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ORIGINAL PAPER

Does the time from spinal cord injury affect the sperm retrieval rate in testicular sperm extraction? A multicenter cross-sectional study

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Summary

***Background:** People with spinal cord injury (SCI) may suffer from anejaculation due to functional obstructive azoospermia (OA). Testicular sperm extraction (TESE) may successfully overcome this problem, even if the optimal timing is controversial.*
***Objectives:** The primary aim of this study was to report our experience with TESE in SCI, focusing on the effect of time since SCI event on the TESE outcomes.*
***Materials and methods:** We included all consecutive people with SCI and functional OA undergoing TESE between January 2011 and December 2021 in four Italian tertiary referral centers.*
We recorded TESE sample parameters, sperm retrieval rate (SRR) and intracytoplasmic sperm injection (ICSI) outcomes. Logistic regression analysis was performed to assess whether time since SCI was significantly associated with these outcomes. The time since SCI was considered in three different ways: (1) continuously; (2) ≤ 9 years vs. > 9 years; (3) ≤ 5 years, > 5 and ≤ 10 years, > 10 years.
***Results:** We included 32 patients with tetraplegia and 75 with paraplegia, undergoing 107 TESE procedures. The median age at surgery and time since SCI were 33 years (IQR 29-38) and 9 years (IQR 3-14), respectively. The SRR was 81.3%. Thirty-three out of 87 patients underwent ICSI, achieving pregnancy in 63.6% after one cycle. The final live birth rate was 90.5%. Logistic regression analyses outlined that the SRR was not affected by considered variables, including time since SCI, considered both continuously and categorically.*
***Conclusions:** Our SRR did not prove to be negatively affected by all considered variables, specially by the time since SCI. Clinicians should not deter SCI patients with functional OA from undergoing TESE after long time since SCI.*

KEY WORDS: *Infertility; Ejaculatory dysfunction; Testicular sperm extraction; Spinal cord injury.*

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INTRODUCTION

Spinal cord injury (SCI) is a life-changing event, associated with a significant impact on patients' lives and healthcare systems (1). SCI is provoked by traumas in more than 90% cases, presenting a male-to-female ratio of 2:1 and a bimodal age distribution with the involvement of young adults and adults over the age of 60 (2). Therefore, SCI may involve young men at the peak of their reproductive needs (3). Indeed, fertility issues represent a significant need to address for all healthcare professionals involved with SCI (4).
This population may experience different-graded alterations in sexual and reproductive functions, presenting with a combination of *erectile dysfunction* (ED), ejaculatory dysfunction and abnormal semen parameters (5). The literature about the fertility of men with SCI has not showed univocal data concerning sperm quantitative and/or qualitative alterations (6). Moreover, the effect of time since SCI is controversial.
In the case of retrograde ejaculation or anejaculation, a condition that leads to a functional *obstructive azoospermia* (OA), a stepwise approach of *penile vibratory stimulation* (PVS), followed by *electroejaculation* (EEJ) may achieve ejaculation in most patients (7). In the rest of cases, a *testicular sperm extraction* (TESE) may be performed to diagnose spermatogenic failure and/or extract spermatozoa for cryopreservation (8).
The aim of our study was to report the data from our SCI patients undergoing TESE, focusing on the effects of time since SCI in TESE outcomes.

METHODS

This study was designed as cross-sectional, retrospective, multicentric. It was conducted in accordance with the

Declaration of Helsinki through all phases. The authors retrospectively collected data from different Italian tertiary referral centers: 1) *Unit of Neuro-Urology, Unipolar Spinal Unit, Niguarda Hospital, Milan, Italy*; 2) *Unit of Neuro-Urology, Città della Salute e della Scienza, University of Turin, Turin, Italy*; 3) *Unit of Neuro-Urology, Careggi University Hospital, Florence, Italy*; 4) *Unit of Urology, Department of Neurosciences, Reproductive Sciences*, and *Odontostomatology, University of Naples "Federico II", Naples, Italy*. We included all patients who underwent TESE from January 1st, 2011, to December 31st, 2021. The biologists of each Fertility Center analyzed all testicular samples, while *assisted reproductive technology* (ART) was not always realized by the same hospital.

Inclusion and exclusion criteria, data collection, variable and outcome definition

We included all consecutive patients with SCI aged more than 18 years old, affected by OA and requiring TESE. Most of them were refractory to PVS. We excluded cases with anemia, immunological and/or coagulation disorders, infections by *human immunodeficiency virus* (HIV), *hepatitis C virus* (HCV), *hepatitis B virus* (HBV), and Treponema pallidum to avoid possible confounding biases. All patients underwent scrotal *ultrasound* (US) and blood test to assess hormonal profile, evaluating total testosterone, follicle-stimulating hormone, luteinizing hormone and prolactin. We included only cases with normal testis volumes and with hormonal values within the laboratory-specific normal ranges to exclude potential biases.

Two authors independently collected the data; each conflict was solved by a senior author. We included couples with only male factor infertility, after a comprehensive gynecological assessment of the female partner. We collected the following pre-operative parameters: SCI level, age at surgery, time since SCI, bladder management, other *genitourinary* (GU) pathologies, smoking status. We also collected data on bladder management screening for *clean intermittent catheterizations* (CICs), *antimuscarinic therapy* (AMT), periodical detrusor muscle injections of *botulinum toxin-A* (BTX-A) (9). We defined bladder management as "not optimal" in case of recurrent *urinary tract infections* (UTIs), *male accessory gland infections* (MAGIs), urine leakages, *vesicoureteral reflux* (VUR) or other possible complications related to the GU system. We collected data on any surgical procedures involving GU system (10). We collected data on the presence of grade III varicocele, which was defined as "*visible and palpable at rest*" according to the standard clinical classification by the examining physician (11). Our aim primary endpoint was to analyze the effect of time since SCI on TESE outcomes. Time since SCI was defined as time from SCI event to the TESE date. We reported the testicular sample descriptive parameters at TESE: number of frozen straws retrieved, number of spermatozoa per *high power field* (HPF), motility and morphology (12). The *sperm retrieval rate* (SRR) was defined as the retrieval rate of spermatozoa suitable for *intracytoplasmic sperm injection* (ICSI). On May 2022, one author performed a phone interview to assess the number of patients who underwent ART up to December 2021, collecting the number of ICSI cycles, the *pregnancy rate* (PR) per couple, and the *live birth rate* (LBR) (13).

Surgical procedure

All patients underwent a conventional TESE, without the use of operating microscope (14). The procedure was performed under general or local anesthesia with Lidocaine 1%. All surgeries were carried out by the experienced surgeon. We performed a small horizontal incision in the median part of the scrotum on the side decided pre-operatively according to dimensions and US evaluations (e.g., vascularization, preserved parenchymal architecture). The skin, dartos muscle, and tunica vaginalis were opened. Once exposed, we performed a 5 mm incision of the tunica albuginea at the middle of the testis. Multiple testicular specimens were excised and sent to our embryologists. We excised very small portions of testicular parenchyma to decrease morbidity in our frail patients, so we sent just the material necessary for ART, and no tissue specimens for histopathological examination (15). Absorbable sutures were later performed to close tunica albuginea, tunica vaginalis and dartos, while the skin was sutured with silk or absorbable thread.

Sperm identification procedure

Sperm identification procedures were very similar across considered centers. By way of example, we reported the procedure at the Niguarda Hospital, Milan. All the testicular samples were preliminarily comminuted with folded needles of tuberculin syringes and then resuspended with 5 mL *human serum albumin* (HSA) and recombinant human insulin medium (*ORIGIO®, Måløv, Denmark*) in the laboratory. Subsequently all testicular samples were subjected to centrifugation at 300 G for 8-10 minutes. Our embryologists carefully examined the samples under the optic microscope to determine the presence of spermatozoa in the pellet (16). In most cases, positive research was completed in about 60 minutes, while extra time was necessary in case of unsuccessful retrieval. The sample was subjected to 1000 IU collagenase (*Gynemed®, Lensahn, Germany*) if spermatozoa were not found immediately. The small sample allowed just a brief description, reporting the number of spermatozoa for 400-fold magnification fields of view (HPF) with microscope and the evaluation of spermatozoa morphology and motility. The collected paillettes were cryopreserved in ORIGIO® medium after freezing with static liquid nitrogen vapor.

Statistical analysis

Data were stored anonymously using Microsoft Excel, Version 15 (*Microsoft Corporation, Redmond, WA, USA*). Another author performed blindly statistical analyses using *Statistical Package for Social Science* (SPSS), Version 28 (*IBM Corporation, Armonk, NY, USA*). We estimated the median and *interquartile range* (IQR) for each quantitative variable when non-normally distributed, while we reported the frequencies for categorical variables.

Patients were stratified into two groups according to the median time between SCI and TESE. Time since SCI was considered continuously and categorically to improve the consistency of our analyses regarding the effects of time since SCI. Initially, we divided our cohort into 3 groups: group A (≤ 5 years), group B (> 5 and ≤ 10 years) and group C (> 10 years). Later, we divided our population into 2 groups based on the median, group D and group

E. Differences between the groups were analyzed using the chi-squared for the categorical variables and the Mann-Whitney U test for continuous variables.
Univariable logistic regression analysis was used to assess the impact of time since SCI on TESE outcomes.
Statistical significance level was determined at p value < 0.05.

Results

We analyzed data from 107 patients (Table 1), affected by tetraplegia (n = 32) and paraplegia (n = 75). The median age at surgery was 33 years (IQR 29-38, range 19-55), while the median time since SCI was 9 years (IQR 3-14, range 0-45). As for bladder management, all patients underwent CICs, 85 (79.4%) took AMT, while 49 (45.8%) patients underwent periodical BTX-A injections into the detrusor muscle. One tetraplegic patient did not tolerate AMT and BTX-A bladder injections. We considered bladder management as not optimal in 39/107 (36.4%) cases.
Ten out of 107 patients (9.3%) reported other GU surgeries in the past: vasectomy (n = 3, 2.8%), hydrocelectomy (n = 2, 1.9%), implantation of adjustable continence therapy (ProACT™) system (n = 1, 0.9%), and endoscopic treatment of vesicoureteral reflux (n = 2, 1.9%) and of stress urinary incontinence with urethral bulking agents (n = 2, 1.9%). We identified (n = 2, 1.9%) cases with severe varicocele. Ten (9.3%) patients reported history of surgery for cryptorchidism. The rate of current smokers was 49.5%.

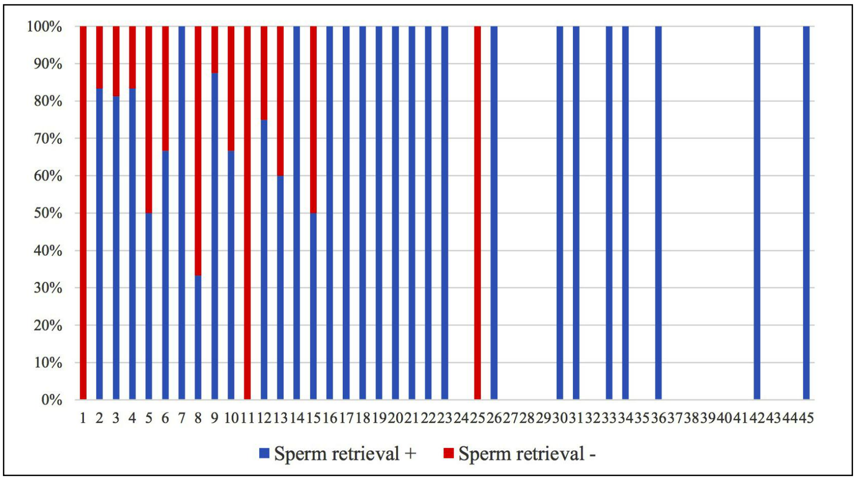
No intra-operative complications occurred in all 107 TESEs. All procedures were performed under local anesthesia but one where general anesthesia was used in a tetraplegic patient suffering from autonomic dysreflexia. No post-operative complications were observed during the considered follow-up.
The overall SRR was 81.3% and we depicted graphically the correlation between the sperm retrieval outcome and the time since SCI by percent stacked barplots (Figure 1).
One paraplegic patient was monorchid due to a testicular abscess and the sperm retrieval was unsuccessful. Another tetraplegic patient presented bilateral testicular microlithiasis and the sperm retrieval was successful.
After dividing our population into 3 groups based on time since SCI, we detected no statistically significant differences (p > 0.05) for all considered baseline characteristics, except for age at surgery (p < 0.001).
Later, we divided our population into 2 groups based on the median of time since SCI: group D (≤ 9 years) and group E (> 9 years). We outlined no statistically significant differences, except for age at surgery (p < 0.001) and cryptorchidism (p = 0.023).
In Table 2 we reported the features in TESE samples and the ART outcomes in case of positive sperm retrieval (n = 87). Spermatozoa presented progressive motility in 31 (35.6%) cases. We observed a normal sperm morphology in 40/87 (46%) samples.
Considering a median follow-up of 76 months (IQR: 57.5-85.5), 33 couples started ART, by use of ICSI.
The PR per couple was 63.6% after a single cycle.

Table 1.
Characteristics and descriptive statistics of patients according to the time since SCI, whose median was 9 years (range: 3-14). We divided patients into different groups based on time since spinal cord injury: group A (≤ 5 years), group B (> 5 and ≤ 10 years) and group C (> 10 years), besides group D (≤ 9 years) and group E (> 9 years). We estimated statistically significant differences among groups A, B and C, and between groups D and E.

Variable	Overall	Time since SCI						
	(n = 107)	Group A (n = 38)	Group B (n = 28)	Group C (n = 41)	p value	Group D (n = 58)	Group E (n = 49)	p value
Level of injury					0.292			0.261
Tetraplegia No. (%)	32 (29%)	12 (31.6%)	11 (39.3%)	9 (22.0%)		20 (34.5%)	12 (24.5%)	
Paraplegia No. (%)	75 (70.1%)	26 (68.4%)	17 (60.7%)	32 (78.0%)		38 (65.5%)	37 (75.5%)	
Age at surgery years					< 0.001			< 0.001
Median	33	30	32	36		31	35	
IQR	29-38	24.5-36.75	28.25-34.25	33-41		27.25-36	32-41	
Range	19-55	19-48	20-45	26-55		19-48	26-55	
Antimuscarinic therapy No. (%)	85 (79.4%)	29 (76.3%)	23 (82.1%)	33 (80.5%)	0.827	45 (77.5%)	40 (81.6%)	0.606
Detrusor injections of BTX No. (%)	49 (45.8%)	19 (50%)	8 (28.5%)	22 (53.7%)	0.098	23 (39.7%)	26 (53%)	0.166
Other GU surgery* No. (%)	10 (9.3%)	3 (78.9%)	3 (10.7%)	4 (9.8%)	0.921	5 (8.6%)	5 (8.3%)	0.779
History of recurrent UTIs and/or MAGIs No. (%)	24 (22.4%)	5 (13.1%)	8 (28.5%)	11 (26.8%)	0.230	9 (15.5%)	15 (10.2%)	0.062
Not optimal bladder management No. (%)	39 (36.4%)	17 (44.7%)	9 (32.1%)	13 (31.7%)	0.417	25 (43.1%)	14 (28.6%)	0.120
Cryptorchidism No. (%)	10 (9.3%)	2 (5.2%)	3 (10.7%)	5 (12.2%)	0.548	2 (3.5%)	8 (16.3%)	0.023
Varicocele – Grade III** No. (%)	2 (1.9%)	None	1 (3.6%)	1 (2.4%)	0.538	None	2 (4.1%)	0.120
Current smokers No. (%)	53 (49.5%)	16 (42.1%)	15 (53.5%)	22 (53.7%)	0.522	26 (44.8%)	27 (55.1%)	0.290
Sperm retrieval + No. (%)	87 (81.3%)	29 (76.3%)	22 (78.6%)	36 (87.8%)	0.387	46 (79.3%)	41 (20.7%)	0.564

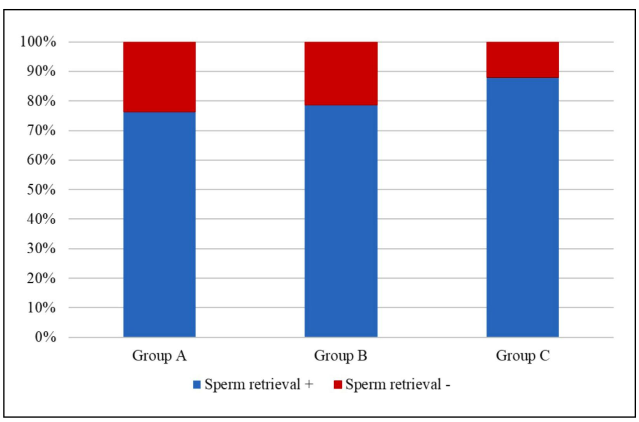
BM: bladder management; BTX: botulinum toxin; GU: genitourinary; IQR: interquartile range; MAGI: male accessory gland infection; SCI: spinal cord injury; SR: sperm retrieval; UTI: urinary tract infection.
** Other GU surgeries were the following: vasectomy (n = 3, 2.8%), hydrocelectomy (n = 2, 1.9%), implantation of adjustable continence therapy (ProACT™) system (n = 1, 0.9%), and endoscopic treatment of vesicoureteral reflux (n = 2, 1.9%) and of stress urinary incontinence with urethral bulking agents (n = 2, 1.9%).*
*** Varicocele defined as grade III was visible and palpable at rest.*

Figure 1.
Percent stacked barplots depicting the relationship between the sperm retrieval after testicular sperm extraction and the time since spinal cord injury, considered continuously and categorically. We assessed no statistically significant statistical differences (p value > 0.05) with each approach.



***A.** The time since SCI was considered as a continuous variable.*

***B.** Our cohort was divided into group A (≤ 5 years), group B (> 5 years and ≤ 10 years) and group C (> 10 years).*



***C.** The considered population was divided based on the median time since SCI into group D (≤ 9 years) and group E (> 9 years).*

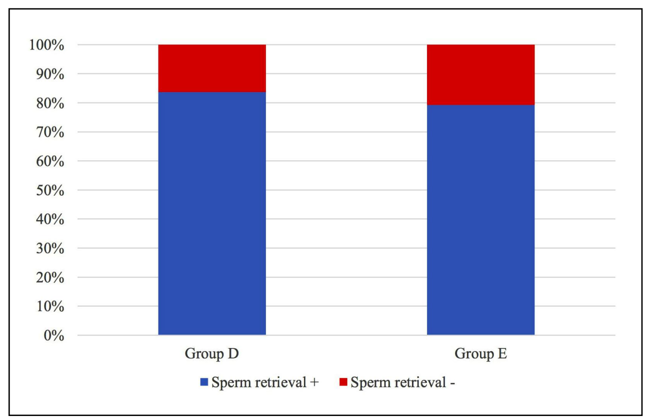


Table 2.
Analysis of the biological reports in case of positive sperm retrieval, besides the outcomes of assisted reproductive technology. We divided patients into different groups based on time since spinal cord injury: group A (≤ 5 years), group B (> 5 and ≤ 10 years) and group C (> 10 years), besides group D (≤ 9 years) and group E (> 9 years). We estimated statistically significant differences among groups A, B and C, and between groups D and E.

Variable	Overall	Time since SCI						
	(n = 87)	Group A (n = 30)	Group B (n = 22)	Group C (n = 35)	p value	Group D (n = 46)	Group E (n = 41)	p value
Frozen straws					0.308			0.394
Median	4	3	4	4		4	4	
IQR	3-4	2-4	2-4	3-4		2-4	3-4	
Range	1-6	1-6	1-4	1-6		1-6	1-6	
Mobility No. (%)					0.004			0.004
Not progressive	56 (64.4%)	22 (75.9%)	18 (81.8%)	16 (44.4%)		36 (78.3%)	20 (48.8%)	
Progressive	31 (35.6%)	8 (26.7%)	4 (18.2%)	19 (54.3%)		10 (21.7%)	21 (51.2%)	
Morphology No. (%)					0.051			0.074
Not optimal	47 (54.0%)	17 (56.7%)	16 (72.7%)	14 (40.0%)		29 (63.0%)	18 (43.9%)	
Normal	40 (46.0%)	13 (43.3%)	6 (27.3%)	21 (60.0%)		17 (37.0%)	23 (56.1%)	
ICSI No. (%)	33 (37.9%)	5 (16.7%)	6 (27.3%)	22 (62.9%)	< 0.001	10 (21.7%)	23 (56.1%)	< 0.001
Pregnancy rate No. (%)	21 (63.6%)	3 (60.0%)	4 (66.7%)	14 (63.6%)	0.974	7 (70.0%)	14 (60.9%)	0.616
LBR No. (%)	19 (90.5%)	3 (100%)	3 (75%)	13 (92.9%)	0.917	6 (85.7%)	13 (92.9%)	0.853

ICSI: intracytoplasmic sperm injection; IQR: interquartile range; LBR: live birth rate.

Table 3.
Univariate logistic regression models predicting positive sperm retrieval.

Variable	OR (95% CI)	p value
Level of SCI		
Tetraplegia	Reference	
Paraplegia	0.749 (0.267-2.097)	0.582
Age at surgery years	0.991 (0.929-1.057)	0.776
Time since SCI years	1.053 (0.981-1.131)	0.154
Time since SCI		
≤ 9 years	Reference	
> 9 years	1.337 (0.497-3.593)	0.565
Time since SCI		
≤ 5 years	Reference	
> 5 and ≤ 10 years	0.448 (0.135-1.482)	0.188
> 10 years	0.509 (0.139-1.869)	0.309
Antimuscarinic therapy		
No	Reference	
Yes	0.729 (0.232-2.284)	0.587
Detrusor injections of BTX-A		
No	Reference	
Yes	2.631 (0.955-7.246)	0.061
Other GU surgical procedures *		
No	Reference	
Yes	1.097 (0.215-5.610)	0.911
Recurrent UTIs and/or MAGIs		
No	Reference	
Yes	0.555 (0.148-2.080)	0.382
Not optimal bladder management		
No	Reference	
Yes	0.701 (0.245-2.004)	0.508
Cryptorchidism		
No	Reference	
Yes	3.375 (0.854-13.336)	0.083
Varicocele – Grade III **		
No	Reference	
Yes	-	0.999
Current smokers		
No	Reference	
Yes	0.800 (0.301-2.122)	0.653

BTX-A: botulinum toxin-A; CI: confidence interval; GU: genitourinary; MAGIs: male accessory gland infections; OR: odds ratio; UTIs: urinary tract infections.
** Other GU surgeries were the following: vasectomy (n = 1), endoscopic treatment of vesicoureteral reflux (n = 2) and of stress urinary incontinence with urethral bulking (n = 2).*
*** Varicocele defined as grade III was visible and palpable at rest.*

The final LBR was 90.5%. Neither complications nor multiple gestations were reported following ART.
Considering the groups based on the previously described different clusters of time since SCI, we observed statistically significant differences ($p < 0.05$) in terms of motility and ICSI.
At univariate logistic regression analysis (Table 3), the following parameters were not associated with positive SRR in a statistically significant way: level of injury (p = 0.582), age at surgery (p = 0.776), AMT (p = 0.587), detrusor injections of BTX-A (p = 0.061), other GU surgeries (p = 0.911), recurrent UTIs and/or MAGIs (p = 0.382), not optimal bladder management (p = 0.508), history of cryptorchidism (p = 0.083), varicocele (p = 0.999), and smoking habit (p = 0.653). Similarly, we considered time since SCI as both continuous and categorical variable without detecting any effects on predicting SRR ($p > 0.05$).

Discussion

Most patients with SCI may experience different-graded alterations in sexual and reproductive functions (17). The fertility assessment should start from evaluating the erectile function. ED may be treated successfully with phosphodiesterase-5 inhibitors (iPDE-5) and, in case of failure, *intracavernous injections* (ICIs) of *prostaglandins* (PGE), reaching satisfactory sexual intercourses in 76% cases with SCI (18).
Patients with SCI may experience anejaculation or retrograde ejaculation. There are several strategies to obtain spermatozoa for ART. The harvest of spermatozoa from the urine may be considered, with an average LBR per transfer equal to 28% (19). According to an 18 year single center experience of 500 men with SCI undergoing PVS, the success rates were 86 and 15% when the patient's level of injury was T10 or rostral, and T11 or caudal, respectively (20).
In PVS-refractory cases, EEJ may achieve success in up to 100%, but the equipment is expensive, the procedure is complex, healthcare professionals' training is lacking, and conscious sedation or general anesthesia may be needed because of significant discomfort or pain (21, 22). For all these reasons, surgical sperm retrieval is usually performed after unsuccessful PVS.
Various surgical techniques have been described over the years, like TESE, *percutaneous epididymal sperm aspiration* (PESA), *microsurgical epididymal sperm aspiration* (MESA), and microsurgical TESE (mTESE) (23, 24). Surgical sperm retrieval combined with *in vitro fertilization* (IVF) ICSI is usually suggested as the first line for infertility in SCI. Indeed, the PR after intravaginal insemination, performed mostly at home, resulted 37.8% in selected couples, so that IVF-ICSI is largely considered in this population (25).
The above-mentioned strategies may lead to obtain low-quality samples because of alterations induced by SCI: production of reactive oxygen species, leucocytospermia with activated T cells secreting inflammatory cytokines (e.g., interleukin-1β, interleukin-12, and tumor necrosis factor-α), antisperm antibodies, derangement in energy substrates and enzymes (e.g., seminal plasma fructose, albumin, glutamic oxaloacetic transaminase, alkaline phosphatase) (26). SCI may severely modify the testicular trophism, decreasing sperm quality at the early post traumatic phase with lower spermatozoid vitality (necrospermia) and reduced motility (asthenospermia) (27). Indeed, a comparison against non-SCI control group suggested that sperm concentration, motility and morphology were significantly decreased in SCI group ($p < 0.05$) (28). However, the PR proved to be not statistically significant different among SCI and OA, but higher with fresh testicular sperm over frozen-thawed sperm for ICSI in SCI (14/22 *versus* 4/16) (29).
In literature, no high *level of evidence* (LE) is available in neurourological patients concerning the efficacy and side effects across different medications (dosages and formulations), procedures and surgical techniques (30). This lack highlights the importance of high-quality studies to draw strong recommendations in an under-represented population, whose prevalence is growing thanks to medical advancements (31).

We reported our experience with TESE in patients with SCI affected by anejaculation in different Italian tertiary referral centers. TESE resulted in a safe procedure with no intra- and post-operative complications reported in our frail patients with SCI.
The different groups based on time since SCI did not differ in terms of baseline characteristics ($p > 0.05$), except for age at surgery and cryptorchidism (in this case, only considering population divided into two groups). As for age at surgery, people with inveterate SCI – obviously – presented as older cases at TESE than patients with recent SCI, justifying this statistical significance.
In Table 2, TESE samples did not differ in terms of number of frozen straws and morphology, but progressive motility appeared to be found more frequently in people with longer times since SCI compared to shorter ones. This data is interesting, as everyone could expect to find worse motility in inveterate SCI. The authors believe that this result is confounding and probably due to a casual accumulation of people with improved motility in groups with longer time since SCI.
ICSI proved to be performed in people with older SCI, as over the years it is more likely to find a partner and start ART compared to young people just after SCI, when TESE is performed to collect and freeze sperm in case of need in the future.
Our study highlighted that none of the variables considered proved to negatively affect the SRR; in particular, the time since SCI did not decrease the SRR. This data is clinically important, specially for infertility counselling, as most patients require TESE as soon as possible after SCI for the risk of future low SRR.
An early TESE after SCI is sometimes performed to anticipate the detrimental effect of time since SCI. However, this issue is not supported by strong-rated scientific evidence (Table 4). There is a void on this issue on current guidelines about this aspect. We performed a literature review looking for studies assessing the effect of time since SCI on spermatogenesis in SCI (32-38). Most studies were characterized by a low LE according to the *Oxford Centre for Evidence-based Medicine* (39).
Sánchez-Ramos et al. prospectively studied 28 men by a *fine needle aspiration* (FNA) biopsy of the testis at 1-, 3- and 6-months following SCI (38). Spermatogenesis was normal in 39, 48 and 80% cases, respectively, at 1, 4 and 6 months after injury, highlighting an early recovery of spermatogenesis.
Iwahata et al. reported the SRR at TESE was significantly

Table 4.
Studies assessing the impact of time since the spinal cord injury on sperm quality and quantity.
The level of evidence of each study was assessed according to the Oxford Centre for Evidence-based Medicine (OCEM).

Reference (year)	Study design (LE)	Population	Intervention	Outcomes
Brackett et al. (1998) (34)	Retrospective analysis of cross-sectional data (4)	125 men examined at 2-year intervals SCI occurred 6 weeks to 26 years earlier	PVS, EEJ and masturbation	No difference in any semen parameters was found as a function of time post injury
Celigoj et al. (2012) (35)	Retrospective case series (4)	7 patients Age at injury: 4.4-11.9 years Age at first evaluation: 24.4-38.1 years Level of injury: T6-L3	PVS or EEJ	Three patients injured before age 9 years: azoospermic One patient injured at age 10 years: subnormal total sperm count Two subjects injured at age 11.9 years: normal total sperm counts
Elliot et al. (2000) (3)	Prospective cohort study (3)	50 men	Testicular biopsy	Spermatogenesis was normal in 28 patients Logistic regression analyses revealed no variable (in particular, post-injury years) was predictive of testis biopsy outcome
Huang et al. (1998) (36)	Prospective case-control study (2)	75 rat models of SCI killed by decapitation 2 weeks-6 months after laminectomy	Testicular histology	Normal spermatogenesis seen in 30% rats at 3 months compared to 47% at 6 months Failure to prevent such effects by exogenous testosterone suggests that nonendocrine factors are involved in the SCI effects on spermatogenesis
Iwahata et al. (2016) (37)	Retrospective case series (4)	52 men with PVS-refractory ejaculation dysfunction	TESE	SRR = 80.7%, PR = 86.5% LBR = 86.5% SRR was significantly better in patients injured within the previous 12 years
Mallidis et al. (1994) (38)	Prospective longitudinal study (3)	7 men	EEJ	Sperm motility and viability decreased towards the pattern of chronic SCI by day 16, so semen storage within the first 2 weeks is recommended
Ohl et al. (2001) (39)	Prospective case-control study (2)	7 dog models of SCI + 6 dogs as controls	EEJ and FNA	Significant changes in sperm at 3 weeks after injury: decreased sperm motility (62.9% to 20.1%), and mean number of spermatids on cross section of the testis decreased compared to controls (13.6 versus 43.9)
Sánchez-Ramos et al. (2017) (40)	Prospective longitudinal study (3)	28 male patients with complete SCI who were evaluated at 1, 3 and 6 months after the injury	FNA	Defective spermatogenesis was found at 1 (61%), 3 (52%) and 6 (20%) months after SCI, suggesting an improvement over time

EEJ: electroejaculation; FNA: fine needle aspiration; LBR: live birth rate; LE: level of evidence; PR: pregnancy rate; PVS: penile vibratory stimulation; SCI: spinal cord injury; SRR: sperm retrieval rate; TESE: testicular sperm extraction.

better in patients injured within the preceding 12 years than those injured longer (p = 0.045).35 Their SRR was 80.7%, and pregnancy was achieved in 32/37 (86.5%) cases undergoing ICSI. We reported similar outcomes, while we did not identify a statistically significant correlation between the SRR and the time since SCI, assessed both continuously and categorically. Indeed, our patients with dated SCI (> 10 years) presented a high SRR (87.8%).

Despite different methodologies, most studies, involving both humans and animal models, reported a rapid decline of spermatogenesis during the first weeks after SCI, followed by its early recovery in the subsequent months. Sperm retrival with a high SRR within the first two weeks after SCI was suggested by *Mallidis et al.* (36). However, surgery is logistically and technically complex when the patient is not completely stabilized in terms of respiratory and/or cardiovascular functions. Therefore, we advocate sperm retrieval after 6 months.

Most patients with SCI were born with a normal GU system and underwent a physiological development, so sperm parameters before the injury were usually comparable to that of the non-SCI population. The real causes of decreased sperm parameters over the years since SCI had not been fully explained, but chronic infections are supposed to play a key role, as they are associated with infiltrating leucocytes producing noxious cytokines (40). In this scenario, testicular sperm is the best candidate for ICSI, as it is protected by testis-blood barrier.

This issue highlights the key role of an optimal bladder and bowel management to reduce the risk for UTIs and MAGIs, preserving testicular parenchyma over the years (41). Our sample included a SCI population followed by tertiary referral centers for rehabilitation. A coordinated, multidisciplinary approach to the SCI rehabilitation was acknowledged as a crucial achievement in outcome improvements (42). An optimal bladder and bowel management is mandatory to slow the deterioration of testicular parenchyma, favoring improved SRRs and ART outcomes. The cohort described by *Iwahata et al.* included patients not uniformly treated in terms of bladder and/or bowel management (35). According to authors, the described declined in SRR could also be a result of chronic infections over time due to inappropriate bladder and/or bowel management, which is the most important aspect to stress with patients during infertility counselling.

Limitations of the study

Our study is not devoid of limitations. To start with, it included case series coming from different centers with a retrospective design (LE = 4), even if the sample was not small, but significantly large considering previous studies concerning patients with SCI.

The histology was not collected for most patients by all centers, so we could not consider this data in our study. This is a significant limitation, especially considering the reduced SRR compared to other series with patients affected by functional OA. Indeed, unsuccessful TESEs could be due with spermatogenesis regression due to different histology modifications (e.g., alterations in Sertoli cells) (43).

We analyzed several outcomes related to ART, because of possible biases due to the modest sample size (n = 33) and different involved centers adopting dissimilar protocols. Some technical features, like ovulation stimulation protocols, were investigated by previous studies, which did not find a statistically significant difference in pregnancy outcomes, supporting the consideration of low-cost protocols for cycle management (44). All these issues should be investigated through future multi-center, long-term studies.

Future directions

Our study represents a contribution to the definition of the effect of time since SCI in the TESE outcomes. Our findings might help other colleagues in the treatment of – usually unfamiliar – patients with SCI and address further research.

Besides an optimal bladder and bowel management, some medicines may improve sperm quality at TESE (45-47). A recent study acknowledged the 4-week-long oral administration of probenecid (known to interfere with the pannexin-1 cellular membrane channel) improved the mean percent of sperm with progressive and rapid linear motility, respectively, from 19% to 26% (p < 0.05) and from 5% to 17% (p < 0.001) (48). Its role is still controversial and further studies are mandatory to recommend its routine use.

Each attempt of improving SRR should be tested in patients with SCI, as fertility concerns proved to significantly impact their health status and should be considered for the management of individuals living in the community (49, 50).

Conclusions

Our experience proved the safety and efficacy of TESE in SCI with a valid SRR, PR and LBR. We highlighted the SRR was not negatively affected by the baseline characteristics, especially by the time since SCI, considered both continuously and categorically. Previously published papers supported early sperm cryopreservation during

Declarations

Ethical approval and consent for participate: *All authors have read and agreed to the published version of the manuscript.*

Availability of data and material: *The data from this study are not publicly available and are subject to privacy restrictions. Summary data may be provided on reasonable request to the corresponding author.*

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the first two weeks or after 6 months from the SCI, highlighting the risk of low SRR over the years. According to our experience, clinicians should not deter SCI patients with functional OA from undergoing TESE after long time since SCI. However, they should encourage an appropriate bladder and bowel management to reduce the risk of UTIs and/or MAGIs, which should damage the testicular parenchyma.

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