile dysfunction	Yes	No
Previous STD	Yes	No
If yes, details:		
Previous surgery	Vasectomy	Varicocele
	Hernia	Testicular torsion
	Undescended testes	
	Other:	
Chemotherapy	Yes	No
If yes, details:		
Previous mumps	Yes	No
If yes, details:		
Number of children		

Hormonal or infertility medication:		
Female age		
Race	Asian	Black
	Coloured	Indian
	White	Other
Height (cm)		
Weight (kg)		
BMI (kg/m^2)		
Co-morbidities, medication:		
Social habits	Smoking	Alcohol
	Recreational drugs	None
	Other:	
Female partner: Gravidity		
Female partner: Parity		
Hormonal or ovulation medication:		
Cause of infertility, details:	Ovarian	
	Uterine	
	Tubal	
	Unknown	
	Other:	

2. <u>FSH level and semen analysis</u>

FSH (ug/dL)		
Semen analysis	Yes	No
Sexual abstinence (days)		
Semen volume (mL)		

Total sperm number (10^6/ejaculate)	
Sperm concentration (10^6/mL)	
Total motility (PR+NP)	
Progressive motility (%)	
Vitality (live spermatozoa %)	
Normal sperm morphology (%)	

3. TESE / ICSI results and outcomes

Sperm retrieval	Yes	No
Histological pattern	N/A	Normal
	Hypospermatogenesis	Maturation arrest
	Sertoli cell only	Seminiferous tubule hyalinisation
	Other/details:	
Number of donor egg cycles		
ICSI performed	Yes	No
Number of ICSI cycles		
Duration between cycles (days)		
Pregnancy	Yes	No
If yes, details:	Biochemical	Clinical
	Other:	
Live birth	Yes	No
If yes, details:	Singleton	Twins
	Other:	

Protocol appendix B: Vitalab consent form for research

The Inner Circle, 159 Rivonia Road, Morningside, 2196 | P.O. Box 652837, Benmore, 2010
Tel: (011) 911 4700 or 0861 882522 | Fax: 0865100951
www.vitalab.com | info@vitalab.com

3.17

3.18 We understand that, should the results of our treatment and/or any aspect of it be published in medical or scientific journals, all possible precautions will be taken to protect our anonymity. We grant permission to Vitalab to publish in professional journals relating to our case, provided our names are withheld.

3.19 In light of our consented participation in the program, we wish to commence with treatment and:

3.19.1 Consent that all information supplied by us in connection with the removal and withdrawal of our gamete(s) be updated in the central data bank;

3.19.2 We have previously made a donation or had similar treatment.

Yes		No	

Initials: _____ _____

If yes, please fill in the details below.

In the event that we have previously made a donation of our gamete(s) (sperm or eggs) or undergone similar treatment, such donation took place on the _____ (date) at _____ (place).

Initials: _____ _____

3.19.3 We consent to a competent person of Vitalab to perform:

3.19.3.1 A physical examination and to complete a full medical history questionnaire;

3.19.3.2 To remove or withdraw the gamete(s) for testing, analysis or any other process as such competent person of Vitalab might deem necessary under the circumstances;

Appendix B: Ethics clearance certificate

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R14/49 Dr N Brits

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M200744

NAME: (Principal Investigator)	Dr N Brits
DEPARTMENT:	School of Clinical Medicine Department of Surgery Division of Urology Medical School University
PROJECT TITLE:	Outcomes of patients undergoing conventional testicular sperm extraction for intracytoplasmic sperm injection in a South African fertility centre
DATE CONSIDERED:	2020/07/31
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Drs Y Unterslak & M Nel, Ms M Marais
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2020/09/04

This clearance certificate is valid for 5 years from the date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the 3rd Floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.
I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **July** and will therefore reports and re-certification will be due early in the month of **July** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature — Date: 25/05/2022

Appendix C: Turn-it-in report

08901071:Brits_Final_for_Turnitin.docx

ORIGINALITY REPORT

10%	5%	8%	1%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Alrabeeah, K., A. Wachter, S. Phillips, B. Cohen, N. Al-Hathal, and A. Zini. "Sperm retrieval outcomes with microdissection testicular sperm extraction (micro-TESE) in men with cryptozoospermia", Andrology, 2015. Publication	1%
2	"Abstracts of the 34rd Annual Meeting of the European Society of Human Reproduction and Embryology", Human Reproduction, 2018 Publication	1%
3	journals.lww.com Internet Source	1%
4	edoc.ub.uni-muenchen.de Internet Source	1%
5	www.fortunejournals.com Internet Source	1%
6	A Gudeloglu, SJ Parekattil. "Update in the evaluation of the azoospermic male", Clinics, 2013 Publication	1%

7	haematologica.org Internet Source	1%
8	Alrabeeah, K., R. Doucet, E. Boulet, S. Phillips, N. Alhathal, F. Bissonnette, I. J. Kadoch, and A. Zini. "Can the rapid identification of mature spermatozoa during microdissection testicular sperm extraction guide operative planning?", Andrology, 2015. Publication	<1%
9	Takashi Imamoto, Hiroyoshi Suzuki, Tomohiko Ichikawa, Haruo Ito, Yoko Kawana, Yoshio Shiseki, Haruo Akama, Masafumi Naito. "Testicular sperm extraction with intracytoplasmic sperm injection for male infertility", Reproductive Medicine and Biology, 2003 Publication	<1%
10	www.nature.com Internet Source	<1%
11	www.thebridgeclinic.com Internet Source	<1%
12	dergipark.ulakbim.gov.tr Internet Source	<1%
13	Alaaeldien Mohamed Abdelmoniem Abdelaal, Hatem Mohamed El - Azizi, Sameh Fayek GamalEl Din, Omar Azzazi, Mohamed Shokr	<1%

	Mohamed. "Evaluation Of The Potential Role Of Shear Wave Elastography As A Promising Predictor Of Sperm Retrieval In Non - Obstructive Azoospermic Patients: A Prospective Study", Andrology, 2021 Publication	
14	"British Fertility Society the University of Sheffield 15–17 April 1998", Human Fertility, 2009 Publication	<1 %
15	Hatsuki Hibi, Tomohiro Taki, Yoshiaki Yamada, Nobuaki Honda, Hidetoshi Fukatsu, Masanori Yamamoto, Yoshimasa Asada. "Testicular sperm extraction using microdissection for non-obstructive azoospermia", Reproductive Medicine and Biology, 2002 Publication	<1 %
16	Luca Boeri, Franco Palmisano, Mirko Preto, Mattia Sibona et al. "Sperm retrieval rates in non - mosaic Klinefelter patients undergoing testicular sperm extraction: What expectations do we have in the real - life setting?", Andrology, 2020 Publication	<1 %
17	Wael Almajed, Mohannad Alharbi, Armand Zini. "Use of mini-incision micro-dissection testicular sperm extraction in men with	<1 %

Appendix D: Plagiarism declaration



PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I Nicholas Brits (Student number: 2079249) am a student registered for the degree of MMed (Urology) in the academic year 2021.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature: _____ Date: 27/02/2022

1

Appendix E: Vitalab consent form

The Inner Circle, 159 Rivonia Road, Morningside, 2196 | P.O. Box 652837, Benmore, 2010
Tel: (011) 911 4700 or 0861 882522 | Fax: 0865100951
www.vitalab.com | info@vitalab.com

3.17

3.18 We understand that, should the results of our treatment and/or any aspect of it be published in medical or scientific journals, all possible precautions will be taken to protect our anonymity. We grant permission to Vitalab to publish in professional journals relating to our case, provided our names are withheld.

3.19 In light of our consented participation in the program, we wish to commence with treatment and:

3.19.1 Consent that all information supplied by us in connection with the removal and withdrawal of our gamete(s) be updated in the central data bank;

3.19.2 We have previously made a donation or had similar treatment.

Yes		No	

Initials: _____ _____

If yes, please fill in the details below.

In the event that we have previously made a donation of our gamete(s) (sperm or eggs) or undergone similar treatment, such donation took place on the _____ (date) at _____ (place).

Initials: _____ _____

3.19.3 We consent to a competent person of Vitalab to perform:

3.19.3.1 A physical examination and to complete a full medical history questionnaire;

3.19.3.2 To remove or withdraw the gamete(s) for testing, analysis or any other process as such competent person of Vitalab might deem necessary under the circumstances;