THE EFFECTS OF REPEATED EJACULATIONS ON THE QUALITY OF SPERMS FOLLOWING SPINAL CORD INJURY

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AUTHOR'S STATEMENT

I, Rizwan Hamid, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm this has been indicated in the thesis.

This work was performed at Spinal Research Centre at the London Spinal Injuries Unit, Royal National Orthopaedic Hospital, Stanmore by me under the supervision of Professor Michael Craggs, Professor of Applied Physiology, University College London.

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I confirm that this thesis has not been submitted elsewhere.

ABSTRACT

Ejaculatory dysfunction after spinal cord injury (SCI) is common with more than 90% of SCI men unable to produce an ejaculate. If the ejaculate is obtained by vibro or electro ejaculation the motility, morphology and forward progression are all subnormal. The exact cause of deterioration of sperms is not known although a number of factors can lead to poor quality semen.

A randomized control trial was designed to evaluate if repeated ejaculation with a Ferticare® vibrator can improve the sperm quality in chronic SCI men. All had a spinal cord lesion above thoracic level 10 with a minimum duration of 6 months. The subjects who vibro-ejaculated (VE) with a Ferticare® vibrator were randomised into the study or control arms. In the study arm VE was applied weekly for 3 months. In the control arm VE was given only once at the beginning and end of 3 months. The semen analysis was performed by two observers according to World Health Organization (WHO) criteria. A paired Student t test was used for statistical analysis.

Forty two of 79 subjects (53%) vibro-ejaculated successfully. Thirty four were randomized into study (n=18) and control (n=16) arms. No serious adverse events were encountered. Only morphology and forward progression on WHO criteria demonstrated significant improvement. There was no statistical improvement in either volume, count or motility.

It is concluded that repeated ejaculation can improve some parameters of sperm in the semen of SCI men. Hence, patients with a SCI above the level of T10 can improve their sperm quality by applying VE weekly for at least 3 months.

It is hoped that larger scale multi-centre studies will be undertaken to confirm the effectiveness of repeated ejaculations.

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LIST OF ABBREVIATIONS

BCR	bulbo-cavernous reflex
C	cervical
EEJ	electro-ejaculation
FDA	federal drug administration
FSH	follicle stimulating hormone
HR	hip reflex
ICSI	Intracytoplasmic sperm insemination
IVF	in vitro fertilization
L	lumbar
LH	luteinizing hormone
PAP	papanicolaou
PSA	prostate specific antigen
SARS	sacral anterior root stimulator
SCI	spinal cord injury
SD	standard deviation
SIC	self intermittent catheterization
SPC	suprapubic catheter
T	thoracic
UTI	urine tract infection
VE	vibro-ejaculation
WHO	world health organization

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CHAPTER ONE

Introduction

1- INTRODUCTION

1.1 Spinal Cord Injury – Introduction

Spinal cord injury (SCI) is a universal condition with an annual incidence of 15 to 40 cases per million(1). The mortality from acute SCI is steadily declining. A patient will spend, on average, 6 months in the first 2 years following the injury in a hospital (1). In the UK there are an estimated 1000 new SCI per annum (www.spinal-injury.net).

More than half of the SCI are related to traffic accidents(2). 55% of the patients sustain injury at the cervical level with the rest divided equally between thoracic, thoracic lumbar and lumbar sacral levels. About half of these patients have complete transection of the spinal cord. However, this trend is changing and now an increasing number of patients sustain incomplete injuries at thoracolumbar level(3;4). The injuries at the thoracic level are more complete than cervical or lumbar ones. About 70% of the SCI are sustained between the ages of 11 and 50 years and crucially 80% to 85% of these are male(2;5;6).

Up to 95% of these men can have ejaculatory failure. Combined with erectile dysfunction and poor sperm quality, which are both very common in SCI as well, this problem takes on significant dimensions. Infertility is a common problem in men following SCI (7). Considering

the younger average age of patients with SCI (7) and the desire to have a family in most men (8) the consequences of not being able to father children can be devastating.

The last century has seen a vast improvement in the length and quality of life after spinal cord injury (1), but unfortunately, infertility remains a major concern(7). The majority of SCI occur in men in their reproductive years (7). Even if the patient is not planning to start a family the loss of ability to procreate is difficult to accept, sexually non-active SCI men have shown decreased ability to engage in vocational training (9).

Male infertility is attributed to erectile dysfunction and poor semen quality. Horne (10) reported erections in 74% of men with SCI whilst only 18% achieving ejaculations. Erections either reflexogenic or psychogenic will be achieved in 85% of men 2 years after SCI (11). The estimated rate of successful sexual intercourse ranges between 5% and 75% after SCI(11). Historically, Bors(12) in 1960 reported an ejaculation rate of only 5% for men with complete upper motor neuron lesions that increased to 32% if the lesion was incomplete. Patients with complete lower motor neuron lesions (conus and complete cauda equina lesions) reported an ejaculation rate of 18% while 70% with incomplete lower motor neuron lesions could ejaculate(12). In line with the observations by Bors, more recently

Sonksen(13) reported an ejaculatory failure rate of up to 90% in men with SCI.

Despite improvements in semen retrieval methods with vibro-ejaculation (VE) and electro-ejaculation (EEJ) the quality of sperms is consistently poor in all series evaluating semen in SCI patients(14-18). Sperm motility and morphology are especially abnormal. It was initially thought that there was no difference in the quality of semen regardless of the technique of sperm retrieval (19). However, later studies demonstrated that the semen obtained by VE is of better quality than that obtained by EEJ (20;21). Although, the various harvesting techniques may yield large numbers of sperm they are generally of very low motility. Overall, 7.5% of SCI patients will have a normal semen analysis according to World Health Organisation (WHO) criteria (22), around 10% will be azospermic with the remainder having abnormal semen quality(23). Hence an overwhelming majority of SCI men will have an abnormal semen quality for as yet poorly understood reasons(24).

As the exact cause of the poor motility cannot be ascertained, at present there is no definite way to improve the quality of sperms. Currently, the only available technique to retrieve semen by the patient himself is VE (25). The semen thus obtained can be used for intravaginal insemination at home by the patient and his partner. Other techniques for semen retrieval like EEJ or testicular

procedures require a physician. The semen obtained by these methods can only be utilised with assisted conceptions techniques in a fertility clinic with all the associated costs. Unfortunately, there is no guarantee that these expensive methods will work effectively. Hence, the procedure is often abandoned much to the disappointment of the patient and results in financial loss.

The semen characteristics of SCI men are unique with a normal sperm concentration. a low motility and denervation of the accessory glands (7). Although, the normal reference range for sperm concentration as per WHO manual in 1999 was 20 X 10^6 per ml or more, there can be considerable variation in the samples from the same man (22). This can be related to the period of abstention, febrile illnesses or medications. However, the variation can be detected even without any apparent cause (22). This variation is all the more marked in SCI men (7). The exact cause of this variation is still not known but is thought to be multifactorial. All these factors lead to an abnormal seminal plasma environment. Overall, the treatments for non SCI infertile men are effective to a similar degree in assisting conception in SCI men(7).

The full impact of SCI on sexual function is not fully appreciated. In a survey of more than 2500 men with SCI the rate of ejaculation with sexual stimulation or masturbation varied from 0-55% with a median of only 15%(13). However, 54% to 95% were able to attain

spontaneous erections to some degree(26). The exact cause of ejaculatory failure is not known but is thought to be related to interruption of the co-ordinated response of the autonomic and somatic nervous system via sympathetic and pudendal pathways respectively.

The rate of spontaneous fertility in SCI patients is about 5%(27). The reported rate with intravaginal and intrauterine insemination is 25% to 61% per treatment cycle (28;29). This drops to about 25% with assisted fertilization techniques(30;31) and is largely dependent on the quality of sperms especially with assisted techniques.

1.2 Normal sperm production and ejaculatory function

The sperms are produced in the testis under hormonal influence from the pituitary gland and are transported to the ejaculatory ducts under the influence of the autonomic and somatic nervous systems.

1.2.1 Normal Sperm Production

Sperm production takes place in the testis under the influence of the pituitary-gonadal hormonal axis. The anterior pituitary secretes follicle stimulating hormone (FSH) which stimulates sertoli cells to activate spermatogenesis, whilst luteinizing hormone (LH) acts on leydig cells to produce testosterone(32). The testicular temperature is

2 – 4 degrees below the rectum due to the counter current exchange mechanism of the blood vessels(33). The spermatogenesis takes place in the germinal epithelium of the testis. The process takes 64 days in humans(34). Spermatozoa at this stage are immotile and only have a limited capability to fertilize the ovum. Final maturation takes place during the epididymal transit which takes between 2 to 11 days(35). The sperms are stored in the caudal part of epididymis for varying lengths of time depending upon the sexual activity. It has been shown that the forward progression of sperms improves during the transit through epididymis (36). The spermatozoa are rapidly transported from the distal epididymis to the ejaculatory ducts just before emission by strong contractions of the vas deferens. This is under sympathetic innervation(37).

1.2.2 Neuro-anatomy of Ejaculatory function

The neural innervation for sexual co-ordination is intricately associated with the limb girdle plexus. This consists of dorsal and ventral rami from the lumbar and sacral plexus along with sympathetic and parasympathetic pathways formed into complicated nerves supplying both the lower limbs and the pelvic viscera. The general outlay of lumbosacral plexuses relevant to ejaculatory function is shown in figure 1.1. The ejaculation is mediated by both autonomic (sympathetic & parasympathetic) and somatic (pudendal) nervous systems.

Figure 1.1: The afferent and efferent nerve fibres involved in ejaculation (38)

1.2.3 Autonomic Nervous System

The sympathetic nervous system participates in ejaculatory function by contributions from 11th Thoracic (T) to 2nd lumbar (L) segments(39). The preganglionic cell bodies are located in the intermediolateral parts of the spinal cord. The fibres pass through to the sympathetic ganglia in the inferior mesenteric and superior hypogastric plexus. The superior hypogastric plexus is situated at the level of the fifth lumbar vertebral body anterior to the aortic bifurcation. From here the inferior hypogastric nerves which are the major conduits of sympathetic innervation into the pelvis pass down into the inferior hypogastric plexus (pelvic plexus) (40). They are joined here by the pelvic nerves carrying fibres from the parasympathetic system. The pelvic plexus lies near the seminal vesicle(41) and several vessels pierce through the plexus on their way to the pelvic viscera (Figure 1.2). The pelvic nerves supply major excitatory stimulus to the vas deferens and seminal vesicle(42). The parasympathetic nervous system contributes albeit to a much lesser extent. The preganglionic cell bodies, like the sympathetic nervous system, originate from the intermediolateral part of the spinal cord from 2nd to 4th sacral nerves. The fibres pass via the pelvic plexus and nerves to the pelvic organs(40).

Figure 1.2: Schematic diagram of pelvic (cavernosal) and pudendal (dorsal) nerves supplying male genital organs (reproduced from Carson 1999)(43)

1.2.4 Somatic Nervous System

The somatic innervation for ejaculatory function is derived from the pudendal nerve. It is formed by the anterior primary rami of the second, third and fourth sacral nerves. The cell bodies for the motor fibres in the pudendal nerve lie in Onuf's nucleus. It is situated in the ventrolateral part of the anterior horn of the sacral segments. It crosses the sacrospinous ligament and passes with pudendal vessels in the pudendal canal. Here it gives off the dorsal nerve of the penis, the inferior rectal nerve and continues as the perineal nerve. The dorsal nerve supplies the corpus cavernosum and the glans. The perineal nerve supplies muscular branches to the sphincter urethra, the bulbospongiosus, the corpus spongiosus and the ischiocavernosus. It also supplies the posterior scrotum(44).

1.2.5 Neuro-physiology of ejaculation

Ejaculation has two phases: emission and true ejaculation. The process of deposition of seminal fluid in the posterior urethra is known as emission. Adequate function of the vas deferens, seminal vesicles, prostate and bladder neck is required for emission. This is initiated by hypogastric nerves via thoracolumbar outflow. There is contraction of ampulla of vas deferens, seminal vesicles and the prostate to propel the emission fluid to the posterior urethra. The bladder neck contracts and the external sphincter relaxes at this

time. The innervation of all these structures is primarily sympathetic(40).

Ejaculation is the second phase in which the fluid is propelled down the urethra towards the external meatus. The rhythmic contractions of the bulbocavernosus and the ischiocavernosus are mediated by the somatic nerve (pudendal). There is also contraction of the periurethral and sphincter muscles(40).

These processes require coordinated autonomic and somatic activity. There appears to be a co-ordination centre for ejaculation from T12 to L1 segments(45). This synchronises the closure of the bladder neck and initiates contractions in the ductus deferens. It leads to the delivery of semen into the urethra. At this time the perineal striated muscles contract rhythmically resulting in expulsion of semen(46).

1.3 Characteristics of Normal Ejaculate

1.3.1 Semen analysis

The normal ejaculate is a combination of spermatozoa suspended in secretions of the accessory glands. This results in a viscous fluid. The semen is analysed for total volume, pH, sperm motility, forward progression, morphology and count. The landmark paper by MacLeod and Gold in 1951(47) established a minimum sperm count of 20 million per cc as a threshold for fertility. The accepted reference

range for semen analysis according to the WHO criteria from 1999 was used as shown in Appendix I(22). It is to be noted that the reference values were reached with some difficulty amongst experts. The relationship of semen quality to fertility is quite complicated, not the least in that it involves the female factor. This can result in men with “abnormal” semen still being fertile whilst men with “normal” semen being sub or infertile. Hence, the use of term reference values instead of normal values by the WHO manual.

1.3.2 Prostatic & Seminal fluid

The normal prostatic secretion contains zinc, citric acid and acid phosphatase The reference range of zinc is 2.4 micro mol per ejaculate(48). Fructose gives an estimation of the secretory function of the seminal vesicles. The reference value of fructose is 13 micro mol per ejaculate(49).

1.4 Patho-physiology of Ejaculatory Failure in SCI patients

1.4.1 Neuro-anatomy

The disruption of corticospinal input following SCI, direct injury to the sympathetic chain or lack of sensory input can all result in lack of emission. If the disruption is not severe then the bladder neck will not close and ejaculation will be in a retrograde fashion. The probability

of ejaculation increases with descending levels of injury and incompleteness. Hence, about 70% of patients with incomplete lower motor neuron injury may ejaculate. On the other hand only about 5% of patients with complete upper motor neuron injury can ejaculate(12) Recurrent urinary tract infections (UTI) can lead to prostatitis and epididymitis in SCI patients. The exact incidence is not known but sub-clinical episodes are even more common. These can lead to the blockage of ejaculatory ducts and might cause testicular atrophy(50).

1.4.2 Neuro-physiology

There seems to be a disturbance of the pituitary-gonadal axis lasting a few months after major spinal cord trauma(51). The hormonal levels generally normalise by the end of one year. Hence, sperm collection should be deferred for at least 6 months following SCI. There can be significant testicular abnormalities at this stage, including hypospermatogenesis, tubular atrophy, fibrosis and maturation arrest(52;53). There is no correlation of these findings with either the level or completeness of SCI. The mechanism is not exactly known but it is suggested that interference with sympathetic innervation of the epididymis, vas and seminal vesicles leads to a failure of co-ordinated action leading to a failure of sperm conduction(54;55).

1.5 Literature Review

There has been a tremendous improvement in the quality of care following SCI. However, infertility remains a major concern. The majority of SCI occur in men who are in their reproductive years. Even if the patient is not planning to start a family the loss of ability to procreate is difficult to accept and can lead to social and psychological problems.

1.5.1 Characteristics of Ejaculate following SCI

It has been shown that about 7.5% of men with a SCI have a sperm concentration greater than 20 million per ml with the remainder having abnormal semen parameters(23). The patients with cervical and thoracic level injuries have better sperm motility than those with lumbar lesions. The sperm motility is superior with incomplete than with complete injuries(18).

There are differences in the seminal fluid of SCI men as compared to able bodied men. The levels of fructose, albumin, glutamic oxaloacetic transaminase and alkaline phosphatase are significantly low whilst chloride levels are significantly higher(56).

1.5.2 Ejaculatory dysfunction in SCI patients

Ejaculatory dysfunction along with poor sperm quality is a major cause of infertility in SCI men. Horne (10) reported in 1948 that only 18% of men with injuries above the sacral cord can ejaculate. Talbot (27) reported an ejaculation rate of less than 10%. In 1960 Bors (12) reported in the biggest study thus far on the evaluation of ejaculate following spinal cord injury. He reported an ejaculation rate of only 5% for patients with complete upper motor neuron lesion that increased to 32% if the lesion was incomplete. Conversely incomplete patients with lower motor neuron lesions reported an ejaculation rate of 70% while 18% with complete lower motor neuron lesions could ejaculate. Brindley(14) suggested that an important prognostic factor for ejaculation with a vibrator is the presence of bulbo-cavernous (BCR) and hip reflex (HR). He suggested that 75% of men with an intact HR will ejaculate. The HR is elicited by scratching the soles of the feet. This evaluates the integrity of the spinal cord between L2 and S2 segments that are needed for ejaculation (57). Szaza and Carpenter in 1989 (58) noted that the absence of BCR predicts a failure to ejaculate with VE in most men with SCI. This observation by two investigators signifies the complexity of problems in this patient group. It appears that the ejaculatory dysfunction in SCI men is a complex phenomenon relying on a variety of factors rather than a single factor abnormality.

1.5.3 Medications for ejaculatory dysfunction in SCI patients

Various investigators have demonstrated that it is difficult to obtain ejaculation in SCI men. Hence, attempts have been made to assist this process. Naturally, the initial endeavours were to facilitate ejaculation with medications. Guttman in 1947(59) reported retrieval of sperms in SCI men following intrathecal instillation of neostigmine. This led to ejaculation in men with SCI. He reported a success rate of around 60% in 70 patients with supra sacral SCI(60). However this was not popularised due to bothersome side effects. There were severe incidents of autonomic dysreflexia with 1 reported death. Later on an ejaculate was obtained by subcutaneous injection of physostigmine followed by masturbation(61). However this was also not popularised due to side effects.

1.5.4 Invasive techniques for sperm retrieval in SCI patients

A variety of techniques have been tried to induce ejaculation in SCI men.

1.5.4.1 Direct aspiration from vas deferens

Spermatozoa can be obtained by direct aspiration from the testis or the vas deferens(62-66). A variety of methods have been employed to this end. These include testicular sperm aspiration or extraction, microsurgical or percutaneous epididymal sperm aspiration or aspiration from the vas deferens. Although successful pregnancies have been reported these are invasive techniques and not particularly liked by the patients. However, it has been demonstrated in more than 100 fertility centres, that a quarter will not offer VE or EEJ as a first line method of sperm retrieval, instead the patient will undergo surgical sperm retrieval(24).The reason for not offering VE or EEJ in these centres was lack of training and education. Surgical methods typically result in a low quality ejaculate committing the SCI men to assisted methods of fertilization like in-vitro fertilization (IVF) and intracytoplasmic sperm insemination (ICSI) and the resulting high costs. Hence, it has been recommended that non-invasive techniques should be tried first(19) and if these are unsuccessful then the patient should undergo a surgical method of sperm retrieval.

1.5.4.2 Electro-ejaculation for ejaculatory failure

The first case of EEJ being used to obtaining human semen was reported by Horne(10) in 1948. He was able to retrieve semen in 8 of 15 patients without any complications. This was, later on,

popularised by Brindley(67). He reported an antegrade ejaculation rate of 43% (total 84 patients) and a retrograde ejaculation rate of 16%. This procedure has been refined overtime by Seager and the success rate of obtaining a sample with EEJ for any level of injury is well above 90%(45;68-72). This is performed with an electrical probe in the rectum. An electrical current is applied to deliver direct stimulation of current to the pelvic nerves via the prostate and seminal vesicle (73). The ejaculate is more an emission than a true ejaculation(67;74). This has to be performed in a hospital by a physician with continuous monitoring of blood pressure as it can induce autonomic dysreflexia(45;67). The main advantage is that EEJ can be used for any level of SCI but patients with an incomplete injury require anaesthesia. The bladder is emptied before the procedure and a sperm friendly medium like Ham's composition is instilled into the bladder to prepare for retrograde ejaculation(20;75). The current is applied in 2-5 volts increments and then switched off. It has been shown that the antegrade flow of semen occurs when the current is switched off (76). A proctoscopy is performed before and after the procedure to rule out any injury to the rectal mucosa. Seager Model 14 Electroejaculator (Dalzell USA Medical Systems, Marshall, VA) is the only Food and Drug Administration (FDA) approved device for performing EEJ (figure 1.3) in humans(7).

Figure 1.3: Seager electroejaculator device with rectal probe

Sperm motility declines rapidly after mixing with urine (77). This can be offset somewhat by instilling Ham's solution into the bladder before starting the procedure. It has been suggested that EEJ is potentially damaging to semen as compared to VE(14).

1.5.4.3 Vibro-ejaculation for ejaculatory failure

VE was first reported by Sobrero(78) in 1965. Again, Brindley (79) is largely accredited for popularising this technique. He was able to ejaculate about 60% of the 81 men with paraplegia of greater than 6 months duration. The incidence of retrograde ejaculation much lower than EEJ (20). The main advantage of VE is that it can be performed in the home environment. T

his allows for a home insemination programme to be instituted, which not only reduces the cost and side-effects of the procedure, but is also more personal. Furthermore, a home insemination programme is closer to natural conception. VE is a safe and cost effective method and yields the highest number of antegrade motile sperms. Hence, it is recommended that it should be employed as the primary method for sperm retrieval in SCI men(75;80). Over the years a variety of devices have been used to perform VE but currently the only instrument approved by the FDA is the Ferticare® personal vibrator (Multicept, Albertslund, Denmark) figure 1.4(7).

Figure 1.4: Ferticare® personal vibrator

The aim of VE is to activate the ejaculatory arc at the thoracolumbar level(73). This requires an intact dorsal penile nerve; a sensory branch of the pudendal nerve. The fibres terminate in the sacral segments 2-4(81;82). SCI men with an injury above T10 can ejaculate in up to 88% of cases, whilst SCI men with injury below T11 report a success rate of only 15%(24). Hence, it is accepted that VE should be tried for patients with a spinal cord lesion above T10. The stimulation is applied to the glans and afferent impulses pass via the dorsal penile nerve. An antegrade ejaculation is obtained in a projectile manner almost similar to normal ejaculation(73). The average time required for obtaining an ejaculate by this technique is between 10 seconds to 45 minutes(17;58;79). If there is no response in 2-3 minutes then the stimulation is stopped. After a gap of 1-2 minutes the stimulation is performed again. The process is repeated until either the ejaculate is obtained or the stimulation has been performed for up to 10 minutes(83). Generally, almost 90% of the SCI men who will ejaculate with VE do so within 2 minutes of starting the stimulation. Additional methods have been employed to facilitate ejaculation in unsuccessful cases. An increase in the success rate has been reported with the application of 2 vibrators, one above and the other underneath the glans(84). This can be employed at the first visit or after multiple unsuccessful attempts with one vibrator. During ejaculation there might be contraction of the abdominal muscles and leg spasms may be seen. A very important consideration for success with VE, as described above is an intact reflex arc at the

thoracolumbar level (14;20;85). However, the success rate with VE is variable and the reported range is between 19-91% (15;17;79;86-88). The highest rate of ejaculation was seen with an amplitude of 2.5mm and a frequency of 100HZ (89). The completeness of injury is not a definite prognostic factor for obtaining semen by VE(14;58). The most common side effect with VE is skin abrasion which does not require any specific treatment. The patients with an injury above the level of T6 can develop autonomic dysreflexia(90). Nifedipine can be given to the susceptible patients. However, there is no need to prescribe this medication prophylactically. It has been shown that sperm motility is better with VE as compared with EEJ(19).

1.5.4.4 Prostatic massage for ejaculatory failure

The sperms are stored in the ampulla of vas deferens and can be sequestered in the seminal vesicle(91). Prostate massage can be employed to obtain sperms by performing a rectal examination and mechanically pressing on the prostate and seminal vesicle. This indeed has been reported to lead to successful pregnancies(92;93). However, the sperms quality is very low. This can be employed in men who have failed VE but cannot undergo EEJ for any reason.

1.5.5 Distinctive semen profile in SCI men

The SCI men have a distinctive semen profile. This is characterised by abnormal semen parameters including a very poor motility and morphology(94). The quality of sperms has consistently been shown to be poor in all series examining semen in SCI men(13;19;95;96). The testes also demonstrate histological abnormalities in a number of studies(10;11;52;97).There is a lower mean spermatid count with a higher Sertoli cell count per seminiferous tubule. Also, there can be a higher Sertoli cell to spermatid ratio as compared to controls (98). However, it is difficult to objectively compare these studies as almost all have used different methods of analysis(99).

1.5.5.1 Semen characteristics during the acute phase following SCI

There is a lack of information regarding semen characteristics during the acute phase after SCI. This may relate to the medical and psychological trauma of SCI and unwillingness of investigators to introduce the subject of fertility during this phase (100). There is a single study in humans in the literature(101) which has addressed the acute effects of SCI on sperm characteristics. In the study EEJ was performed in 7 men starting from the 2nd day following a SCI. There was no abnormality in the sperm characteristics up to the 16th day. Thereafter sperm motility deteriorated rapidly. This study has

never been replicated in humans. However, Ohl and colleagues (100) studied the acute effects of SCI on the semen quality and spermatogenesis in a canine model. Seven dogs underwent surgical T7 SCI. There were 6 controls. EEJ was performed at baseline and then twice weekly for 3 weeks. They demonstrated that sperm motility and spermatogenesis decreased significantly but not before 3 weeks in SCI dogs. The similarity of motility changes between the two studies (100;101) demonstrates that the canine model can mimic the human situation. There have been other attempts at evaluating the acute changes in spermatogenesis (102) and epididymal sperm motility (103). However, these later investigations lacked the ability to evaluate the serial changes in spermatogenesis and ejaculated semen quality (100). There are many other issues to consider in the immediate period following SCI. The surgical stabilization of the spine with the management of associated injuries takes precedence over evaluation of fertility potential. Most of the patients are also not in the right frame of mind to undergo EEJ immediately after SCI.

1.5.5.2 Semen characteristics during the chronic phase following SCI

The patho-physiology and the mechanisms which lead to the deterioration of sperms following SCI are largely unknown. In most series evaluating the semen from SCI patients, the ejaculate was obtained by vibration, chemical or electrical stimulation. The sperm

count was less than 20 million per ml in more than 85% of the cases and the motility was less than 40% in almost all the cases(45;67;104;105). The reported motility varied, with some studies reporting 0.5% whilst others reported a motility of more than 40% (45;67;104;105). It was also demonstrated that the motility was better with incomplete injuries and in SCI men who had cervical and thoracic lesions as compared to complete injuries and lumbar lesions (18). It has therefore been suggested that a host of factors in combination lead to the deterioration of sperm quality(99).

The various suggested causes of poor sperm quality following a SCI include:

1- Stasis of prosthetic fluid(106;107)

2- Testicular hyperthermia(108)

3- Recurrent urinary tract infections(109;110)

4- Type of bladder management(18)

5- Abnormal testicular histology(97)

6- Changes in pituitary testicular axis(68;111;112)

7- Possible antisperm antibodies(111;113)

8- Long term use of various medications(12;114)

A number of investigators have suggested that the stasis of semen in the ejaculatory ducts leads to a deterioration in sperm motility (106;107). Brindley has shown a progressive increase in sperm motility with repeated EEJ(14). Francois et al have demonstrated an increase in sperm motility of a man with repeated ejaculations from 0% to 30% (107).

Some investigators have reported that frequent VE for 3-6 months will improve the quality of sperms following SCI(15;17). Interestingly, Ohl et al(18) reported no improvement in semen quality in patients who had intermittent spontaneous ejaculations but showed improvement in semen quality in SCI patients who underwent repeated electo-ejaculation 2-4 times under their care. On the other hand, Sonksen in 1999(115) reported no beneficial effects of repeated ejaculations on the quality of sperms. He performed VE weekly for one year in a series of 18 patients and did not find any significant improvement in any of the semen parameters.

Increased scrotal temperature has been implicated as a factor for decreased motility although this has not been proven. Brindley(108) demonstrated a significant correlation between high scrotal temperature with lack of motile spermatozoa in paraplegics sitting in wheelchairs. However, other investigators have found no correlation between high scrotal temperature and semen characteristics in SCI men and non-injured subjects as controls(116;117).

UTI are not uncommon in SCI men(118). They are thought to adversely affect fertility. It has been noted that 60% to 70% of the patients with SCI can have bacteriuria on routine examination of the urine(119). Recurrent UTI can lead to epididymo-orchitis resulting in obstruction of the ducts(120). It has been shown that patients who have urinary reflux on video-urodynamics might have chronic

infections leading to deterioration of sperm quality(18). A lower sperm count and motility has been demonstrated in men with chronic prostatic infection(109). The urine itself is thought to be extremely detrimental to sperm motility(77;121). It has been suggested that sperm motility is halved after mixing with urine for only 5 minutes(77). On the other hand no significant difference was seen in ejaculates from SCI men with infected urine as compared to non-infected urine(122).

Poor sperm quality is also thought to be related to the atrophy of semineferous tubules(123). There appears to be a higher sertoli cell count per seminiferous tubule. However, as with most theories investigators have found both normal and abnormal testicular histology in SCI men with no significant correlation to the level of injury, the duration of SCI or to the number of urinary tract infections(52;68;97).

It has also been suggested that hormonal changes occur following SCI and the level of FSH and LH and the production of testosterone is altered(111). Initial studies showed that the levels of FSH and LH were elevated but testosterone levels were normal. Most of the studies have shown conflicting results with hormonal levels both above and below normal values(124;125). It has been suggested that the reason for the inconsistency can be the pulsatile nature of the

release of the gonadotrophins and it may be difficult to pick up the subtle changes in the hormonal levels with any consistency(126).

Another theory is the production of antisperm antibodies following SCI(113). Two conditions are thought to be associated with antisperm antibody production. One is the obstruction of ejaculatory ducts secondary to epididymitis(68), but the reason for the development of antibodies is not known(126;127). The second possibility is recurrent UTIs leading to a prostatic immune reaction(128). Again, various investigators have documented both the presence and absence of antisperm antibodies in SCI men(129-131). No significant correlation has been established between antisperm antibodies and sperm quality but it is thought that antibodies in the seminal plasma might exert a deleterious effect on sperm quality(132). It has been demonstrated that up to 70% of the men develop antisperm antibodies after undergoing vasectomy(126) suggesting that obstruction might lead to development of antisperm antibodies that play some role in causing a poor sperm quality in SCI men.

The method of bladder emptying is also thought to play some part in the deterioration of sperm quality. It has been shown that SCI men who empty the bladder with SIC or have a sphincterotomy have better sperm motility (27%) than those emptying the bladder by reflex voiding or with an indwelling catheter (5%)(18). However, even those

voiding spontaneously did not have normal sperm motility indicating that the method of bladder drainage cannot be solely responsible for the deterioration of sperm characteristics after SCI. Rutkowski(133) demonstrated that SIC is superior to other methods of bladder drainage in terms of delivering motile sperms after both VE and EEJ in SCI men.

Men with SCI are not infrequently on a variety of medications. A number of them will have been taking various medications for 10 years or more. There are no studies demonstrating the safety or otherwise of medications on sperm quality(19). Nitrofurantion causes spermatogenic arrest in rats, which is reversible(134). Similarly prostaglandins, used as pain killers, can lead to infertility in men(135). Alpha blockers can lead to bladder neck relaxation and are also thought to affect the smooth muscle contractility of the vas deferens(136). We do not know the long term effects on spermatogenesis of some commonly used medications in SCI men like trimethoprim, quinolones and diazepam(137). Nifedipine is given for prevention of autonomic dysreflexia. In a study, this has been shown to inhibit the contraction of isolated human seminal vesicle tissue (138). There are no other studies specifically looking at the effect of Nifedipine on human ejaculatory tissues. However, in rats it has not been shown to delay ejaculation in vitro (139). Hence, the exact role of Nifedipine in relation to human ejaculatory function is not known.

The method of obtaining ejaculate has also been studied as a potential cause of poor sperm motility in SCI men. It was initially thought that the there was no difference in the quality of semen regardless of the technique of sperm retrieval (19). However, later studies demonstrated that motility is better with VE (24%) as compared to EEJ (12%)(20;21) but it is still well below 50% of what is considered normal by WHO criteria. The biochemical composition of EEJ semen of SCI men differs from that of the masturbated semen of normal men (56;140).

Leukocytes are seen in almost all semen samples from SCI men(141;142). They have been associated with male infertility in able bodied men but the treatment of infections in SCI men has not been shown to improve the sperm motility in this group(122). Interestingly, the SCI men have demonstrated a large number of activated T lymphocytes which secrete cytotoxic cytokines(143). Some studies have shown improved sperm motility once these cytokines were neutralised(144;145). However, it is not clear if leukocytospermia plays a significant role in the deterioration of sperm quality in SCI men.

1.5.5.3 Role of seminal fluid in poor quality semen following SCI

There is abnormal prostate and seminal vesicle function in men with SCI(146-148). Recent investigations have suggested that seminal plasma may play a crucial role in causing deterioration of sperm motility and forward progression. As a result of SCI there can be denervation of accessory glands (prostate and seminal vesicle) that might contribute to an abnormal semen quality(7). The semen of SCI men is brown in colour in more than 25% of the cases. This was previously thought to be related to stasis due to infrequent ejaculations. However, microscopic examination has revealed that the discoloration of semen might be related to a dysfunction of the seminal vesicle. The SCI men with brown ejaculate have a lower semen volume and a thinner consistency compared to men who do not have a brown semen(149). It has been shown that seminal plasma obtained with the ejaculate from SCI men inhibits the motility of normal sperms. Conversely the sperms from SCI men improve their motility if they are mixed with seminal plasma from able bodied individuals(150). It has also been shown that the sperms of men with SCI demonstrate significantly better sperm motility and viability when aspirated from the vas deferens as compared to sperms obtained with VE or EEJ(151). In 12 SCI men the mean sperm motility aspirated from the vas deferens was 54.4% while in the healthy non SCI age matched controls it was 77.5%. The mean sperm motility in the ejaculated specimen of SCI men decreased to 14.1% while there

was no change in the non SCI age matched controls. A large number of poor quality sperms were found in the seminal vesicle of SCI men(91). These are the sperms comprising a major portion of the ejaculate with VE and EEJ. Another study demonstrated lower levels of fructose and albumin in the semen of SCI men pointing to the accessory gland being a factor in causing the deterioration of sperms after SCI(56). There is also prostate gland dysfunction in SCI men. The PSA is lower in the semen(147;152) but higher in the serum(147) of SCI men when compared with age matched controls. Interestingly, the prostate of SCI men are smaller than age matched controls(153;154). The significance of this is unknown. A number of studies have demonstrated abnormal concentrations of various substances in the seminal plasma of SCI men as compared to able bodied controls. The concentration of platelet activating factor acetylhydrolase(155), reactive oxygen(156), and somatostatin(157) is higher in SCI men than controls. Whilst, the concentrations of fructose, albumin and alkaline phosphatase(56) are lower in SCI men than in controls. Recently, in a very elegant experiment Maher et al employed nuclear magnetic resonance spectroscopy to analyze seminal fluid metabolite profiles in 10 men with and 8 men without SCI above T10 (158). They demonstrated that uridine is likely to be an essential precursor to metabolites required for capacitation and is a potential marker for the prognosis of post-SCI functional fertility recovery. A new term "seminal oligouridinosis" was coined to describe this newly identified condition. The above data provides

evidence that seminal plasma is probably a major contributing factor to the deterioration of sperms following SCI, however the exact correlation between seminal fluids and abnormal sperm characteristics in SCI men remains to be determined.

1.5.6. SCI men achieving successful fatherhood

A number of investigators have reported that SCI men have been successful in achieving fatherhood. This has been reported with both home insemination programme and assisted fertility techniques.

1.5.6.1 Home insemination programme with intra-vaginal insemination

This is a viable option for SCI men who can either ejaculate spontaneously or with the help of VE. The patients and their partners are taught the technique of collecting semen in a non-spermicidal container and vaginal delivery via a 10 ml syringe(57). Brindley in 1984(14) reported seven pregnancies with the birth of 5 healthy babies after VE and vaginal self insemination. Several others have also reported successful pregnancies with VE and self insemination programmes(28;29;72;159). Most utilised multiple ovulation cycles and the overall pregnancy rate per couple was 25 – 61%. In a recent trial in 3 countries at 4 large centres 43% of the partners of SCI men achieved pregnancies with intra-vaginal insemination(7).

1.5.6.2 Insemination programme with intrauterine insemination

Intrauterine insemination is recommended if the intravaginal technique has failed. Ohl et al(160) reported a per cycle pregnancy rate of 8.6%. They recommended that if the total sperm count was less than 4 million motile sperm then the patient should proceed directly to IVF as the chance of pregnancy per cycle was only 1.1%. On the other hand if the motile sperm count was more then 40 million then the success rate per cycle increased to 17.6%.

1.5.6.3 Assisted techniques for conception

Success has been reported with assisted reproductive techniques including IVF and ICSI utilising sperms obtained by VE or EEJ (11;28;29;30;72;159). The success rate in SCI men with assisted techniques is 25% per cycle(11;30;72;159;161) which is comparable to pregnancy rates achieved with these techniques in able bodied men(162). Surgical sperm retrieval has also been used to obtain sperm in SCI men as a first line treatment without trying VE or EEJ(24). The reason cited is the lack of training of physicians in these techniques due to the small number of patients with SCI. However, it is the physicians responsibility to inform the patient of all the options available even if he or she cannot offer all the treatments(163) and to explain why a particular option is futile(164).

1.5.7 Techniques to improve semen quality in SCI men

Various studies have been carried out to improve the quality of sperms in men with SCI. However, the only technique employed to improve the quality of sperms has been to perform repeated ejaculations either with VE or EEJ. Some studies have substantiated this hypothesis(15;17;107) while others have found no appreciable improvement in sperm characteristics with repeated ejaculations(45;115;165). However, none of these studies were randomized with a control group. Interestingly, it has been shown that SCI men might stop ejaculating with repeated ejaculation after initial success with VE(15). The cause of this phenomenon is not yet ascertained.

Siosteen and Beretta have suggested that the quality of semen can improve after repeated VE(15;17). 30 men vibrated on a weekly basis at home for 3 to 6 months. They compared the first ejaculate with that produced after 3 to 6 months of repeated ejaculation. Both the authors reported that the sperm quality improved after repeated VE. There was improvement in motility and concentration but importantly there was a decrease in abnormal sperm morphology after a prolonged use of a vibrator. Siosteen found that the total count of motile sperms and the level of fructose also increased. He suggested improved function of the seminal vesicles and the prostate. Additionally, the sperm penetration capacity reached almost normal levels after repeated ejaculation. Mallidis(166)

demonstrated an improvement in sperm motility and viability with repeated EEJ for 4 consecutive days. On the other hand Sonksen (115) performed repeated VE for 19 SCI men over 1 year and did not find any significant improvement in any of the semen parameters. Similarly, Das et al performed 3 successive EEJ in 16 patients at 2 -4 week interval and did not find any improvement in semen parameters(167). Hence, there is no conclusive evidence either way that the quality of sperms can be improved objectively. Additionally, Sarkarati(45) demonstrated that the quality of the semen is not good during the first 6 months. Thereafter there is no appreciable decline in sperm quality(168).

1.5.8 Deficiencies in the literature

It can be derived from the above data that no single factor is responsible for poor quality sperms following SCI. It is the combination of factors described above that collectively lead to the deterioration of semen quality post SCI. However, it is surprising that no controlled studies in humans have been performed to demonstrate the exact cause of poor semen quality. There have few studies in humans on this subject but they have mostly looked into stasis as a cause of poor sperm quality(106;107). None of these studies were conducted as a randomised controlled trial with a control group. The only controlled study performed to evaluate the acute changes in semen quality was undertaken by Ohl and

colleagues in a canine model (100). They demonstrated a significant decrease in sperm motility but not before 3 weeks in SCI dogs. Although, this study closely mimics the human situation after SCI no long-term studies are available to test the progression of these changes into the chronic stages of SCI. There have been some studies evaluating the role of seminal plasma in poor quality semen but again these have mostly been observational studies(56;150;151). Although all investigators agree that the semen quality is poor and the cause is multifactorial no suggestions have been put forward to identify the most critical factors leading to the deterioration of sperms in SCI men. Hence, further studies are required to focus on ejaculatory dysfunction, seminal vesicle and vas deferens function and thorough evaluation of seminal plasma. This might shed more light on the causation of poor semen quality in SCI men.

1.6 Aim of this study

There is a gap in the literature as to why the SCI men have poor quality semen. Several theories have been put forward as discussed earlier, but no definite answers have been found. Certainly, it is agreed there is no single factor responsible for all the changes leading to poor sperm quality in SCI men. Previous studies have attempted to determine whether one of the suggested factors; repeated VE can improve the quality of sperms in SCI men. The results of these studies so far have been contradictory with some

demonstrating that repeated ejaculations can improve the sperm quality, whilst others indicative of that this particular intervention does not make any appreciable difference to the quality of sperms in SCI men. Importantly, no human study was a randomised control trial.

As there is no consensus as to the exact cause for poor quality semen, no effective treatment can be suggested to improve the sperm quality in a SCI man.

Hence, a randomised controlled study was designed to study the effect of repeated ejaculation using VE in patients with a SCI. The aim was to evaluate if this particular factor can lead to an improvement of semen parameters in SCI men.

1.6.1 Null Hypothesis

To evaluate if repeated ejaculations improve the quality of sperms in SCI men the experiment was designed to test the null hypothesis. This will state that any intervention like repeated ejaculations will have no significant impact on the quality of sperms in SCI men.

1.7 Summary

The exact cause of the deterioration of the quality of sperms following SCI is not known. A host of factors have been described in

the pathophysiology of poor sperm quality following SCI. However, there is no conclusive evidence that there is a single factor causing the deterioration of sperms in SCI men. With the establishment of IVF and ICSI techniques the emphasis is now on obtaining a single or few sperms and utilising these assisted fertility techniques to achieve pregnancy. This is a reasonable approach with a good chance of success. However, one has to keep in mind that these techniques require a considerable amount of time, and more importantly, money. Hence, if a technique could be demonstrated that would improve the quality of sperms in a non-invasive manner then the patients could try to improve the quality of sperms in the home environment. If this was successful, a home insemination programme could be instituted. This would not only be cheap but would also be close to the natural method of conception. This would enhance the self-esteem of SCI men and help in their re-integration within the community. In the event that this technique failed, then assisted fertility techniques could be tried to achieve a successful pregnancy.

The best possible chance of ensuring a successful fertility programme is through a Multi-Disciplinary Team. This should include rehabilitation physicians, urologists, gynaecologists, psychologists, nurse co-ordinators and a reproductive endocrinologist.

CHAPTER TWO

Materials & Methods

2 MATERIALS AND METHODS

2.1 Overview of the project setting

The study was designed and performed at the London Spinal Cord Injury Centre, Royal National Orthopaedic Hospital, Stanmore. A dedicated room was set up at the premises for the performance of VE. The equipment for the evaluation of semen was placed in one corner of the laboratory. The invitation for participation in the study was extended by all members of the unit including urologists, clinical scientists, rehabilitation physicians and specialist nurses both during in-patient and out-patient consultations. Additionally, it was publicised through the SCI charity (ASPIRE). All invited subjects were under the care of the unit for spinal rehabilitation.

2.2 Regulatory requirements

The local research and ethics committee approved this study. The trial was carried out in accordance with the "Recommendations guiding physicians in biomedical research involving human subjects - Declaration of Helsinki(169).

2.3 Study design

The subjects who successfully ejaculated antegradely in the clinic with help of the Ferticare® personal vibrator without any apparent contra-indications were randomised into either the study or control groups. The study group was required to VE weekly and semen analysis was performed at baseline and then on a monthly basis for a total of 3 months. The subjects in the control group were ejaculated at baseline and then at the end of 3 months. The control group was advised to abstain from ejaculation by any method.

2.4 Subject selection

The subjects were invited to the hospital for screening and baseline evaluation of the ejaculate. The inclusion and exclusion criteria are given in appendix II. All aspects of the study were discussed with them according to the patient information sheet as described in appendix III. The subjects were given adequate time to discuss the protocol with their partners if they so wished and then were given an opportunity to ask questions. They were encouraged to come back at a later date if they so wished. Once they understood the requirements of the study and were happy to proceed, all subjects signed the consent form (appendix IV). A total of 79 subjects agreed to participate in the study.

2.5 Subjects

The patients with an injury level above T10 were invited to participate in this study. Subjects with both complete and incomplete injuries were eligible to take part. The minimum duration since the time of injury was set at 6 months in line with previous studies by Brindley(14) and Siosteen(15). It was considered to randomise patients separately by completeness of injury and with method of bladder drainage but it was concluded that it would be almost impossible to recruit sufficient numbers of patients for adequate statistical analysis of results.

The mean age of the subjects at injury was 28 years (range 11- 47 years) and the mean age at procedure was 42 years (range 26 - 63 years). The mean duration since injury was 7.7 years (range 0.5 – 44 years). Of the 79 subjects, 39 had injury at cervical level, 25 subjects had injury at thoracic levels T1-T7 whilst 15 had an injury level between T7-T10. There were 41 complete and 38 incomplete injuries.

45 subjects were performing SIC for bladder management. 17 subjects had a SPC, 8 were voiding on urge, 8 were reflex voiding whilst 1 patient had a sacral anterior root stimulator (SARS).

An attempt was made to evaluate if BCR and HR could predict a successful outcome of the procedure. However, on analysis of full data it was discovered later on that clear documentation for these

was only performed in 33 subjects by both the observers. Hence, this could not be included in final analysis.

2.6 Materials and equipment

The following materials were required to perform the project successfully.

2.6.1 Microscope

A Zeiss Compound microscope was used for evaluation of the ejaculate (Figure 2.1). The microscope had magnifications of 10, 20, 40 and 100 (oil immersion) with an ocular piece of 10X. For the purpose of assessment of motility, forward progression and for sperm counting the magnification of 10, 20 or 40 was used. Sperm morphology was analysed with a 100 magnification under oil immersion.

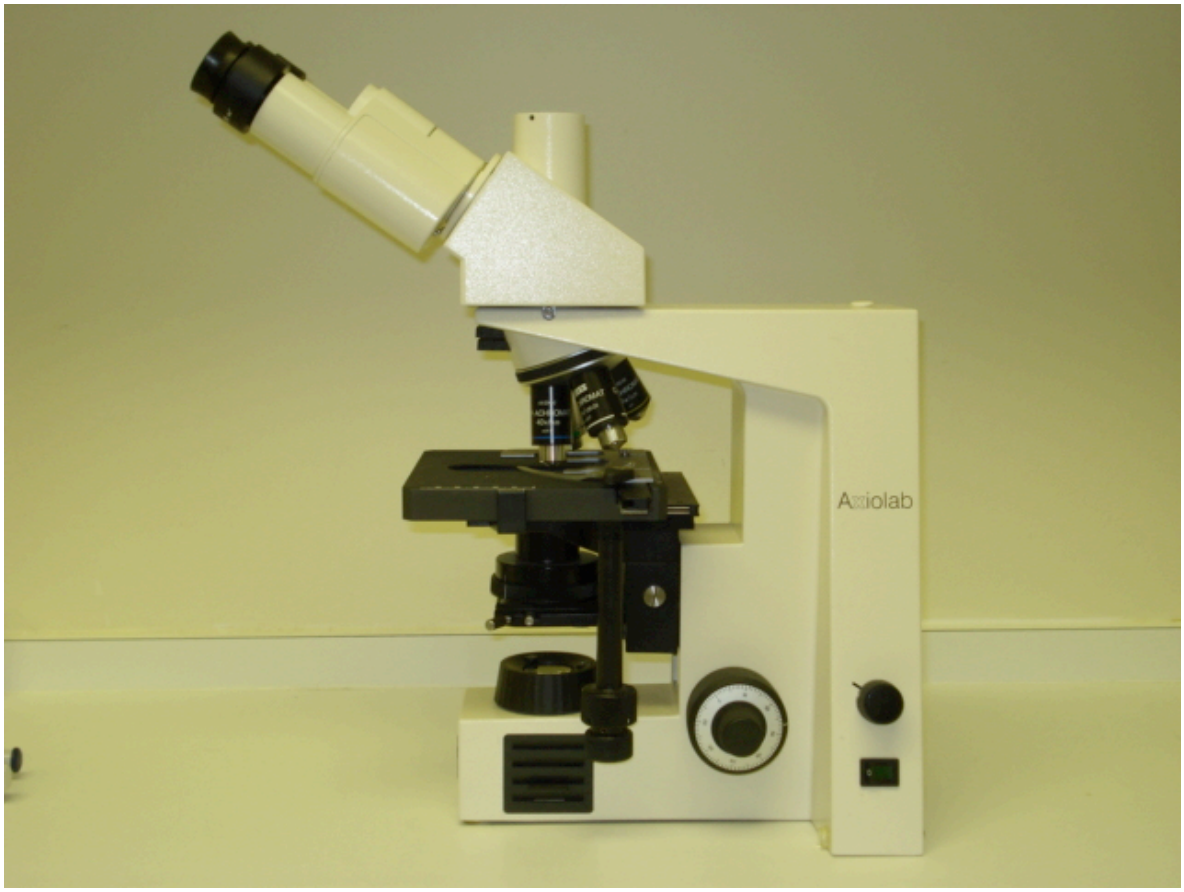

Figure 2.1: Zeiss microscope

2.6.2 Mini-therm stage warmer

A mini-therm stage warmer (Hamilton Thorne Research, Beverly, MA, USA) was used to analyse the motility and forward progression of sperms at 37° C. The stage was pre-heated to 37° C. The temperature was checked with the control slide. When the colour of the control slide changed from black to green, it signified the achievement of the desired temperature. The mini-therm stage fits easily onto the slide holder of the Zeiss compound microscope. There is a slot within the mini-therm stage warmer to accommodate a standard slide. The mini-therm stage warmer was continuously heated so as to keep the temperature constantly at 37° C for accurate assessment of sperm motility and forward progression at body temperature (Figure 2.2).

Figure 2.2: Mini-therm stage warmer with control slide

2.6.3 Microscope slides and cover slips

Standard pre-cleaned frosted slides were used for performing all microscopic evaluations. A 1 inch cover-slip was used on top of the slides for the assessment of motility and forward progression.

2.6.4 Pipettes

1- Positive Displacement Pipette: A Positive displacement pipette (Eppendorf) was used for accurate assessment of the sperm motility (Figure 2.3). It withdraws a fixed volume of semen (10 micro litres). It is often used when the fluid being examined is viscous. A disposable chamber was used which was discarded after each evaluation.

2- Normal Pipette: A normal pipette (Eppendorf) was used for evaluation of the sperm count. It withdraws a pre-determined volume of formalin which is added to a fixed volume of semen for fixation of sperms to evaluate accurately the count (Figure 2.4). The Eppendorf pipettes have plastic connectors which are single use and were discarded after single use.

3- Plastic Pipettes: Normal single channel plastic pipettes were used with adjustable volume setting. They were used for withdrawing semen from the containers. They were discarded after each use.

Figure 2.3: Positive displacement pipette

Figure 2.4 : Normal pipette

2.6.5 pH strips

The pH strips from Bayer® were used for the assessment of the pH.

2.6.6 Plastic bottles

Open mouthed plastic containers were used for the collection and evaluation of semen during the study. They were single use and discarded after each study.

2.6.7 Haemocytometer

A haemocytometer with neubauer chamber was used for counting sperm (Figure 2.5). It was washed in running water and cleaned with alcohol wipes after every use.

Figure 2.5: Haemocytometer

2.6.8 Solutions used for assessment of morphology

The following solutions were used for the assessment of morphology:

1- Ethanol

2- Distilled water

3- Haematoxolin

4- Eosin

5- Alcohol

6- Tap water

The details of the solutions and the preparation of slides are given in appendix V.

2.6.9 Ferticare® personal vibrator

The ejaculation was performed using a Ferticare® personal vibrator (Multicept, Albertslund, Denmark). The vibrator had to be charged overnight for 16 hours before adequate usage. It had 2 settings, 1 for amplitude and the other for frequency. The vibrating disc was on the top (Figure 2.6). The on/off button was on the side. The device had to be used with care so as not to overload the system. The subjects in the study arm were loaned the vibrator and explained the technique of performing ejaculations once a week.

Figure 2.6: Ferticare® personal vibrator

2.7 Performance of vibratory stimulus

2.7.1 Pre-procedure preparation

The baseline ejaculation was performed in the laboratory. It was ensured that a blood pressure monitor and an emergency resuscitation apparatus were available in the room. All subjects prone to autonomic dysreflexia were given prophylactic Nifedipine 10mgs. Otherwise it was ensured that this medication was available in the laboratory in the event that the subject developed autonomic dysreflexia.

After discussing the patient information sheet (Appendix III) with the subjects and answering all the questions, consent was obtained on the form approved by the ethics committee (Appendix IV). The subject relevant pre-operative and post-operative history was recorded in the data collection sheet (Appendix VI).

He was then seated comfortably, ensuring that the facility was available to elevate the back rest in case of the subject going autonomic. The temperature of the laboratory was set at 25° C. The partner, if accompanying was seated in the corner. For the subject performing SIC the bladder was emptied with the catheter. In case of a SPC the leg bag was emptied. The condom sheath was removed for those on this method of bladder drainage. A careful assessment of the glans and penile skin was then made for signs of any abrasion or skin lesion. The urine samples were not routinely tested to check for infection as those subjects who were complaining of symptoms of

a urine infection were not brought for screening unless the infection was cleared.

2.7.2 Demonstration of the Ferticare® personal vibrator

A Ferticare® penile vibrator (Multicept, Albertslund, Denmark) was used to collect the samples. A demonstration of the vibrator was performed to familiarise the subject with the equipment, especially to acquaint him with the typical noise produced by the vibrator. The vibrating disc was also placed against one of his fingers to give the subject a feeling of what to expect. The settings were then explained to the subject and the partner. The subject was told to expect spasms in the lower part of the abdomen and the upper part of the thighs. He was reassured that this was quite normal and that the spasms would increase as he was about to ejaculate.

It was explained to the subject that the initial vibrations would be performed for 2 to 3 minutes or until he ejaculates. The settings would then be increased and further attempts would be performed. This would continue until the procedure was successful, the subject requested to stop or he developed any adverse symptoms. A maximum number of attempts were not set before the start of the project but it was expected that no more than 6 attempts would be made in one evaluation.

2.7.3 Use of Ferticare® personal vibrator to obtain semen sample

The principle investigator stood on the right side of the subject with the collector on the left side. The collector held an open mouthed plastic container to ensure that the complete sample was collected. A blood pressure (BP) monitoring equipment was kept available in the procedure room but continuous monitoring of the BP was not performed. The initial settings on the vibrator were calibrated to a frequency of 100 Hz with an amplitude of 2.5mm as suggested by Sonksen et al(89). The foreskin was retracted for uncircumcised subjects and the Ferticare® vibrator started at the optimised settings as above for the initial attempt at VE. The vibrations were applied to the lower part of the glans and frenulum on the ventral side of the penis (Figure 2.7). The subject was warned that a feeling of constriction in the chest or a throbbing headache were signs that he might be going autonomic, in which case the investigator must be alerted at once as the procedure must then be stopped immediately. The subjects were offered Nifedipine before the procedure but most declined. However, it was ensured that it was available in case a subject developed autonomic dysreflexia.

If the first attempt at VE was unsuccessful a gap of 1 to 2 minutes was given and then the process was repeated. The parameters on the Ferticare® vibrator were then increased to a frequency of 110 Hz and the amplitude to 3.5mm. The procedure was repeated again for

2 to 3 minutes. A maximum of 6 cycles were performed if ejaculation did not occur. However, no maximum number of attempts were set if the initial attempt was unsuccessful. The penile skin was evaluated for damage before each set of vibrations. Obviously, if there were any adverse effects i.e. to the skin or the patient generally feeling unwell then the procedure was stopped immediately. All the adverse events were documented. It is to be emphasised that only antegrade samples were collected. Hence, if the subject felt spasms in the lower limbs and abdomen which are thought to be a sign of ejaculation no attempt was made to evaluate if the subject had ejaculated in a retrograde fashion. This was undertaken to ensure that all the collected and hence, evaluated semen samples are not mixed with urine for standardization of results. Also, this was in keeping with line with previous studies by other investigators(15;17;45;107;115;165)

The baseline and the final evaluation at 3 months for all subjects, both in study and control arms was performed in the laboratory. In addition, the monthly evaluations at one and two months for the subjects in the study arm were also undertaken in the laboratory.

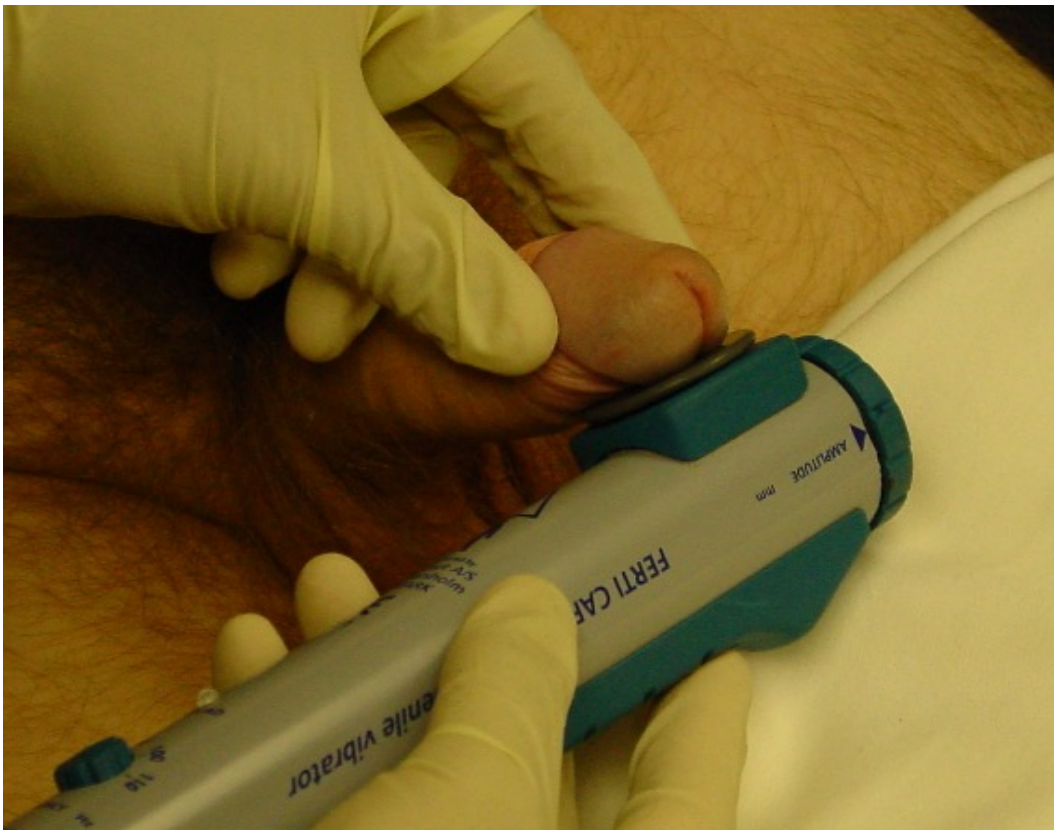

Figure 2.7: Vibro-ejaculation with the investigator on the patient's right side and the vibrator placed underneath the glans

2.7.4 Collection of semen specimen

The assistant standing on the left side with a wide mouthed container collected the ejaculate. To ensure accurate evaluation, the assistant made sure that the whole of the sample was collected. The patients were offered to wear a condom before starting VE but most refused.

2.7.5 Safe handling of the semen sample

The subjects were not screened for infectious diseases (Hepatitis, Acquired Immunodeficiency Syndrome or Herpes Simplex). Hence, the semen sample was thus treated as a biohazard. Gloves were worn whilst handling the samples. The semen sample was immediately processed in our laboratory and the specimen was discarded according to the hospital protocol. The safety guidelines for the Andrology laboratory were strictly observed as per WHO guidelines (Appendix VII).

2.7.6 Loan of Ferticare® personal vibrator

The subjects who were randomised to the study arm were supplied with the Ferticare® vibrator. This was an expensive piece of equipment and hence, the subjects were asked to sign a form stating that they would be charged if they lost the vibrator.

2.8 Semen analysis

The semen analysis was performed according to the WHO recommendations(22). The semen underwent both macroscopic and microscopic evaluations as described below.

2.8.1 Macroscopic examination

The following parameters were evaluated during the macroscopic examination.

2.8.1.1 Liquefaction

Normally the semen sample liquefies within 15 minutes. However it may take up to an hour for it to liquefy, especially in SCI patients. The liquefaction time was set at 30 minutes in the laboratory as a cut off between normal and abnormal liquefaction. If the sample contained jelly like grains it was stirred gently so as to reduce errors in determining sperm concentration(170) but it was not vigorously shaken. However, if the sample did not liquefy within 30 minutes this was documented. No additional measures such as mechanical mixing or enzyme digestion were used to facilitate liquefaction.

2.8.1.2 Appearance

This was examined after the liquefaction had been assessed. A normal sample should be grey and homogenous. Any change in colour i.e. black, brown, or red was noted.

2.8.1.3 Volume

This was assessed by pouring the semen into a calibrated container. As plastic syringes are thought to affect sperm motility they were not used for the assessment of volume.

2.8.1.4 Viscosity

The viscosity of the semen was assessed by gently aspirating it into the plastic pipettes. It was then allowed to drop by gravity and the pattern of the droplets was observed to assess the viscosity. Normal semen should drop as discreet drops. If the viscosity is abnormal then a long thread is formed which extends from the pipette all the way down to the base of the collecting flask. It is generally recommended that the length of the thread should be no longer than 2cms. Like liquefaction, if the sample was viscous this was documented but no additional measures were used to reduce viscosity.

2.8.1.5 pH

The pH was assessed by putting a drop of the semen on the pH strip. Strips from Bayer® were used for this purpose. It was read after 30 seconds according to the calibrations guide supplied with the pH strips.

2.8.2 Microscopic examination

The motility, forward progression, count and morphology of the ejaculate were assessed with the help of a compound microscope.

2.8.2.1 Preparation of sample for analysis of motility and forward progression

It has been suggested that the size of the cover slip should be the same for the purpose of standardisation. A 10 micro litres volume of semen was placed in the centre of a clean slide with a positive displacement pipette. This was covered with the standard cover slip. It was ensured that the cover slip spread the drop of semen evenly onto the slide. It was always confirmed that no air bubbles were trapped between the cover slip and the slide. In case this was noted the slide was discarded and a fresh one prepared. Two slides were made, one for each observer. It was ensured that the two observers did not study each other's preparations.

Once the slides were satisfactorily prepared, they were left to stand for 1 minute. During this time the microfilm heated stage was warmed to 37° C. The laboratory temperature was set at around 23 - 25° C. Once the heated stage reached a temperature of 37° C it was checked with the control slide. It changed its colour from black to green. At this point the heated stage warmer was placed onto the slide holder of the compound microscope. The prepared slide was seated in the stage warmer. The initial viewing was performed at a 100 magnification (10 X objective & 10 X ocular). Once a general view was obtained it was then decided to set the magnification for final assessment of motility and forward progression depending upon the sperm agglutination and the evenness of spermatozoa on the slide.

2.8.2.2 Assessment of sperm motility

A simple grading system was used for the assessment of sperm motility. This was performed very carefully. At least 5 microscopic fields were randomly assessed, half a centimetre from the edge of the cover slip. Two slides with 10 micro litres of semen were prepared. The motility was assessed independently by 2 observers (Figure 2.8). A total of 200 spermatozoa were classified by each observer. Sperm motility was expressed as a percentage of the total sperms present (0% - 100%). The average percentage and the

difference between the percentages as assessed by the 2 observers were plotted on the graph (appendix VIII) as suggested in the WHO manual. For example if percentage motility by the 2 observers were 60% and 35%. The average would be 47.5% and the difference would be 25%. Once this is plotted on the graph it would appear that for 200 spermatozoa a 10% difference could be expected. This difference here was 25%, so it cannot be accepted. These slides were discarded and fresh slides were prepared.

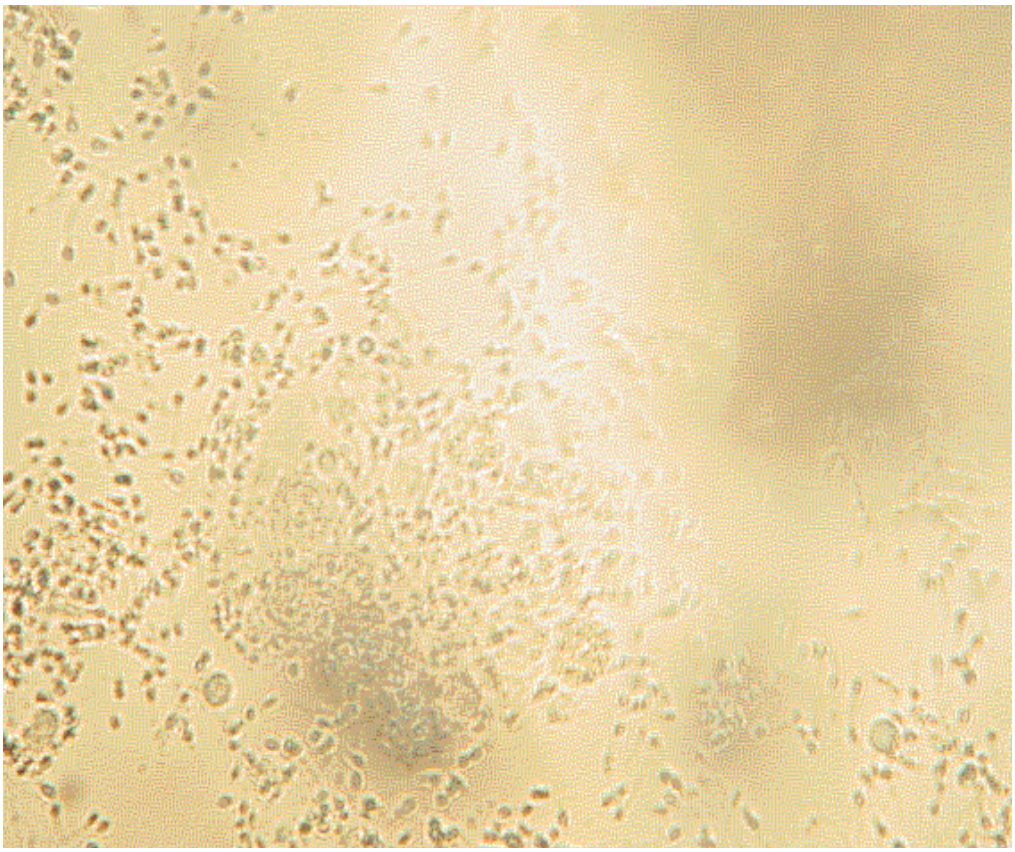

Figure 2.8: Slide to assess the motility and forward progression of sperms (under microscope)

2.8.2.3 Assessment of forward progression

The forward progression of the sperms was graduated on a scale of 0 to 3. The progression was classified as non-progressive - 0, sluggish -1, good forward progression -2 and a rapid progression -3. The forward progression was assessed at the same time as motility assessment. This was assessed in the same 200 spermatozoa along with the motility. It was performed independently by 2 observers. If there was a difference between the 2 observations then this was disregarded and fresh slides were prepared.

2.8.2.4 Assessment of sperm count

The sperm count analysis was performed with a haemocytometer after adequate dilution and fixation with formalin based solution. Two separate preparations were made on either side of the haemocytometer. A 1 to 20 dilution was usually performed with a normal displacement pipette (Eppendorf). The diluent is prepared by adding distil water, sodium bicarbonate and formalin (WHO manual). The neubauer chamber of the haemocytometer was fixed with a cover slip. The cover slip was pressed firmly so as to develop newton rings between the 2 glass surfaces. The semen mixed with formalin was allowed to stand for 5 minutes and stirred gently so as to get a uniform concentration. Then 10 micro litres of the mixed specimen were added to each part of the counting chamber of the

haemocytometer with a pipette. A small drop was placed on the side and with a capillary action it spread underneath the cover slip. The counting was performed either at 200 or 400 magnifications. Only complete spermatozoa with both a head and tail were counted (figure 2.9). All other forms i.e. pinheads and tailless were eliminated.

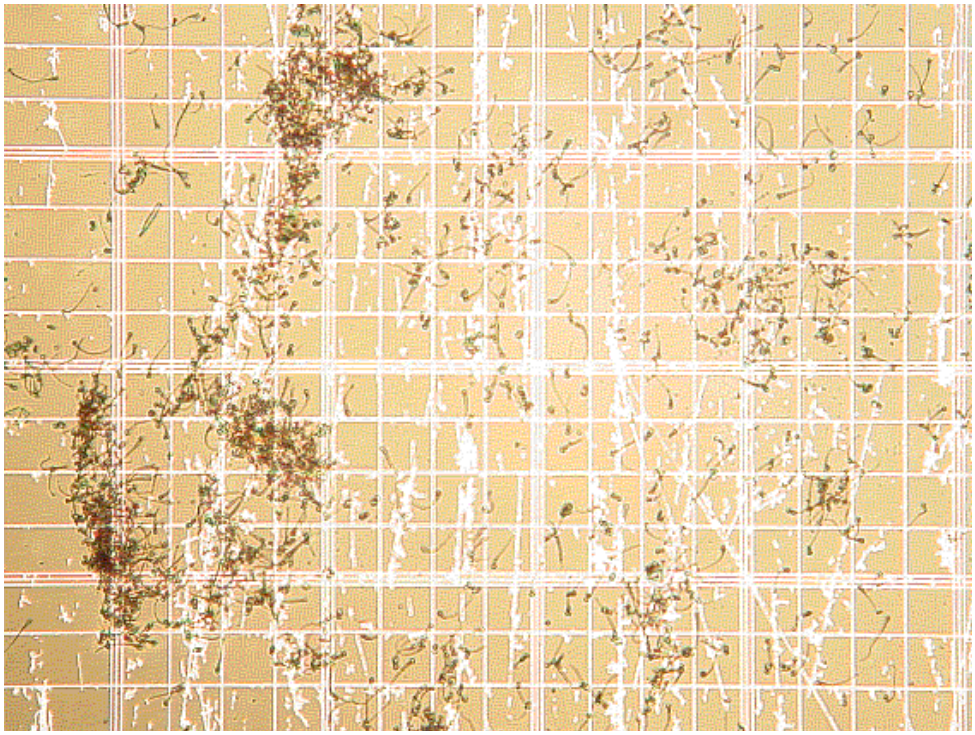

Figure 2.9: Assessment of sperm count (under microscope)

The central scale of the neubauer haemocytometer contained 25 large squares. Each of these squares was divided into16 small squares (Figure 2.10). If there were less than 10 spermatozoa in a large square then all the 25 squares were counted. If there were 10 to 40 spermatozoa then 10 large squares were assessed and if the sample contained more than 40 spermatozoa in a large square then 4 outer and 1 central square was counted. As a rule, if the sperms were crossing the lines only the ones on the upper and left hand side were counted.

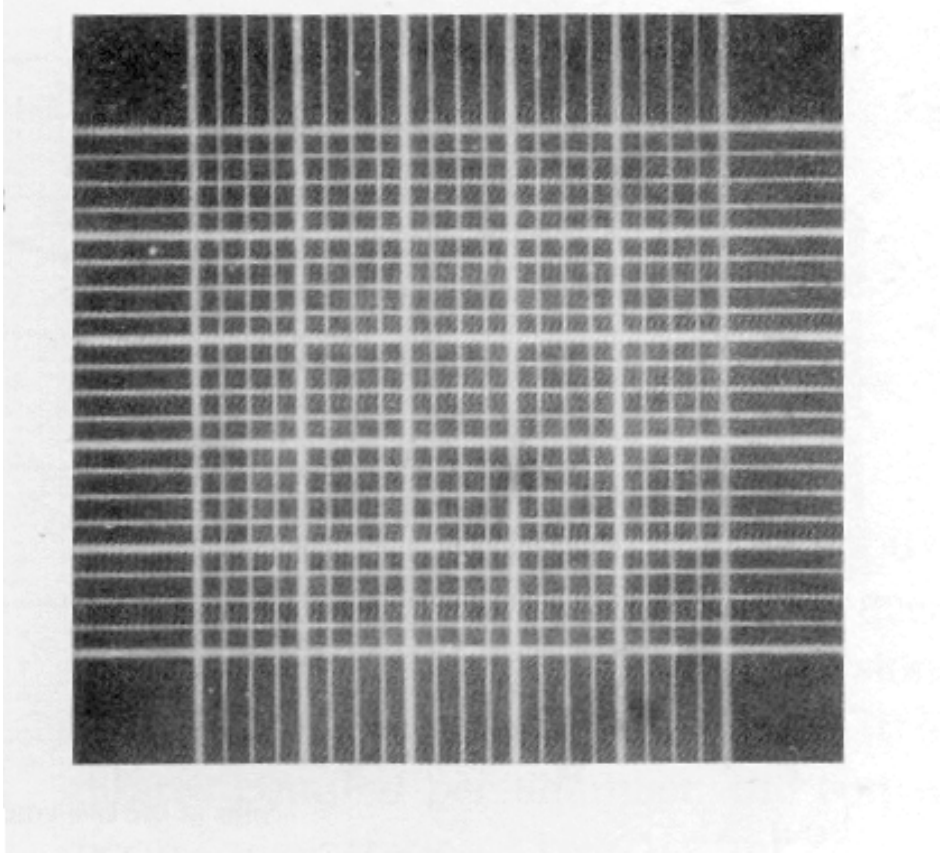

Figure 2.10: Neubauer chamber of a haemocytometer

An attempt was made to count duplicates of 200 spermatozoa by 2 independent observers on either side of the neubauer haemocytometer. The count was calculated according to the dilution and conversion factors for the neubauer haemocytometer as specified in the WHO manual (figure 2.11). The average count was checked across the table for a given dilution i.e 1;20. Then the conversion number was noted from the number of the large squares counted. This provided the count in millions per ml. This was performed separately for both observers. The sum of the 2 counts by the observers and the difference between them was calculated. This was plotted on the graph used for accurate calculation of sperm count as per WHO guidelines (appendix IX). If the difference was greater than expected from the graph values then this was discarded and fresh slides were prepared. No special processing techniques were utilised if the number of the spermatozoa was low. The sample was not centrifuged.

Figure 2.11: Dilution and conversion factors for analysis of sperm count- adapted from WHO manual (22)

2.8.2.5 Assessment of sperm morphology

Two slides were prepared for duplicate analysis of sperm morphology. The slides were washed in ethanol and dried. 10 micro litres of sperms were dropped on the slide. A second slide was placed on top so that the drop spread evenly between the 2 slides. It was ensured that there were no air bubbles between the 2 slides. The slides were gently pulled apart and 2 smears were obtained simultaneously. The slides were allowed to dry in air and then fixed. Papanicolaou (PAP) staining: A PAP stain was used for the staining of the spermatozoa. It stained the head, the acrosome along with the central piece, the cytoplasmic droplet and lastly the tail. The composition of the PAP stain is shown in appendix V. The head is stained in pale blue in the acrosomal region. The post-acrosomal region is stained dark blue. The mid-piece is stained red and the tail is bluish. The cystoplasmic droplet is stained green (figure 2.12).

No additional techniques were employed to improve the sperm concentration or decrease the viscosity by removal of seminal plasma or addition of normal saline. The slides were prepared with the available sample.

The classification for assessment of sperm morphology was:

1- The head should be oval.

2- The acrosome should comprise 40 to 70% of the head area.

3- The mid piece should be 1 ½ times the length of the head and attached axially to the head.

4- The cytoplasmic droplet should be less than half the size of the head.

5- The tail should be straight, uniform and uncoiled.

All the borderline forms were classified as abnormal(171). The various abnormalities noted are shown in appendix X.

For counting the morphology only the spermatozoa with complete head and tail were evaluated.

Once the slides had been prepared for morphology and dried they were placed in the slide chamber underneath the Ziess microscope. 100 oil immersion lens was used. 200 consecutive spermatozoa were counted and the percentage of the normal sperms by the strict criteria described above was noted separately. This was performed on two slides by two independent observers. The average percentage of the 2 observers and the difference between the percentages was calculated. This was plotted on the same graph used for assessment of motility (appendix VIII). The final calculation was performed in a similar fashion. If the difference was outside the expected range according to the WHO guidelines(22) then these were discarded and fresh slides prepared.

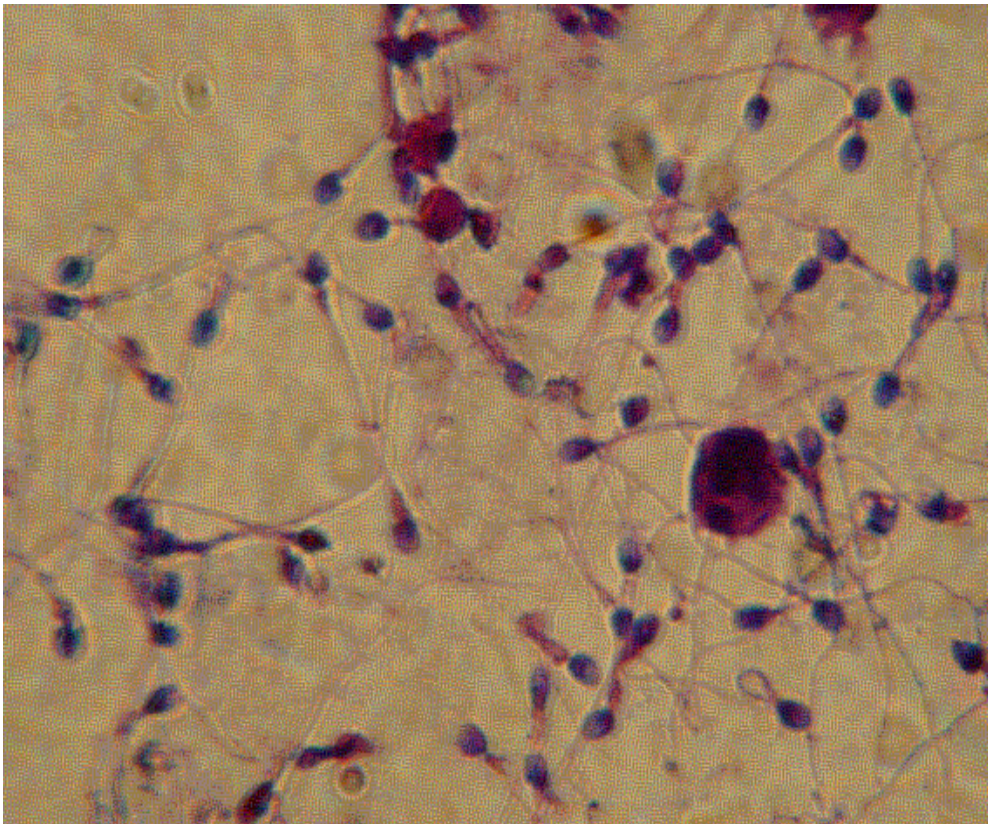

Figure 2.12: assessment of sperm morphology under microscope

2.9 Statistical design to test the hypothesis

The study was set up to test if repeated VE would improve the quality of sperms following SCI in men. There are various semen characteristics that can be evaluated according to the WHO guidelines including volume, count, morphology, motility and forward progression. Various investigators (15;17;106;107;115) have evaluated count, morphology, motility and forward progression whilst investigating the effects of repeated ejaculations on the quality of sperms in SCI men. It was decided to evaluate the same characteristics but choose motility as the primary factor for statistical analysis on advice of the statistician. Only one parameter had to be selected to ensure that sufficient number of subjects can be recruited in both arms for the study to be adequately powered to detect a difference in this parameter.

It was determined that a difference in motility of 20% between the 2 arms would be considered significant. There was no reference guidance available from the previous studies above to base our assumption, hence it was thought that if this difference was detected it could be considered as significant. The statistician calculated that to rule out the probability of showing no difference when one exists with a power of 80% (type II error) and to rule out a probability of showing a difference when none exists (type 1 error) with 95% confidence it would require 9 patients in each arm.

A paired Student t-test was used to calculate the statistical significance between the baseline and 3 months semen parameters in both the study and control groups. A p value of <0.05 was taken as significant. Contingency tables were used to evaluate other data with Chi-square test for significance.

CHAPTER THREE

Results

3 RESULTS

3.1 Overall account of subjects

A total of 79 subjects agreed to participate in the study. Forty two (53%) subjects were successfully ejaculated with the Ferticare® personal vibrator. The remaining 37 were unsuccessful in producing an antegrade ejaculate even after multiple attempts. Eight of the 42 successful subjects decided not to participate after baseline evaluation.

The remaining 34 were randomised into study and control arms. The 37 non-responders were referred for EEJ after appropriate counselling about whether they wanted to explore further the status of their sperms.

The mean age of the subjects at injury was 28 years (range 11- 47 years) and the mean age at procedure was 42 years (range 26 - 63 years). The mean duration since injury was 7.7 years (range 0.5 – 44 years). Thirty nine subjects had injury at cervical level, 25 subjects had injury at thoracic levels T1-T7 whilst 15 had an injury level between T7-T10. There were 41 complete and 38 incomplete injuries.

45 subjects were performing SIC for bladder management. 17 patients had a SPC, 8 were voiding on urge, 8 were reflex voiding whilst 1 patient had a SARS.

3.2 Comparison between responders and non-responders

The 37 subjects who did not ejaculate in an antegrade fashion were labelled as non-responders for the purpose of this study. All the non-responders were given an opportunity to re-try the procedure. The majority had a minimum of 2 attempts. One subject had 6 attempts, whilst 7 had only 1 attempt. Of the 7 in whom only 1 attempt was made, 1 developed autonomic dysreflexia, whilst the remaining 6 did not find the experience comfortable. Some of the non-responders were brought back after a few days to have a second attempt. However, of the subjects who were unsuccessful initially none managed to produce an antegrade sample with repeated attempts. The probable reasons for this will be discussed later on.

3.3 Predictors for successful ejaculations

The demographics were compared between the responders and the non-responders to evaluate the predictors of success. There was no statistical difference between the age at injury, age at procedure or the time since injury. The time since injury varied between 0.5 to 27 years in both groups. This is summarized in the table 3.1 below. A parametric non paired, two tailed test was used to evaluate for significance.

Parameter	Responders	Non-Responders	p value
Age at Injury	28.02 (± 7.55)	29.54 (± 8.96)	0.42
Age at procedure	42.62 (± 8.56)	43.04 (± 7.06)	0.83
Time since injury	6.52 (± 5.82)	8.96 (± 9.65)	0.17

Table 3.1: Comparison of age at injury, age at procedure and time since injury between responders and non-responders

The level of injury, completeness of injury and the bladder management were compared between the responders and non-responders by Contingency tables. A Chi-Square test was used to evaluate for significance. The results are described for level of injury in table 3.2, completeness of injury in table 3.3 and bladder management in table 3.4 below.

Table 3.2: Level of Injury

	C1-C7	T1-T7	T7-T10
Responders	20	13	9
Non-responders	19	12	6

Chi-square value 0.35, p=0.84

Table 3.3: Completeness of injury

	Complete	Incomplete
Responders	17	25
Non-responders	24	13

Chi-square value 4.69 p=0.03 *

Table 3.4: Bladder Management

	SIC	SPC	Urge	Reflex	SARS
Responders	28	7	3	3	1
Non-responders	17	10	5	5	0

Chi square value 5.372, p=0.25

It appears that the only statistically significant factor influencing success with VE in an antegrade fashion was the completeness of injury. An incomplete injury afforded a better chance than a complete injury for success with VE.

It was also tried to evaluate if the BCR or the HR could predict a successful outcome of the procedure. Unfortunately, these 2 tests could only be performed in 33 subjects by both the observers and hence these were not evaluated on the final analysis. These were performed in 17 responders and 16 non-responders. The BCR was positive in 16/17 responders and 12/16 non-responders. The HR was positive in 13/17 responders and 9/16 non-responders. There was a trend for both the reflexes to be positive in responders but regrettably no definite conclusion could be drawn as full data could not be obtained.

3.4 Summary of total subjects attending for screening

Forty two from a total of 79 subjects (53%) successfully VE and were invited to participate in the study according to the protocol. However, 8 of these subjects declined to take part in the study. The majority attended for screening as they wanted to find out if they would ejaculate with this method. Some subjects, after discussion with their partners, decided not to enter the study as they felt that they would not be able to fulfil the requirements of the study protocol.

The remaining 34 subjects agreed to participate in the research project. Eighteen subjects were randomised to the study and 16 to the control arm. Of these 34 subjects 25 completed the entire study according to the protocol. 12/18 subjects completed the study arm whilst 13/16 subjects completed the control arm of the project.

3.5 Demographics of subjects entering the project

A total of 34 subjects entered the study. Eighteen subjects were randomised to the study and 16 to the control arms. The demographics details of these subjects is summarised in table 3.5.

Table 3.5: Demographics of 34 subjects randomised to the project (next page)

Pt no	Age at injury (yrs)	Age at procedure (yrs)	Time since injury (yrs)	Level of Injury	Complete / Incomplete	Bladder Management	Project status
1	34.0	48.0	5.75	C6	Incom	SIC	Study completed
2	29.0	47.0	9.0	T2	com	SIC	Study completed
3	35.0	47.0	4.0	T4	com	SPC	Study completed
4	47.0	55.0	1.5	T5	com	SIC	Study completed
5	37.0	45.0	0.6	C5	Incom	SIC	Study completed
6	17.0	43.0	17.0	C4	Incom	SIC	Study completed
7	39.0	54.0	5.0	T5	incom	Urge voiding	Study completed
8	29.0	38.0	1.5	T8	com	SIC	Study completed
9	21.0	31.0	2.5	T4	incom	SIC	Study completed
10	30.0	52.0	15.0	C5	com	SIC	Study completed
11	29.0	53.0	19.0	C5	com	SARS	Study completed
12	31.0	46.0	7.0	T7	com	SIC	Study completed
13	19.0	30.0	3.5	T6	incom	SIC	Control completed
14	32.0	42.0	2.4	T1	com	SIC	Control completed
15	24.0	37.0	4.2	C6	com	SPC	Control completed
16	31.0	47.0	8.0	C4	incom	Urge voiding	Control completed
17	26.0	44.0	10.0	C5	Incom	SPC	Control completed
18	41.0	52.0	1.5	T5	Incom	SIC	Control completed
19	33.0	45.0	4.0	C5	incom	SPC	Control completed
20	38.0	50.0	4.4	T3	Com	SIC	Control completed
21	35.0	45.0	1.5	C5	Com	SPC	Control completed
22	13.0	34.0	13.0	C4	Incom	SPC	Control completed
23	21.0	29.0	1.5	T5	incom	SIC	Control completed
24	34.0	47.0	7.0	C5	com	Reflex void	Control Completed
25	21.0	31.0	4.0	C6	incom	SIC	Control Completed
26	36.0	47.0	3.0	T5	com	SIC	Drop out – Study
27	23.0	41.0	10.0	C4	incom	SIC	Drop out – Study
28	20.0	29.0	1.25	T10	com	SIC	Drop out – Study
29	18.0	52.0	27.0	T5	incom	SIC	Drop out – study
30	18.0	26.0	0.5	C5	incom	SIC	Drop out – study
31	28.0	36.0	2.0	T7	com	SIC	Drop out – study
32	17.0	32.0	7.0	T10	com	SIC	Drop out – control
33	24.0	40.0	7.2	T7	com	SIC	Drop out – control
34	17.0	29.0	4.5	C5	incom	SIC	Drop out – control

25 of the above 34 subjects completed the study. The study and control arms appear to be well matched as shown in table 3.6 below. There was no significant difference between the study and control group with regard to the patient's age, time since injury, level of injury, completeness or bladder management.

Parameter	Study Group	Control Group	p value
Number	12	13	
Age at Procedure (years)	46.58 (± 6.95)	41.00 (± 7.89)	0.07
Time since injury (years)	7.32 (± 6.38)	5.0 (± 3.54)	0.27
Level of Injury			
C1-C7	5	9	Chi-square 4.109
T1-T6	5	4	P=0.13
T7-T10	2	0	
Complete	6	5	Chi-square=3.23
Incomplete	6	8	P=0.58
Bladder Management			
SIC	9	6	Chi-square=5.24
SPC	1	5	P=0.26
Urge	1	1	
Reflex	0	1	
SARS	1	0	

Table 3.6: Subject Demographics – study and control arms (25 patients completing project)

3.6 Analysis of subjects dropped from the project

Nine of 34 subjects (26%) did not fulfil the entire research protocol. Six of 18 subjects (33%) in the study arm dropped out after entering the study. The majority of these 4/6 (66%) could not continue to ejaculate regularly after entering the study at various intervals. They were all trying to use the vibrator as per the instructions. We did not try to evaluate if they were having retrograde ejaculations as it would not have been possible to objectively compare the ejaculate with the antegrade samples. One subject was unwell and could not continue with repeated ejaculations on weekly intervals as per protocol. The 6^{th} subject neither attended for monthly evaluations nor for the final analysis at 3 months. He could not be contacted even after repeated attempts. It could not be ascertained if he was following the protocol.

In the control arm 3/16 (18%) subjects dropped out. One was admitted to another hospital with long term illness and was deemed unsuitable to travel for the 3 month evaluation. The remaining 2 patients could not be contacted in spite of repeated attempts to request them to come for the final analysis at 3 months.

3.7 Results of the experiment in study and control arms

Twenty five subjects (12 in study and 13 in control arm) completed the project. The changes in various parameters studied are depicted in the graphs below.

3.7.1 Volume

The changes in the volume of the ejaculate in the study arm (figure 3.1a) and control arms (figure 3.1b) are depicted in the graph below. There does not appear to be any significant change in volume over the 3 months in either the study or the control groups. This is shown in figure 3.2.

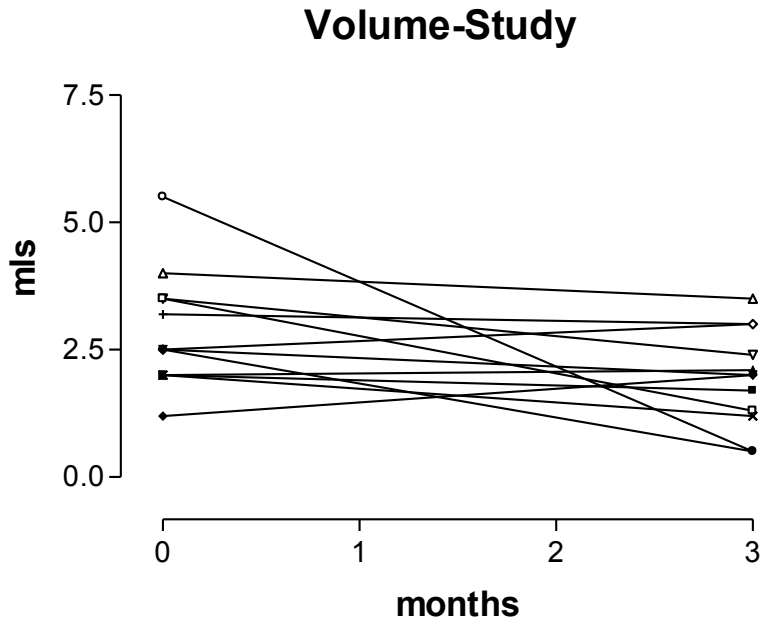

Figure 3.1a: Individual change in volume baseline and 3 months in study group (n=12)

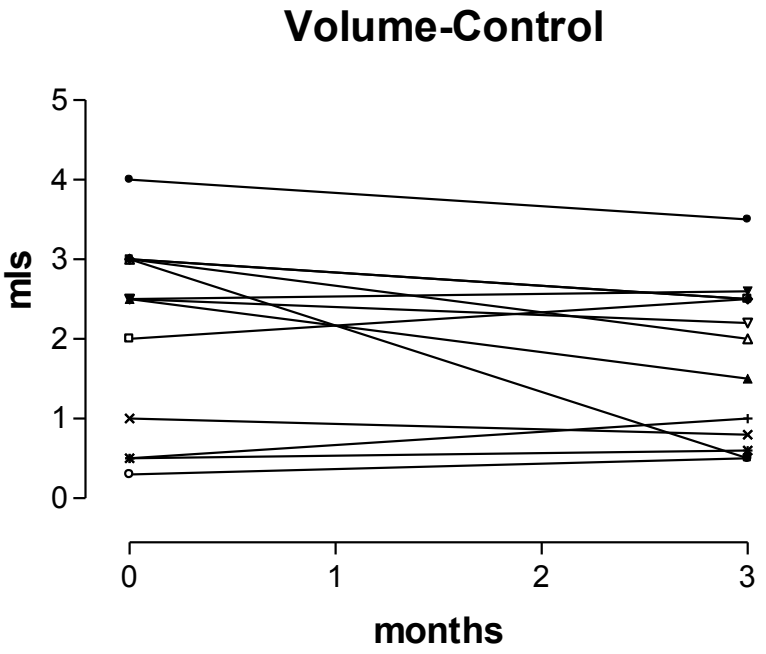

Figure 3.1b: Individual change in volume baseline and 3 months in control group (n=13)

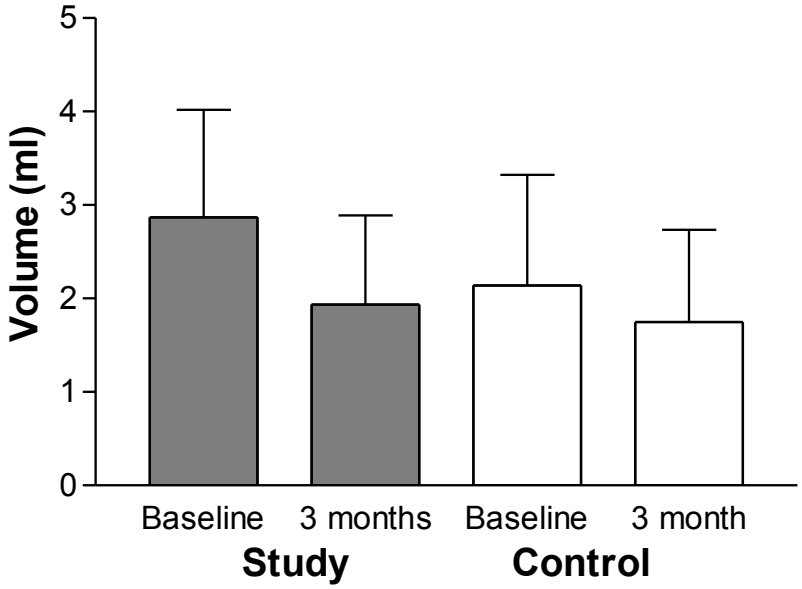

Figure 3.2: Graph denoting mean volume changes (error bars denote SD) in study and control arms at baseline and 3 months

3.7.2 pH

The pH of the ejaculate in the study (figure 3.3a) and the control (figure 3.3b) arms depicted a tendency to increase over the duration of the project. On statistical comparison there was no significant difference in the pH in the study arm over 3 months. However, there was a statistical increase in the pH of the control group at the end of the study period as shown in figure 3.4 below.

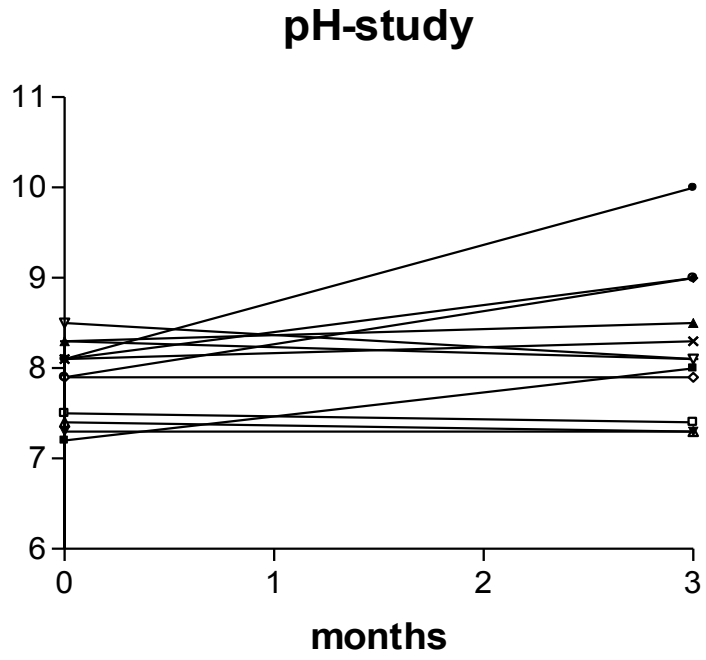

Figure 3.3a: Individual change in pH baseline and 3 months in study group (n=12)

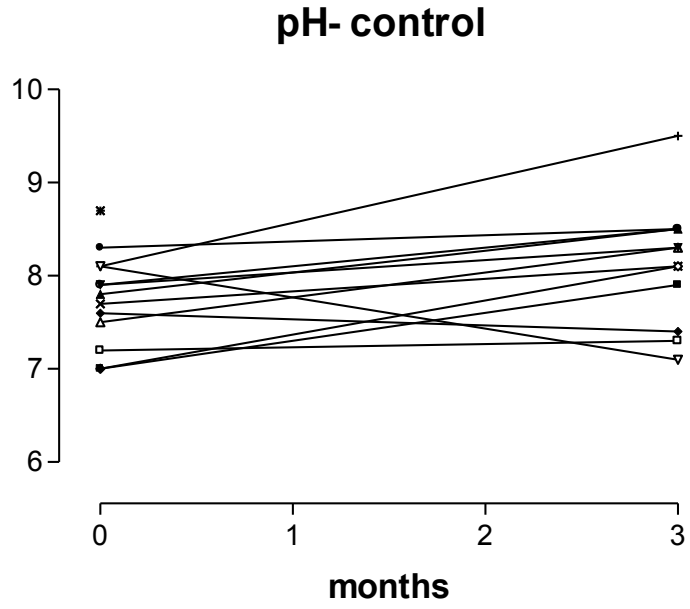

Figure 3.3b: Individual change in pH baseline and 3 months in control group (n=13)

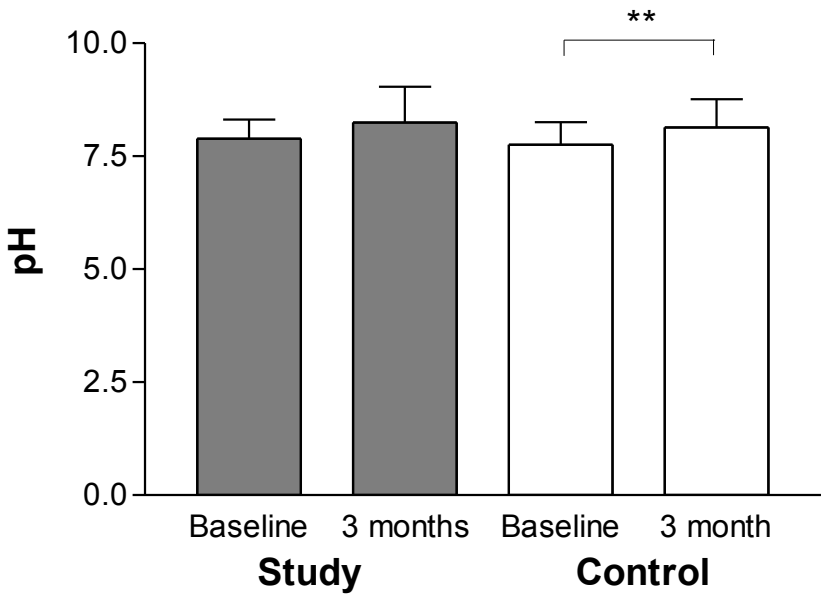

Figure 3.4: Graph denoting mean pH changes (error bars denote SD) in study and control arms at baseline and 3 months

3.7.3 Motility

The motility of sperms in the study group (figure 3.5a) changed for most of the subjects. This did not seem to a follow a definite pattern. The motility in the control group (figure 3.5b) was also variable without any definite pattern.

Overall, the motility of sperms did not change significantly in either the study or control arms on statistical comparison between the baseline and the 3 months sample in each group as shown in figure 3.6.

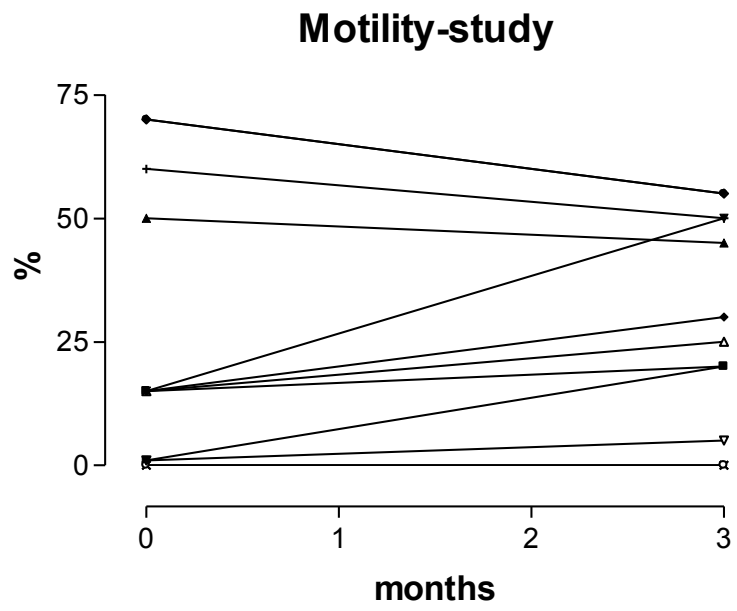

Figure 3.5a: Individual change in motility baseline and 3 months in study group (n=12)

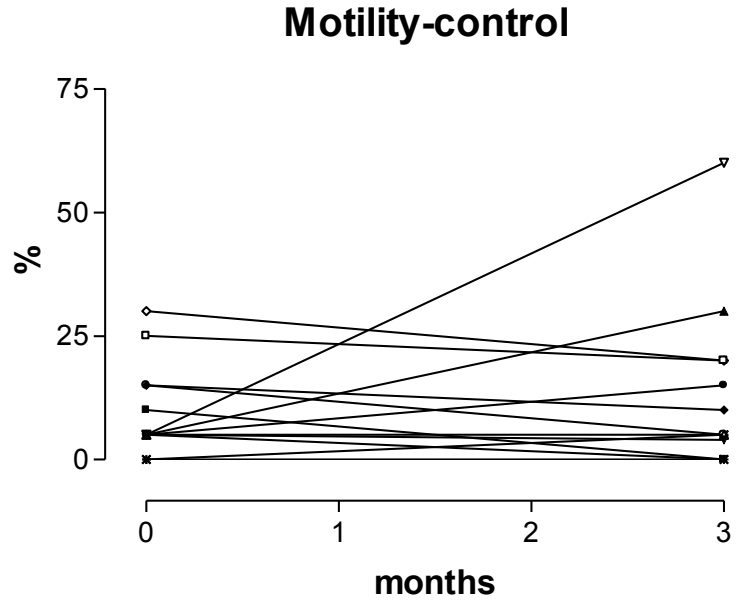

Figure 3.5b: Individual change in motility baseline and 3 months in control group (n=13)

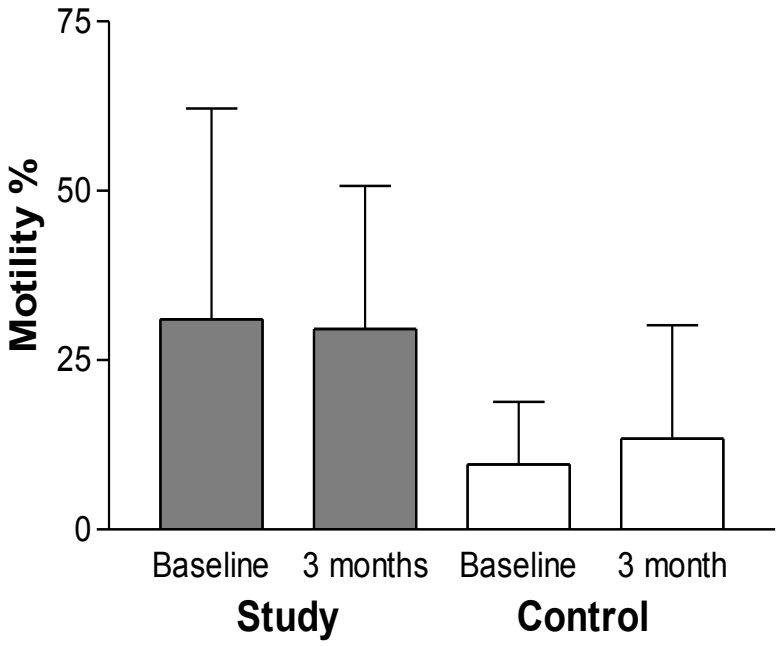

Figure 3.6: Graph denoting mean changes in motility (error bars denote SD) in study and control groups at baseline and 3 months

3.7.4 Forward progression

The forward progression of sperms improved in the study arm (figure 3.7a) with repeated ejaculations over the duration of the study period. The control arm (figure 3.7b) did not demonstrate any significant improvement in forward progression between baseline and the end of the study period. At 3 months there was a statistically significant increase in forward progression in the study arm but there was no significant change in the control arm as shown in figure 3.8.

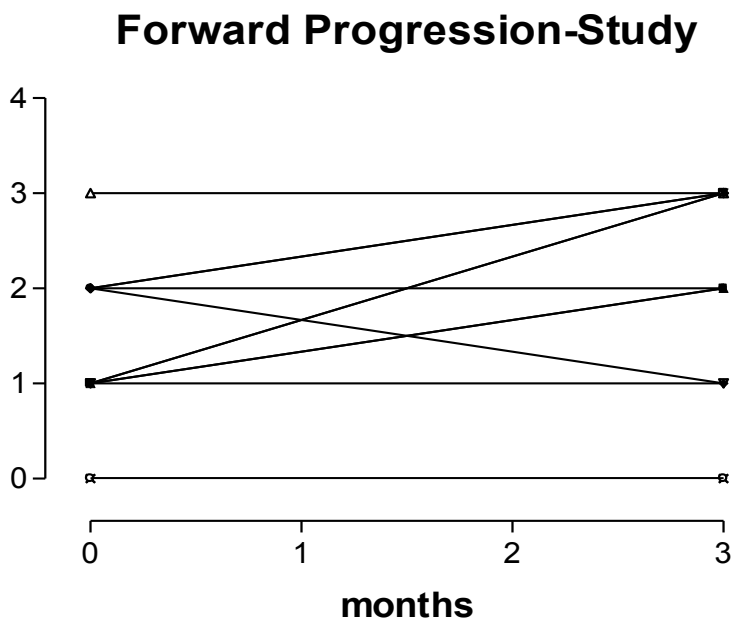

Figure 3.7a: Individual change in forward progression baseline and 3 months in study group (1-2:(**2**);1-3;(**2**);2-1:(**1**);2-2:(**1**);3-3(**1**);1-1:(**1**);2-3:(**2**);0-0:(**2**):– n=**12**)

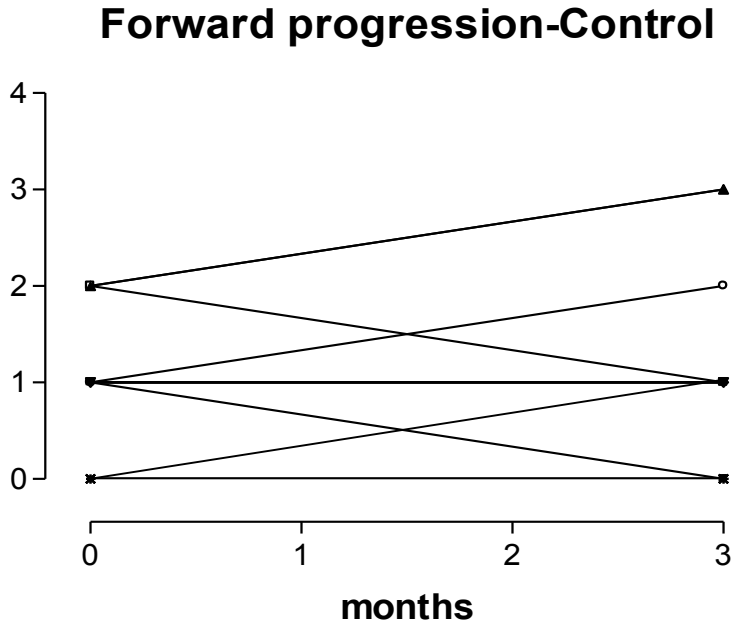

Figure 3.7b: Individual change in forward progression baseline and 3 months in control group (2-3:(**2**);1-0:(**1**);1-1:(**5**);2-1:(**1**); 1-2:(**1**); 0-0:(**2**); 0-1:(**1**):- n=13)

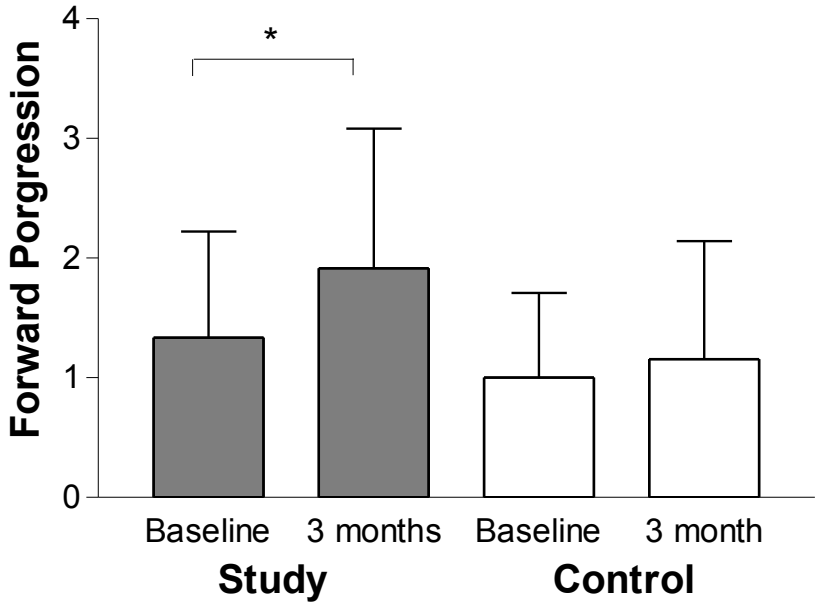

Figure 3.8: Graph denoting mean changes in forward progression (error bars denote SD) in study and control groups at baseline and 3 months

3.7.5 Morphology

The changes in morphology of sperms in the study group are shown in figure 3.9a. The morphology of the control group is depicted in figure 3.9b. There was a statistically significant improvement in the morphology of sperms in the study group over the 3 month study period. On the other hand, the morphology did not change significantly in the control group over this time as shown in figure 3.10.

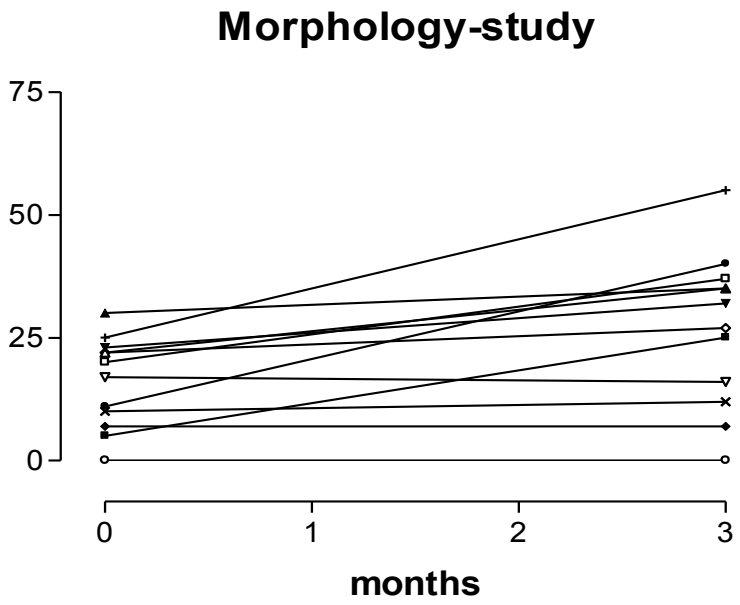

Figure 3.9a: Individual change in morphology baseline and 3 months in study group (n=12)

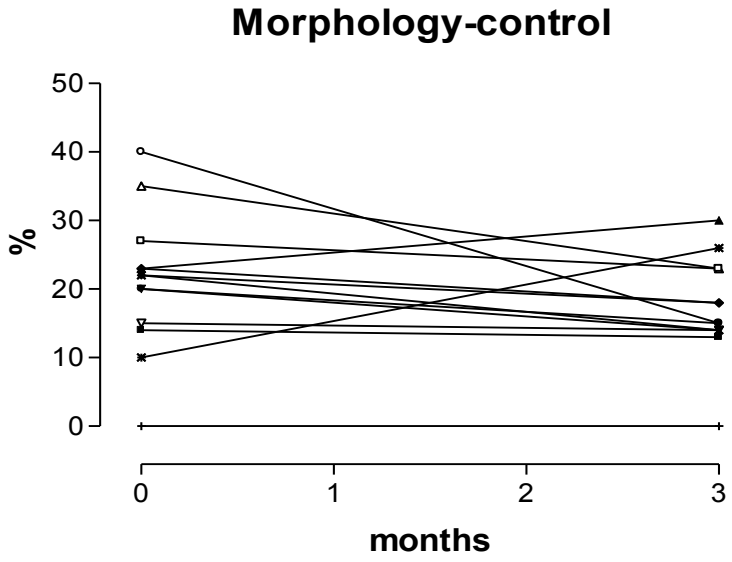

Figure 3.9b: Individual change in morphology baseline and 3 months in control group (n=13)

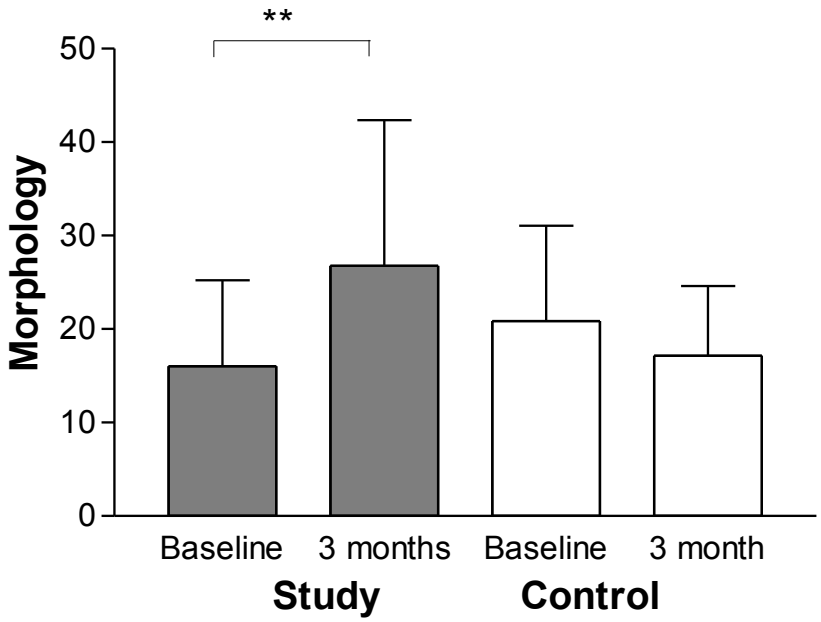

Figure 3.10: Graph denoting mean changes in morphology (error bars denote SD) in study and control groups at baseline and 3 months

3.7.6 Count

The count in the study group (figure 3.11a) changed considerably between different subjects but this did not seem to follow a definite pattern.

The count in the control group (figure 3.11b) also appeared to change without any definite pattern.

Overall, there was no significant change in the sperm count in either the study or control groups over the 3 month study period as shown in figure 3.12.

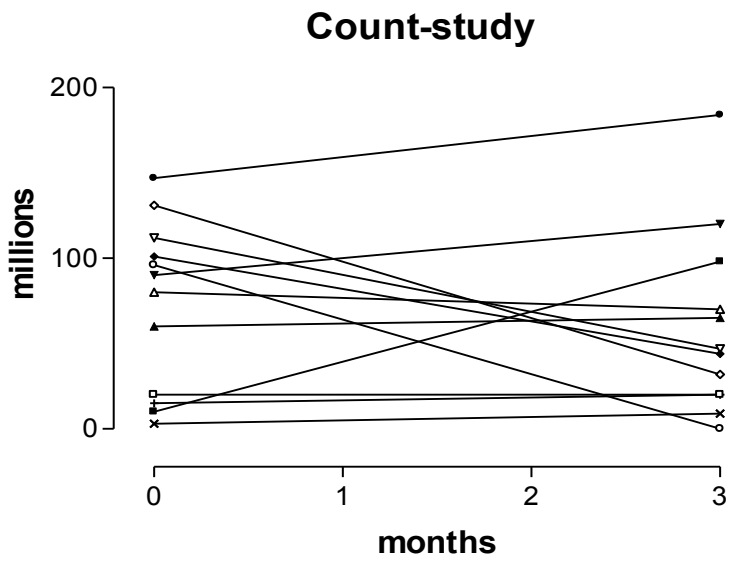

Figure 3.11a: Individual changes in the count baseline and 3 months in study group (n=12)

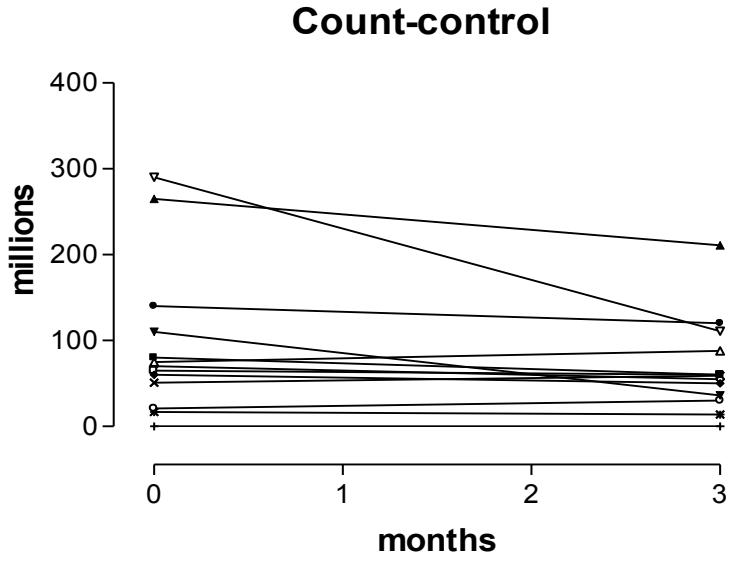

Figure 3.11b: Individual change in count baseline and 3 months in control group (n=13)

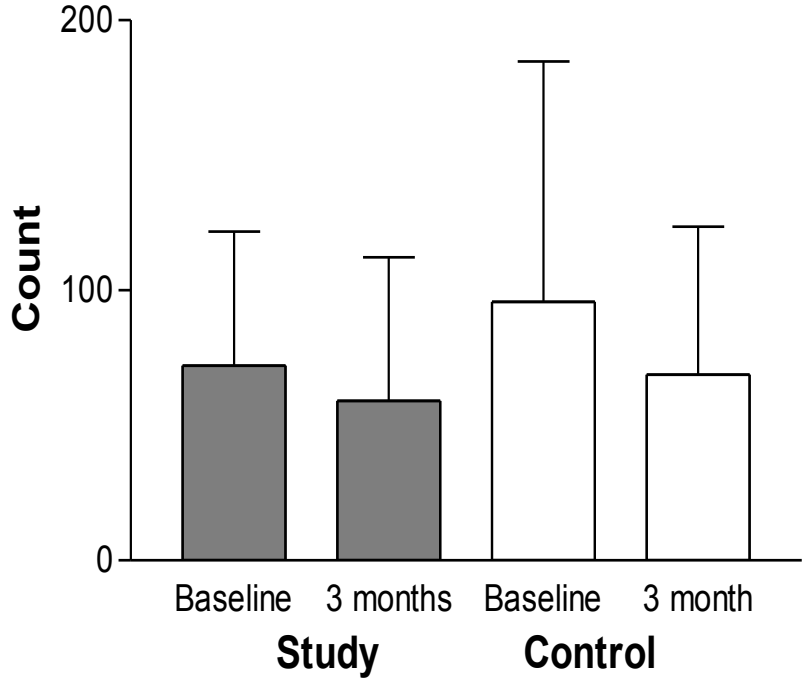

Figure 3.12: Graph denoting mean changes in count (error bars denote SD) in study and control arms at baseline and 3 months

3.7.7 Liquefaction

Overall, the semen samples in the project liquefied satisfactorily during the study period. There was no significant difference in the liquefaction time between the study and the control groups (figure 3.13).

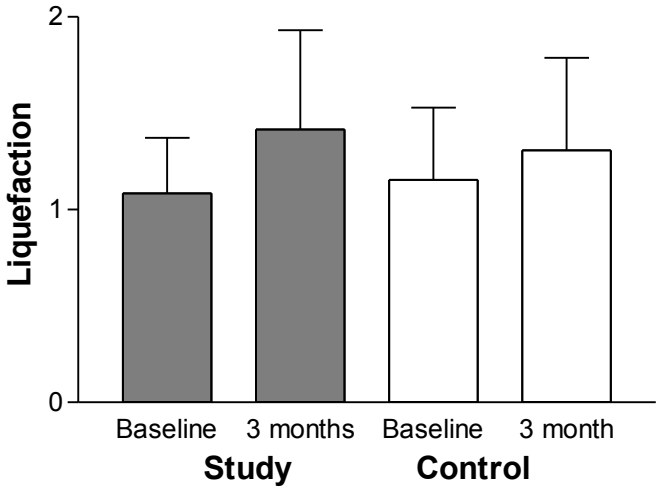

Figure 3.13: Comparison of liquefaction- study and control groups at baseline and 3 months

3.8 Detailed analysis of semen parameters in the study arm at monthly intervals

As there is a huge variation in various semen parameters in SCI men, further analysis were performed in the study group at monthly intervals to record the rate of change of various parameters. This was particularly important to evaluate if the improvement in some parameters was consistent over the duration of the study as one would expect with repeated ejaculations or is it that the change was a matter of chance.

A regression analysis was performed for each variable. A linear regression was undertaken. The r2 value suggests how good a fit the data is to the straight line, the closer one gets to 1 the better the fit. The significance value is whether the slope is significantly not horizontal. If it is horizontal there is no relationship between the X and Y data. If it is significantly not zero then there is some relationship between the X and Y data.

3.8.1 Volume

The volume in the study arm shows some variation but overall it does not appear to vary widely in most subjects (Fig 3.14). However, on linear regression curve (Fig 3.15) there seems to be a significant decrease over the 3 months. On statistical comparison with each subject separately there was no significant decrease in volume as shown in table 3.7.

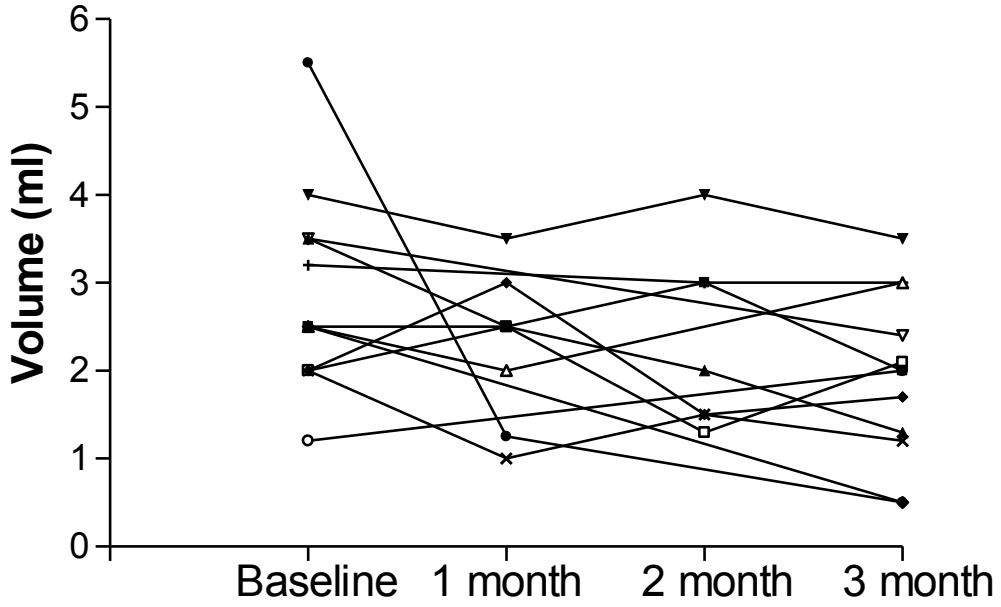

Figure 3.14: Volume in study arm – monthly over 3 months

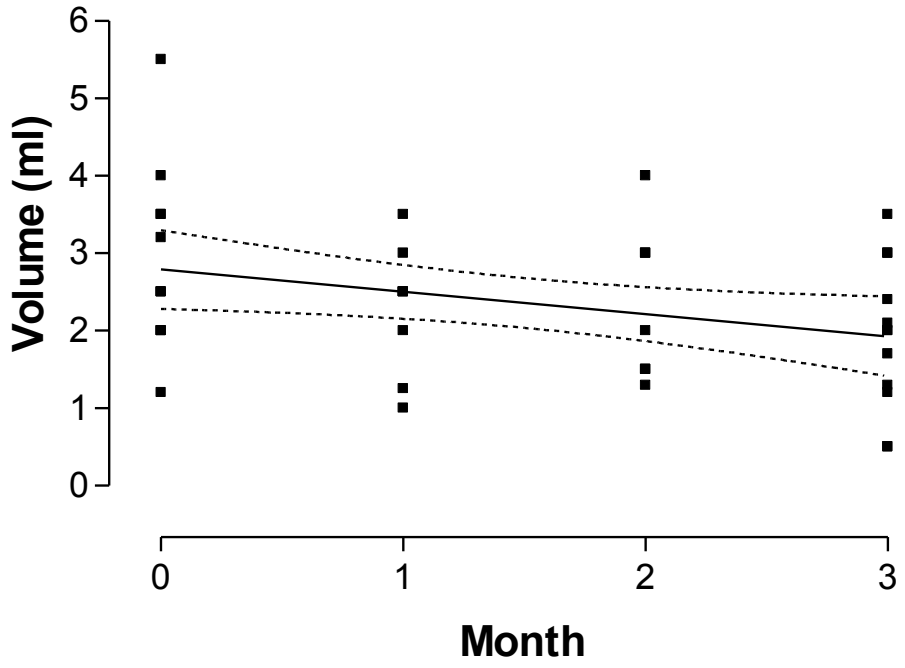

Figure 3.15: Volume in study arm with linear regression curve (r2 = 0.0116. Slope significantly non-zero p=0.034

3.8.2 pH

There was no particular pattern on the individual curves for this parameter (Fig 3.16) with some increasing and others decreasing. This was confirmed on the linear regression curve which did not reveal any significant change over 3 months (Fig 3.17). This is consistent with the statistical analysis in individual subjects between baseline and 3 months period as shown in table 3.7.

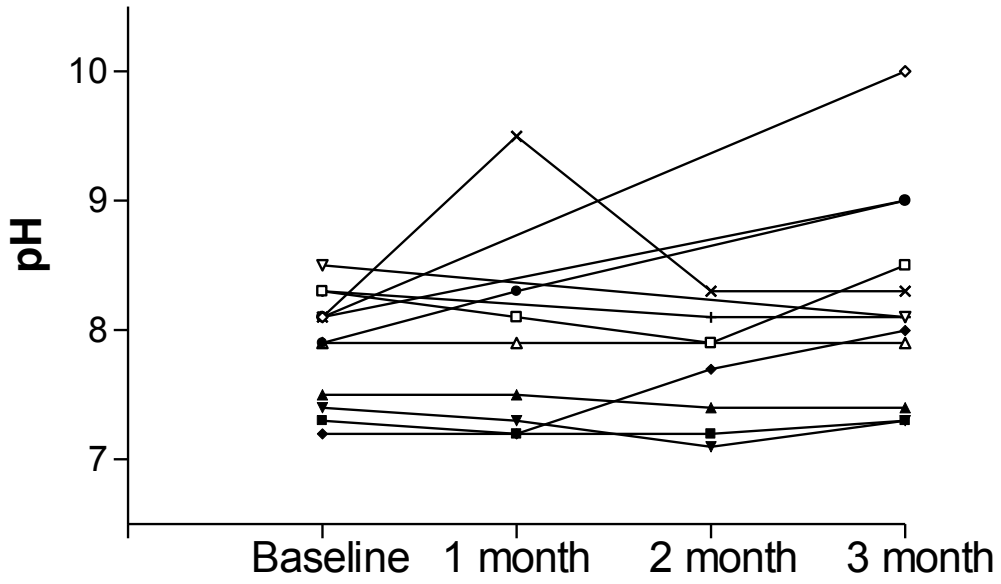

Figure 3.16: pH in study arm – monthly over 3 months

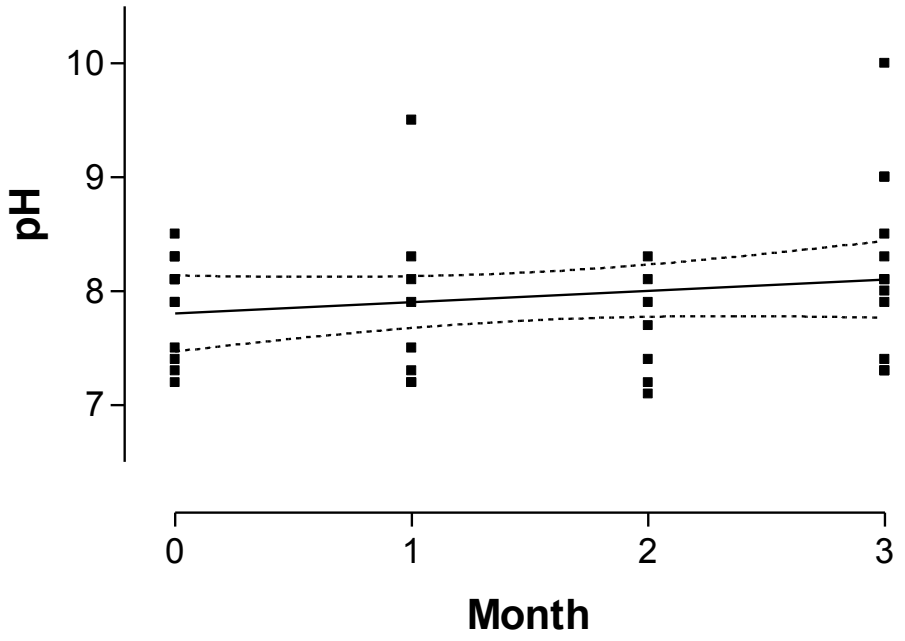

Figure 3.17: pH in study arm with linear regression curve (r^2=0.0035)

3.8.3 Motility

There appears to be quite a variation in the motility of individual subjects. This appears to both increase and decrease over 3 months. Also, it seems that in a number of individuals it changes on a month to month basis. The cause of this variation is difficult to ascertain as there was no change in the management of these individuals. There was no significant change seem in the linear regression curve in the motility over the 3 month period. This is consistent with findings of statistical analysis between individual subjects at baseline and 3 months as shown in table 3.7.

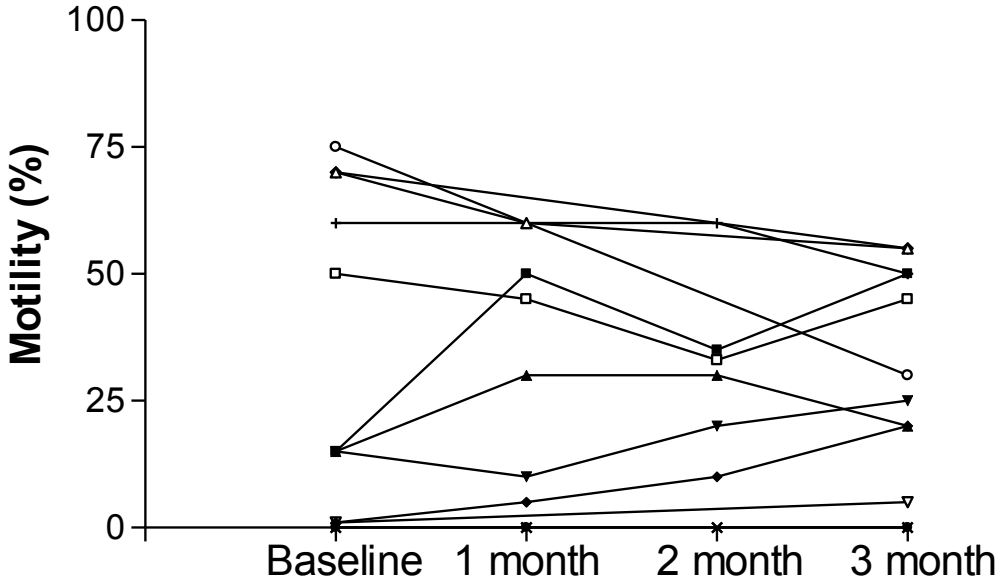

Figure 3.18: motility in study arm – monthly over 3 months

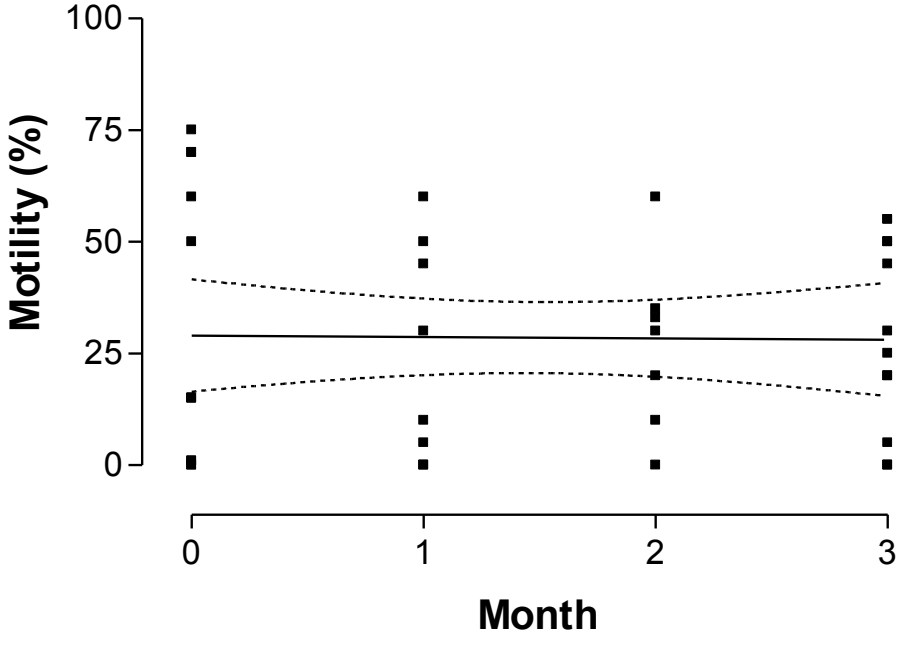

Figure 3.19: Motility in study arm with linear regression curve (r^2 = 0.0002)

3.8.4 Forward progression

There appears to be a wide variation in the forward progression of individual subjects on a monthly basis. However, there does not seem to be a pattern to individual changes with some increasing and others decreasing on a monthly basis as shown in Figure 3.20. The accompanying linear regression curve does not demonstrate any significant change (fig 3.21). This is in contradiction to the individual changes in subjects between baseline and at 3 months as shown in table 3.7. On reason can be that the monthly variation resulted in picking up the difference at set points rather than there was an overall increase in forward progression of the sperms. Alternatively, as this was measured in numbers (1-4), this led to the difference not being detected on the linear regression curve.

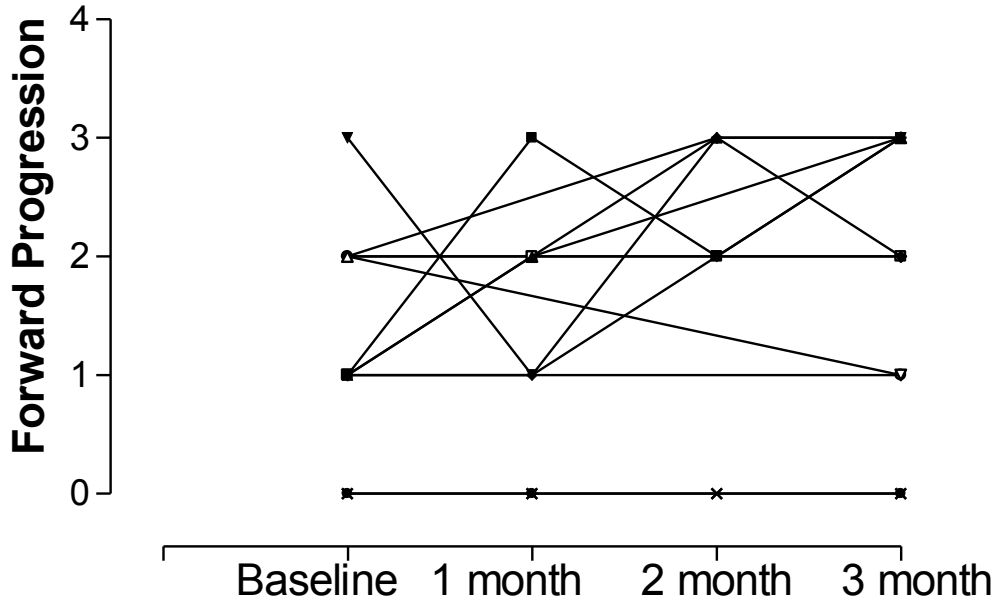

Figure 3.20: forward progression in study arm – monthly over 3 months

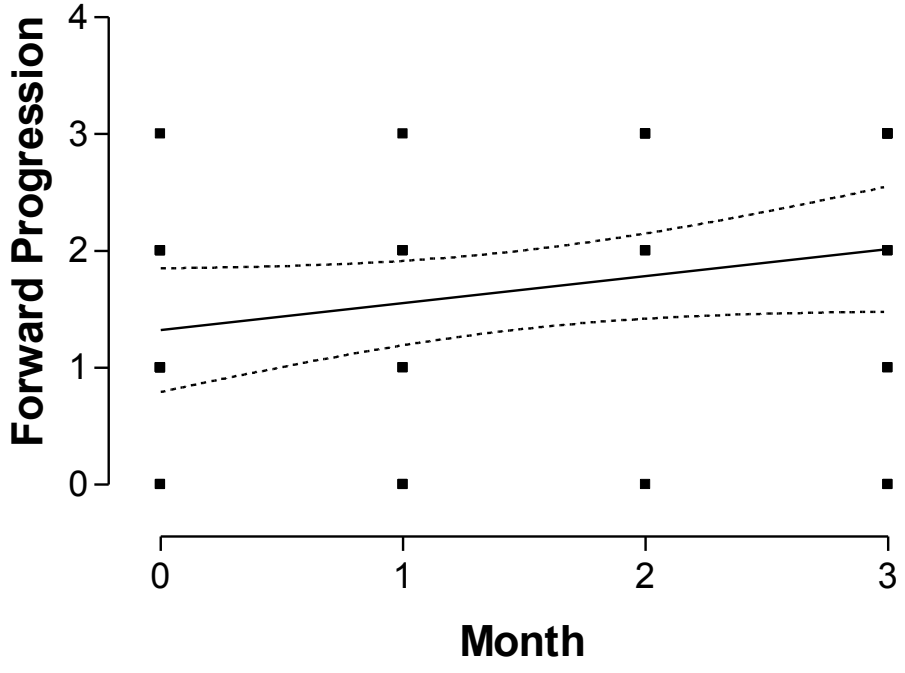

Figure 3.21: forward progression in study arm with linear regression curve ($r^2 = 0.072$)

3.8.5 Morphology

The morphology also showed considerable variation in individual subjects over the study period (Fig 3.22). It appears that mostly it increased but again this was not consistent in every subject. The accompanying linear regression curve does reveal a significant improvement in this parameter over 3 months (Fig 3.23). This is in agreement with the individual analysis of morphology in study and control arms between baseline and 3 months as shown in table 3.7.

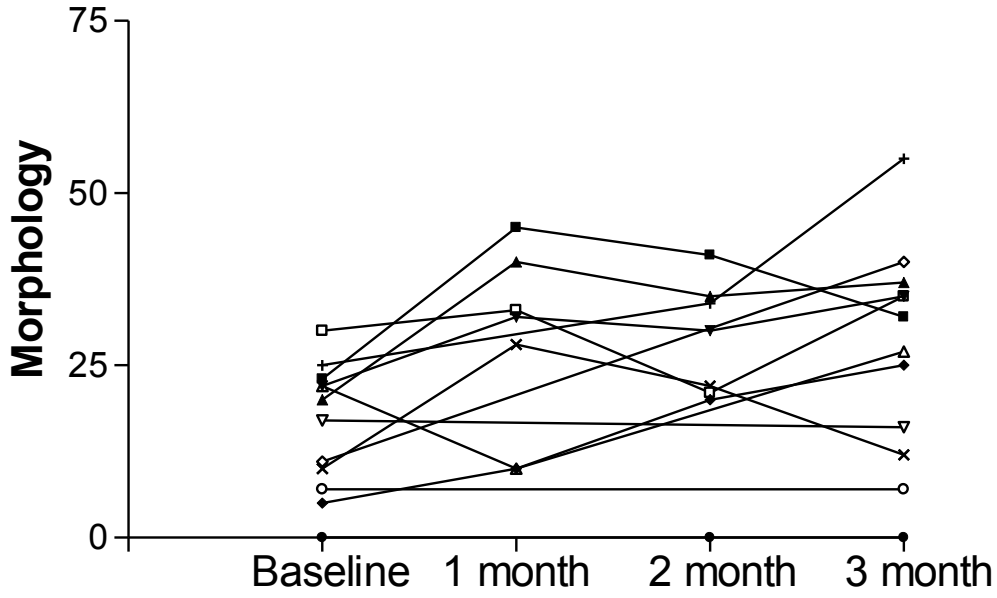

Figure 3.22: morphology in study arm – monthly over 3 months

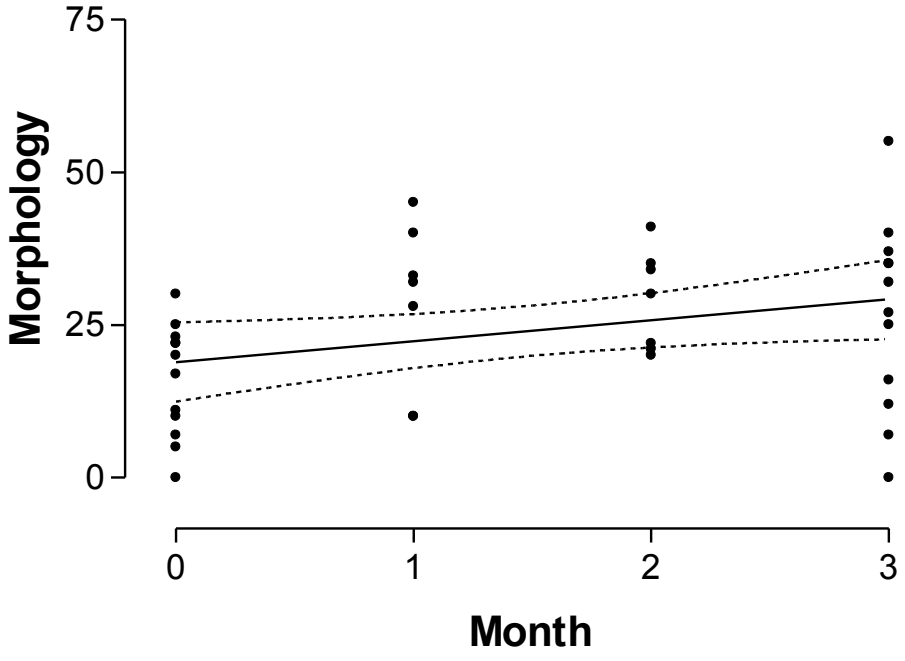

Figure 3.23: morphology in study arm with linear regression curve (r^2 = 0.106. slope significantly non-zero p=0.04)

3.8.6 Count

The count demonstrated a wide variation in individual subjects on a monthly basis with a decrease in most cases (Fig 3.24). The linear regression curve reflects this pattern by revealing no significant change over the 3 month period (Fig 3.25). This is consistent with the statistical analysis in individual cases at baseline and 3 months as shown in table 3.7.

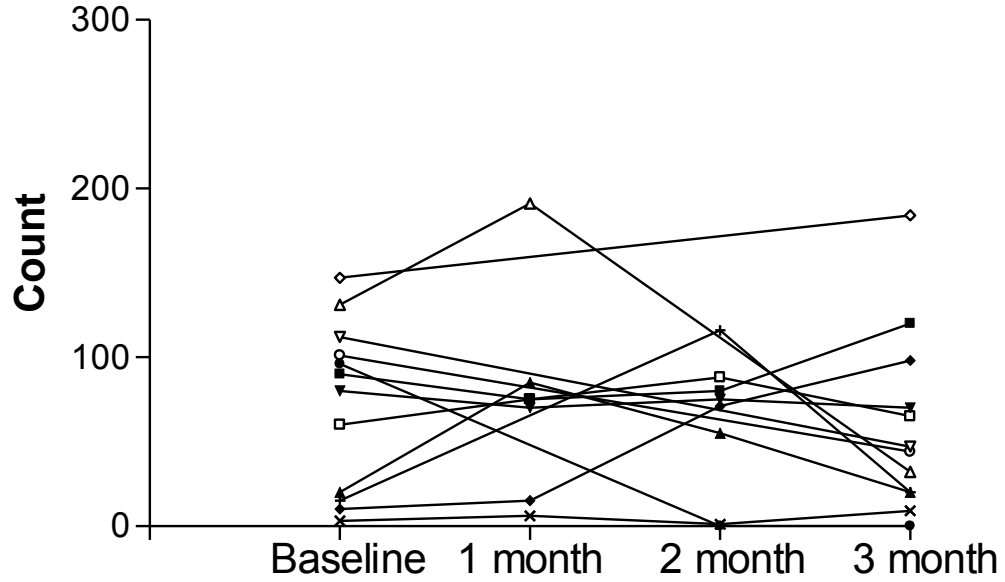

Figure 3.24: count in study arm – monthly over 3 months

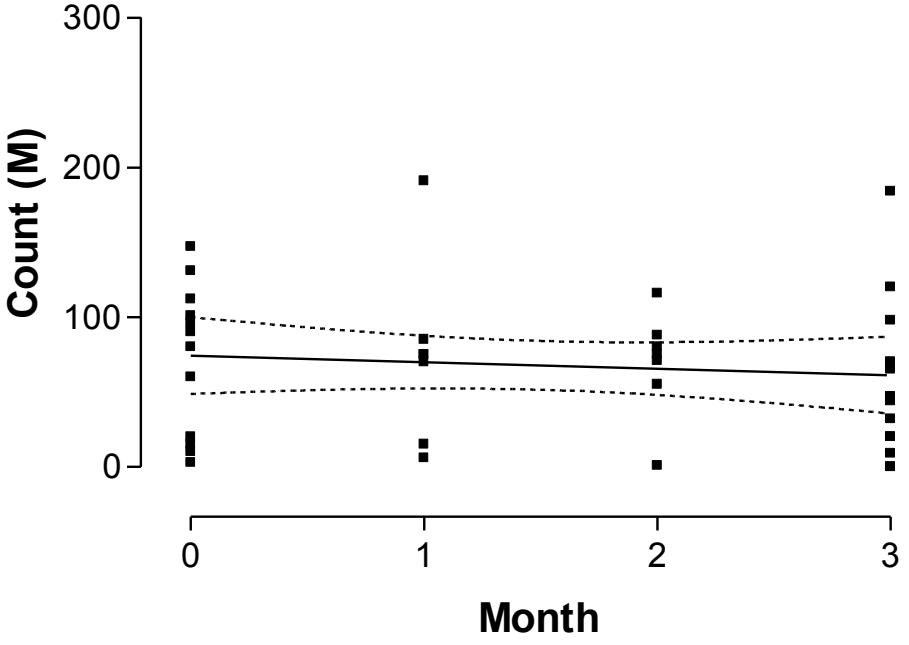

Figure 3.25: count in study arm with linear regression curve ($r^2 = 0.012$)

3.9 Statistical comparison of results between study and control arms

Two parameters, morphology and forward progression showed a statistically significant increase in the study arm but not in the control arm. There was a significant increase in the forward progression of the sperms. Additionally, the morphology of sperms improved significantly over the study period. The analysis was performed with a paired Student's t test, 2 tailed and a significance of <0.05. All the other variables, including motility, did not improve significantly. Surprisingly, the pH increased significantly in the control group. The results are summarised in the table 3.7 below.

Parameter	**Study Group**			**Control Group**		
	Baseline	3 month	P value	Baseline	3 month	P value
Volume (ml)	2.87 (± 1.15)	1.93 (± 0.96)	NS	2.14 (± 1.19)	1.75 (± 0.99)	NS
pH	7.88 (± 0.43)	8.24 (± 0.80)	NS	7.75 (± 0.5)	8.14 (± 0.62)	**0.0027 ****
Motility	31.0 (± 31.14)	29.58 (± 21.16)	NS	9.61 (± 9.23)	13.38 (± 16.8)	NS
Forward Progression	1.33 (± 0.89)	1.92 (± 1.17)	**0.05 ***	1.00 (± 0.71)	1.15 (± 0.99)	NS
Morphology	16.0 (± 9.22)	26.75 (± 15.62)	**0.006****	20.85 (± 10.21)	17.15 (± 7.44)	NS
Count	72.08 (± 49.74)	59.08 (± 53.21)	NS	95.69 (± 89.01)	68.77 (± 54.85)	NS
Liquefaction	1.08 (± 0.29)	1.42 (± 0.51)	NS	1.15 (± 0.38)	1.31 (± 0.48)	NS

Table 3.7: statistical analysis in study and control arms at baseline and at 3 months (Within group Student's paired t test, 2 tailed, significance $p<0.05$)

3.10 Statistical comparison of results within study and control arms

As already described in section 3.5 above no significant difference between the study and control group with regard to the patient's age, time since injury, level of injury, completeness or bladder management was detected. Further analysis were performed to check if the groups were also comparable at baseline and at 3 months in respect to semen characteristics. This is summarised in table 3.8 below.

There was no statistical difference within the groups at baseline and at 3 months for any parameter except motility. There was a significant difference between sperm motility of the subjects. This was evident both at baseline and at 3 months. It is difficult to explain the isolated difference in this particular parameter as the groups were comparable in all other parameters including demographics. One explanation can be that there is a wide variation in sperm characteristics and this became evident during a controlled study. However, as there was no significant improvement in motility within the groups it is felt that this particular problem did not affect the conclusions of the study.

Parameter	**Baseline**			**3 months**		
	Control	Study	P value	Control	Study	P value
Volume (ml)	2.14 (± 1.19)	2.87 (± 1.15)	NS	1.75 (± 0.99)	1.93 (± 0.96)	NS
pH	7.75 (± 0.5)	7.88 (± 0.43)	NS	8.14 (± 0.62)	8.24 (± 0.80)	NS
Motility	9.61 (± 9.23)	31.0 (± 31.14)	*** 0.0266**	13.38 (± 16.8)	29.58 (± 21.16)	*** 0.0442**
Forward Progression	1.00 (± 0.71)	1.33 (± 0.89)	NS	1.15 (± 0.99)	1.92 (± 1.17)	NS
Morphology	20.85 (± 10.21)	16.0 (± 9.22)	NS	17.15 (± 7.44)	26.75 (± 15.62)	NS
Count	95.69 (± 89.01)	72.08 (± 49.74)	NS	68.77 (± 54.85)	59.08 (± 53.21)	NS
Liquefaction	1.15 (± 0.38)	1.08 (± 0.29)	NS	1.31 (± 0.48)	1.42 (± 0.51)	NS

Table 3.8: Statistical analysis in the study and control groups at baseline and at 3 months. (Between groups (non-paired t-test significance > 0.05)

3.11 Dropout due to non-compliance with protocol

There was only 1 subject in the study group and 2 in the control group who did not comply with the protocol without any valid reason. All the other drop outs 5/9 could not complete the study either due to being unwell or unable to follow the protocol despite trying i.e failure of VE.

3.12 Adverse events

No major complication was noted during the study apart from 1 patient who developed severe autonomic dysreflexia during the procedure which was immediately abandoned. This patient did not ejaculate till he developed dysreflexia and was assigned to the failed group.

Three subjects (25%) in the study group developed minor penile abrasions secondary to repeated ejaculations but did not require any treatment. They continued to perform repeated VE as per protocol.

One subject reported mild symptoms of dysreflexia but did not require any treatment.

Two subjects in the control group developed mild symptoms of dysreflexia during VE. However, they did not require any active treatment and the symptoms subsided after ejaculating.

CHAPTER FOUR

Discussion

4- DISCUSSION

4.1 Summary of results

This study evaluated 79 subjects for success with VE. Forty two of the 79 (53%) subjects were successfully VE with the Ferticare® vibrator. Thirty four subjects agreed to participate in the project and were randomised into study and control arms. The 37 subjects who did not ejaculate in an antegrade fashion were labelled as non-responders. All the non-responders had a minimum of 2 attempts. Some of the non-responders were brought back after a few days to have a second attempt. In this study none of the subjects who were unsuccessful initially managed to produce an antegrade sample at the second visit.

The success of the procedure was not dependent on the age at injury, age of procedure or the time since injury. The average age at injury was 28 years and the average age at procedure was 42 years. The time since injury varied between 0.5 to 27 years in both groups. There were 39/79 (49%) subjects with cervical level injury. Twenty five (31%) had upper thoracic (T1-T6) and 15 (19%) had a lower thoracic (T7-T10) level injury. However, there was no significant difference between the responders and the non-responders according to the level of injury.

In the study the method of bladder management did not influence the success with VE. Thirty five of the 42 responders (83%) and 27/37

non-responders (73%) were emptying the bladder by either SIC or with a SPC. There was no significant difference between the two groups.

However, there was a significant difference between the responders and non-responders according to the completeness of the injury. In this study an incomplete injury afforded a significantly better chance of obtaining an antegrade ejaculate as compared to a complete injury.

A total of 34 subjects were entered into the study protocol. Eighteen were randomised to the study and 16 to the control arm. Twenty five of 34 subjects (74%) completed the entire study. Twelve of 18 (66%) subjects in the study arm completed the entire study whilst 13/16 (81%) in the control arm completed the study. At the end of the study there was no statistically significant difference in liquefaction, volume, pH, count and motility in the study arm. However, there was a statistically significant improvement in the morphology and forward progression of sperms in the study arm. On the other hand there was no difference in any of the parameters in the control arm at the end of the study period apart from pH which increased significantly.

Hence, this study has shown that repeated VE results in some improvement of quality of sperms following SCI in men. The characteristics that improve significantly are morphology and forward progression. However, the primary variable i.e. motility, thought to be one of the main factors in the quality of sperm, did not show a significant improvement. Additionally, a little more than half of SCI

patients with injury above T10 produced an antegrade ejaculate. The only significant factor determining success of VE in this group was the incompleteness of SCI.

4.2 Comparison of results with significant improvement

This study has shown a statistically significant improvement in two of the parameters evaluated. The morphology and forward progression of sperms improved over the study period of 3 months in the study arm but there was no significant change in these parameters in the control arm. These results confirm the findings of some previous investigators. Beretta in 1989(17) performed repeated VE in 15 patients weekly for 3 months. He reported a significant improvement in motility, morphology and sperm count over the study period. There was no comparable increase in volume. Similarly, Siosteen in 1990(15) performed VE on a weekly basis for more than 4 months in 16 SCI men. She concluded that the semen volume increased significantly as did the forward progression of sperms but there was no significant improvement in sperm morphology and motility. She also noticed a rise in semen fructose and acid phosphatase suggesting an improved function of seminal plasma. Brindley in 1983(106) also concluded that repeated ejaculations improve semen quality in SCI men. On the other hand Sonksen(115) performed the longest study to date with VE in SCI men. Nineteen SCI men underwent a weekly programme of VE for 1 year. There was no

improvement in volume, count, motility or morphology over the period of the study. Some investigators have tried repeated EEJ to evaluate if this technique improves the quality of sperms in SCI men. Mallidis in 2000(166) performed daily EEJ for 4 consecutive days in 9 SCI men. He reported improvement in sperm motility and viability by an average of 23%. However, Das in 2006(167) performed EEJ in 16 SCI men at 2-4 weekly intervals. He carried out 3 consecutive attempts. He did not find any significant improvement in sperm motility or morphology but noticed a significant decrease in semen volume. It is interesting to note that none of the studies mentioned above provide conclusive evidence of whether repeated ejaculations in SCI men improve the quality of sperms. Furthermore in studies demonstrating an improvement in some parameters, the improved parameters were not consistent across studies. This reveals the complexity of the problem in SCI men but does confirm the fact that poor quality semen in SCI men is a multifactorial problem. There are a significant number of studies in the literature evaluating the sexual dysfunction in SCI men(99;172-177). A number of studies have investigated the causes of ejaculatory dysfunction and abnormal semen characteristics(13;19;96) in SCI men. However, they have mostly concentrated on obtaining an ejaculate(14;17;58;74;85;120) and identifying the probable causes of poor semen quality like stasis of semen, role of urine infections and bladder management rather than suggesting remedial steps to improve the semen characteristics. There are only a handful of studies as described above which have

tried to improve the quality of sperms in SCI men but none were set up as a randomised control study. This is the first study that has evaluated the effects of repeated ejaculations in SCI men in a controlled setting. It has shown that there is an improvement in sperm morphology and forward progression but not motility. It has to be emphasised that the study was not powered to evaluate the statistical difference in forward progression or morphology. As in previous studies, it is somewhat difficult to explain why only morphology and forward progression improved and not motility, which is thought to be an important parameter in sperm characteristics. It can be argued that with stagnation and probable mixing of sperms with seminal plasma the quality of sperms deteriorates. Once the patient starts to perform repeated VE and the ejaculatory ducts are cleared then over a period of time the sperm characteristics might improve. It can be postulated that not only the individual sperm characteristics but the environment in which they are stored (seminal plasma) is equally if not more important. Indeed, this can be hypothesised from some acute and chronic studies. In Ohl et al (100) canine model serial examination of semen profile and spermatogenesis indicated a decline in spermatogenesis and sperm motility at 3 weeks but not before this time. It was argued that if this continues in chronic phase one would expect a decrease in total sperm numbers over time. However, we know that although, the motility of sperms in SCI deteriorates over time, there are a significant number of sperms in ejaculated specimens from chronic

SCI men (21). Hence, it is not clear whether spermatogenesis recovers or there is a disorder of sperm storage in seminal vesicle of SCI men (91). Furthermore, it has been demonstrated that seminal plasma of SCI men is toxic to sperms from able bodied men (150) whilst, the motility of sperms of SCI men improved when mixed with seminal plasma from able bodied men. Additionally, the sperm motility is higher when obtained from vas deferens than from ejaculated specimen in the same SCI men (178). The sperms need a normal shape and form (morphology) to have a normal motility and forward progression. The spermatogenesis takes place in the germinal epithelium of the testis and the process takes 64 days in humans (34). However, the spermatozoa at this stage are immotile and only have a limited capability to fertilize the ovum. The final maturation takes place during the epididymal transit which takes between 2 to 11 days (35). The sperms are stored in the caudal part of epididymis for varying lengths of time depending upon the sexual activity. It has been shown that the forward progression of sperms improves during the transit through epididymis (36). It can be postulated that with repeated VE the ejaculatory ducts can be cleared of senescent sperms and over time the quality might improve. It can be argued that had the study been carried on for longer, there could have been a different impact on the motility of sperms. However, it is somewhat different to postulate whether there would have been an improvement or the motility might have not changed or indeed could have decreased. It has to be admitted that

Sonksen in 1999(115) did carry on with VE for a year and did not find any significant improvement in sperm characteristics, but the study was not randomised. Also there were patients (number not specified in the paper) who had an injury for less than 6 months in his study. It has been suggested by some investigators that the quality of sperms deteriorates following SCI and does not stabilise until 6 months after injury (45). It is possible that including patients from this group might have skewed the results of the study.

The pH in the control arm increased significantly in this study. There was a trend in the study arm towards increased pH but this did not reach statistical significance. The reason for this phenomenon could not be ascertained. It is understood that if the pH is acidic with azoospermia then obstruction of the ejaculatory ducts is a strong possibility (WHO manual). However, the significance of basic pH as in our study is not known. It was not possible to compare this particular factor with other studies in the literature as pH was not measured in previous studies.

4.3 Comparison of results with no significant improvement

In this study there was no significant improvement in volume, motility and count with repeated ejaculations. However, Beretta in 1989(17) reported a significant improvement in motility and sperm count with repeated VE over 3 months. There was no significant increase in

volume. On the other hand Siosteen in 1990(15) demonstrated significant increase in semen volume but no improvement in sperm motility. Sonksen in 1999(115) did not find any improvement in volume, count or motility over repeated VE for 1 year. Mallidis in 2000(166) reported improvement in sperm motility on an average of 23%. Das in 2006(179) did not find any significant improvement in sperm motility but noticed a significant decrease in semen volume. In the current study the volume decreased considerably but did not reach statistical significance, unlike the Das study above where the volume decreased significantly. On the other hand Siosteen reported a significant increase in semen volume over a 3 month repeated VE programme. The decrease in volume in this study might be related to repeated ejaculations but, importantly, like other studies it did not evaluate if there was any retrograde ejaculation. Hence, it is a possibility that some of the ejaculate was in a retrograde manner, accounting for a decrease in volume but this needs to be confirmed in later studies.

The sperm count in this study decreased as demonstrated by Sonksen(115) although it did not reach significance in either study. The count increased significantly in the study by Beretta(17).

It is difficult to ascertain the exact cause of decrease in the count. However, it has been demonstrated previously that the ejaculations might cease after a period of repeated VE(15;96). Certainly, in this study 3 of 18 subjects in the study arm stopped VE after a few successful initial attempts. It is possible that this phenomenon might

in some way account for a decrease in semen volume leading to a decrease in sperm count.

4.4 Predictors for success with Vibro-ejaculations

The success rate for VE in this study was 53% (42/79). This is somewhat lower than the reported success in previous studies. Brindley(79) who popularised this technique reported a rate of about 60%. There is a large reported range of success in the literature varying between 19-91%. One of the reasons can be that over years several non-medical vibrators have been used with varying output(14;15;17;45;87;88). Sonksen suggested an amplitude of 2.5mm and a frequency of 100Hz as a predictor of success with VE(89). He reported a success of 96% but this included both antegrade and retrograde ejaculates. Ohl in 1996 reported a success of 65% using the same parameters(16).

A number of factors were evaluated to determine the predictability of success with VE. There was no statistical difference between the responders and non-responders when considering age at injury, age at procedure or the time since injury, method of bladder drainage or level of injury (assuming the level of injury is above thoracic level 10). The only significant factor in this study determining success was the incompleteness of injury. Sonksen in 1994(89) reported no predictors of success as above including the completeness of injury. The only factors in his study determining success were the optimised

parameters with an amplitude of 2.5 mm and a frequency of 100Hz. Unlike the current study the completeness of injury was not a definite prognostic factor for obtaining semen by VE according to Brindley and Szasz(14;58). Bird et al in 2001(180) reported, like the current study, that there is no difference between responders vs non-responders with regards to age or time since injury. However, they carefully investigated the BCR and HR and concluded that if both reflexes were present then the success rate for VE was 80%. On the other hand it was only 8% if both reflexes were absent. Similarly, Brindley(14) noted that the most important factor determining success was the presence of HR. Szasz in 1989(58) reported BCR as a predictor of success with VE. In the current study the reflexes could only be performed in 33/79 patients. The BCR was positive in 94% and HR in 76% of the responders. Although there was a trend for both the reflexes to be positive in the responders, regrettably no definite conclusion could be drawn from this study as the full data could not be obtained due to the fact that the 2 observers did not document the evaluation of both these reflexes independently in all subjects.

4.5 Role of seminal fluids in poor semen quality post SCI in men

It has been shown in previous studies and confirmed by the current one that no single factor seems to be responsible for the deterioration of sperm quality following a SCI. It appears that a

combination of various factors leads to a poor sperm quality. Lately, attention has turned to the evaluation of seminal plasma as a cause of deterioration in sperms following SCI. It is known that there is an abnormal prostate and seminal vesicle function in men with SCI(146-148). It has been demonstrated in another study that lower levels of fructose and albumin are present in the semen of SCI men pointing to an accessory gland factor in causing deterioration of sperms after SCI(56). More evidence that seminal plasma may play a crucial role in causing deterioration of sperm quality was demonstrated when it was shown that seminal plasma obtained with ejaculate from SCI men inhibits the motility of normal sperms from able bodied men. Conversely the sperms from SCI men improve their motility if they are mixed with the seminal plasma from able bodied individuals(150). It has also been shown that the sperms of men with SCI demonstrate significantly better sperm motility and viability when aspirated from the vas deferens as compared to sperms obtained with VE or EEJ(178). A large number of poor quality sperms in the seminal vesicle of SCI men have been documented(91). These, obviously comprise a major portion of the ejaculate with VE and EEJ. More recently, Maher et al(158) employed a nuclear magnetic resonance spectroscopy to analyze seminal fluid metabolite and demonstrated that uridine is likely to be an essential precursor to metabolites required for capacitation and is a potential marker for the prognosis of post-SCI functional fertility recovery. A new term "seminal oligouridinosis" was coined to describe this newly identified condition.

The above data provides evidence that seminal plasma is also a major contributing factor to the deterioration of sperms following a SCI.

4.6 Methodological weaknesses

This study was conducted in a hospital out-patients setting with SCI men. These patients had complex needs and were looked after by a multidisciplinary team. There were a number of limitations observed during the study which had to be taken into account to successfully complete the project.

4.6.1 Uncontrollable variables

SCI patients are a very heterogeneous population and their care is quite complex with input from doctors and specialist nurses. There are a number of factors that were difficult to control during the experiment as explained below.

4.6.1.1 Enrolment of subjects

All eligible subjects were informed of the study at the institution. However, the subjects decided whether they wanted to attend the screening. Overall, younger subjects with partners were more

interested in evaluating their fertility status. It is difficult to ascertain if this in anyway affected the subject sample and affected the outcome.

4.6.1.2 Effect of medications on sperm quality

The SCI men are generally on a variety of medications.There are a number of medications which can affect sperm quality. It was accepted that it would not be possible to stop the medications for the duration of the study. Hence, this limitation was accepted and it was anticipated that randomization would minimise the risk of the results being affected by this potential restriction. A record of all the medications was not kept for the subjects. Furthermore, a comparison between the study and the control arm was not made in this respect. However, this factor has not been deliberated upon in any of the previous studies evaluating semen quality in SCI men.

4.6.1.3 Impact of urine infections on the project

UTIs are not an uncommon occurrence in SCI men. This could have become a problem for the study if active subjects had developed epididymo-orchitis. Fortunately, no subject in our study developed epididymo-orchitis. Subjects who were suffering from a urinary infection were not enrolled in the study. However, it was decided that if subjects developed a UTI during the study, they would be prescribed the appropriate antibiotics but not withdrawn as long as

they could VE weekly. A few who developed UTI did not develop any systemic signs precluding them from performing weekly VE to fulfil the protocol requirements.

4.6.1.4 Weekly vibro-ejaculations at home

It was accepted as part of the protocol that the subjects randomised to the study arm would be relied upon to perform weekly VE at home. It was expected that the subjects would not only fulfil this requirement but also would not try to ejaculate more than once a week. Although, the subjects were instructed to try to VE on a set day, it had to be accepted that this would not always be possible due to multiple reasons including being unwell, hospital appointments or simply forgetting the procedure. However, when they came for their monthly appointments it was confirmed with every subject that they had ejaculated 4 times since their last visit and that it was generally on a weekly basis.

4.6.1.5 Monthly visit for study arm subjects

All subjects in the study arm were required to attend for monthly evaluations of their semen and to ensure that they were following the protocol. However, a number of subjects lived at some distance from the hospital. Hence, it was not always practical for them to come to the hospital. A total of 6 out of 12 subjects in the study arm did not come for the monthly visit. However, all attended for the final visit at 3 months and confirmed that they had been performing VE on a weekly basis for the duration of the study.

4.6.1.6 Drop out of subjects

A total of 9/34 (26%) subjects dropped out from the study. This rate may be considered somewhat high but as mentioned above the SCI men have complex needs and require regular input from health care personnel. Only 3 of these subjects did not comply with the protocol without any valid reason. All others were either unwell or stopped ejaculating.

4.6.2 Preparation and analysis of semen samples

The semen from SCI men can be very thick and lumpy and may take long time to liquefy. However, no significant problems with

liquefaction were encountered. An additive medium was not used to facilitate this process as this might have affected the sample. Similarly, sperm count in SCI men is much below normal. It is recommended in WHO guidelines that if the sperm concentration is very low then the sample can be centrifuged and a known volume of seminal plasma removed. Sperm count is then performed but the final concentration is corrected to adjust for the removal of seminal plasma. Since the facility centrifugation was not available, it was decided to perform sperm count on the sample available. As this was consistent for all the cases in both arms, it was felt that this would not affect the outcome of results.

Sperm motility and morphology could also be performed in the centrifuged sample. In this study, however, the analysis was performed on the original sample after preparation according to the WHO guidelines but without centrifugation in any case.

4.6.3 Blinding of the observers

It was not possible to blind the observers examining the semen samples as this was performed by the investigators. It was not possible to devise a way to collect the semen sample anonymously as the subjects were booked to come for VE under the supervision of the investigators. The semen sample was analysed in the laboratory by the investigators soon afterwards. As the WHO guidelines were followed in preparing 2 slides by two separate observers. They

evaluated the semen samples independently and the final result was reached as per the WHO recommendations. It was felt that adhering to the guidelines would minimise the bias in the final results.

4.6.4 Success with vibro-ejaculation

The success rate for VE in the study was 53%. This is lower than 65% and above in other studies. One of the reasons could be that this study did not look for retrograde samples. This is possible and indeed, some of the subjects labelled as non-responders did report a milky white discharge at the next urine sample. However, it was decided for the purpose of the study that only the subjects who demonstrated clear evidence of antegrade ejaculation would be recruited for 2 main reasons: there would be certainty that the subject had ejaculated and more importantly, there would not be a need to deal with a urine contaminated semen sample whilst performing the analysis. This could have caused problems with standardising the results. Hence, it is accepted that the reported success rate with VE might be lower than other studies, but it is emphasised that this was to ensure production of an antegrade sample of semen.

4.7 Future implications

This is the first study in the literature evaluating the effects of repeated ejaculations following SCI in men in a randomised controlled fashion. A semen analysis was performed for 3 months like most of the previous studies apart from Sonksen in 1999(115) who performed the study for 1 year.

4.7.1 The importance of finding statistical changes

In this study, morphology and forward progression improved significantly over the study period. Sperms have to be normal morphologically to demonstrate good motility. It is known that sperm motility and viability is much higher if aspirated from the vas deferens of SCI men(178). It has also been shown that sperms from able bodied men are inhibited when mixed with seminal plasma of SCI men (150). On the other hand, the same study demonstrated that seminal plasma from able bodied men improve the motility of sperms from SCI men. Hence, it can be postulated that stagnation of sperms in the accessory glands during the maturation phase in the epididymis and storage in the seminal vesicles can lead to deterioration of quality of sperms. It can be hypothesised that repeated ejaculations might improve the quality of sperms by decreasing the exposure of sperms to this abnormal environment.

4.7.2 Significance of unchanged parameters

It is well known that the most important characteristic of sperms is motility with forward progression if successful fertilization of an ovum is to take place. It remains to be seen if motility can be improved with repeated VE over a longer period in randomised controlled studies, although the literature is unclear on this issue. Hence, it is felt that a large multicentre trial should be undertaken to answer this question.

4.7.3 Role of seminal plasma in poor sperm quality in SCI men

There is growing evidence that seminal plasma plays a major role in the deterioration of sperms following SCI in men. However, only a few studies have tried to investigate this issue. This institution, in collaboration with others, has recently pointed out specific biochemical abnormalities especially uridine(158) in the seminal fluid of SCI men. A comparison has also been made between the biochemical substances in the seminal fluid of infertile men with SCI and age-matched controls(181).

It is felt that newer techniques of imaging like nuclear resonance imaging should be utilised to evaluate the seminal plasma in SCI men at various stages following injury to gain insight into the causes of poor sperm quality

4.7.4 The role of repeated ejaculations in improving sperm quality within home settings – yet undefined

It is recommended that the SCI men should start with VE and home vaginal insemination programme. The first step in this direction is to determine that they have sperms in the ejaculate. It has been recommended that there should be at least 4 million motile sperms in the ejaculate (160). However, recently, Sonksen et al (182) have reported that men with a motile count of less than 1 million can also achieve home pregnancies with home insemination programme. It can be suggested that these SCI men should undergo repeated VE as the initial step in the management of poor quality sperms over a period of time that is still to be determined. The home insemination programme should be instituted in all cases where a reasonable quality ejaculate can be obtained. The success of this method can help with the psychological well being of SCI men and help them to achieve some sense of normality. This can be an important aspect of the rehabilitation process.

4.7.5 Cost implications

A doctor's prime responsibility is always to provide the best possible treatment for the patients. In the current age of rising costs of medical care this takes on an additional importance. It is felt that if sperm quality can be improved with repeated VE then the cost of

achieving pregnancy in a home environment is much less as compared to the assisted techniques to which one has to resort if this method fails. Although, it has to be emphasised that the best quality health care is the one that is tailored to individual patients' needs and desires. This has particular relevance for fertility issues as the age of the partner is of critical importance in deciding the best method of achieving pregnancy in childless couples.

4.7.6 Need for a large multicentre randomised control trial

It is proposed that a large multicentre randomised control trial should be undertaken. It is suggested that the study should evaluate the effects of repeated ejaculations on the semen in SCI men. Furthermore, it is recommended that the seminal plasma should be evaluated by nuclear resonance imaging to see if the constituents change with repeated ejaculations. It is felt that this experiment will provide critical insight into understanding the basis of poor semen quality in SCI men.

4.8 Conclusions

The SCI men have unique semen characteristics with a normal count but very low motility and abnormal morphology. The situation is compounded by denervation of accessory glands probably contributing to further deterioration of sperm quality. The sperms can be obtained by either VE or EEJ in more than 95% of cases. These techniques should be employed before resorting to surgery.

This is the first randomised control trial evaluating the effects of repeated vibro-ejaculation in SCI men. It has demonstrated that repeated ejaculations on a weekly basis for 3 months in SCI men significantly improves the morphology and forward progression of sperms. However, there was no significant improvement in the primary variable evaluated; sperm motility. The volume of semen and count of sperms also did not demonstrate any significant changes. The success rate for VE with optimised parameters was 53% but it has to be stressed that this was with a production of an antegrade sample. The only factor predicting a successful outcome with VE was an incomplete injury. The age of the patient, level of injury and the method of bladder management did not predict a successful outcome for VE. There were no significant adverse events encountered during the duration of the study.

Hence, it is recommended that VE should be the initial method of sperm retrieval in SCI men. If the sperms are of poor quality then the patient could undergo a period of repeated VE on a weekly basis, the duration of this is yet undefined and the benefit not certain at the time, but is cost effective and is close to the natural process of procreation. If this fails then other assisted fertility techniques can be applied. Thankfully, the pregnancy rate is similar in SCI men as that from non SCI men with male factor infertility.

4.9 Future Research

It is strongly recommended that further research needs to be undertaken in earnest in large multicentre randomised trials to establish the exact cause of deterioration of sperms in SCI men. Importantly, the role of seminal plasma and accessory glands need to be critically evaluated in this respect.

Appendices

APPENDIX I

Reference Range for normal semen analysis

Semen parameter	Value
Volume (ml)	2.0 or more
PH	7.2 or more
Count (million/ml)	20 or more
Motility (%)	50% or more
Morphology (%) normal	50% or more

APPENDIX II

Inclusion Criteria

• Patient should be willing to participate in the study.

• All participants in the study arm shall be willing to perform vibro-ejaculation at weekly intervals for the duration of the study.

• They will agree to come to hospital on a monthly basis for semen analysis.

• The spinal cord injury should be of more than six months duration.

• They should be able to ejaculate with vibro-ejaculation only.

• The level of injury is above T10.

Exclusion Criteria

• Subjects requiring methods other than vibro-ejaculation i.e electro-ejaculation for ejaculation.

• Subjects getting repeated “Autonomic Dysreflexia.”

• Subjects getting Recurrent Urinary Tract Infections.

• Subjects who have had ejaculations since the injury.

• The level of injury is below T10.

• The duration of injury is less than six months.

APPENDIX III

PATIENT INFORMATION SHEET
FOR
PARTICIPATION IN A CLINICAL STUDY TO EVALUATE THE EFFECTS OF REPEATED EJACULATIONS ON THE QUALITY OF SPERMS IN PATIENTS WITH SPINALCORD INJURIES

Many thanks for taking time to read this information sheet regarding participation in this clinical trial.

As you know fertility is generally poor in patients with spinal cord injuries. Only 5-10% of the patients can ejaculate after spinal injury regardless of the level. Even after assisted ejaculation the quality of the sperms is consistently poor. This is thought to be the most important factor for low fertility rate in spinally injured patients. Some investigators have suggested that “repeated ejaculations can improve the quality of sperms in patients with spinal cord injuries.”

We are planning to test this theory in a randomised controlled trial.

We are inviting people who have been injured for more than six months and the level of injury is above T10 to participate in this study. They should not have ejaculated after the injury.

As you fulfil these basic criteria we hope that you will agree to take part in this study.

If you are willing to participate in this study, we will have the following expectations of you:
1- Randomly assigned to either the treatment or the control group.
2- If in the study group vibro-ejaculate at weekly intervals for three months.
3- If in the control group then no ejaculations till the end of study.
4- Consent to semen analysis regardless of the group.
5- You will be required to come to the hospital for semen analysis at monthly intervals if in the study group, but at the start and the end of the trial if in the control group.
6- consent for semen to be analysed to ascertain associated causes of infertility in spinal cord injuries.

You will be paid the travelling expenses when coming for the semen analysis and visits associated with this project.

The potential side effects can be “Autonomic Dysreflexia” or Bruising of the penis with the Vibrator alongwith pain or discomfort. We plan to give you Nifedipine 20-30 minutes before the procedure to

minimise the risk of going autonomic. Moreover we perform vibroejaculate with a condom on reduce the chances of bruising.

You will appreciate that low fertility rate is a very important issue in spinal cord injured patients. If it can be proven that with this simple method we can improve the quality of sperms then it can have major impact in increasing the fertility rate.

We hope that you will agree to participate in this project. However we would like to make it clear that if you decide not to participate or withdraw at any time after enrolling in the study without assigning any reason, your normal care and management will not be affected. Moreover this study may not benefit you directly.

If you have any queries or want to discuss any part of the project please do not hesitate to contact me on telephone 020 954 2300 ext. 5608 or 5606. You can also contact Sister Liz Banbury, our specialist Nurse for sexual health via the switchboard.

Once you decide to enter the study then we will provide you with a direct telephone number for use in emergencies related to this project.

I hope to hear from you soon.

Yours sincerely

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APPENDIX IV

CONSENT FORM

Agreement to participate in a clinical investigation

CLINICAL STUDY TO DEMONSTRATE IF REPEATED EJACULATIONS IMPROVE THE QUALITY OF SPERMS IN PATIENTS WITH SPINAL CORD INJURIES.

Investigators: Professor MD Craggs, Director of Spinal Research Centre
Mr PJR Shah, Clinical Supervisor & Consultant
Dr F Middleton, Consultant in Rehabilitation Medicine
Mr R Hamid, Clinical Research Fellow in Neuro-urology
Sr Nurse H Bywater, Clinical Nurse Specialist

1. I have read the information sheet concerning this study and I understand what will be required of me if I take part in this study.

2. My concerns regarding this study have been answered by.....

3. I understand that at any time I may withdraw from this study without giving a reason and without affecting my normal care and management.

4. I understand that the information from this study may be published in scientific journals, but that I will not be identified.

5. I agree to take part in this study.

Patient's signature .....

Name in BLOCK LETTERS .....

Date .....

Doctor's signature .....

Name in BLOCK LETTERS .....

Date .....

APPENDIX V

Papanicolaou staining procedure modified for spermatozoa (adapted from WHO manual)

The Papanicolaou stain distinguishes clearly between basophille and acidophilic cell components and allows a detailed examination of the nuclear chromatin pattern. Although this method has been used for routine diagnostic cytology, the standard Papanicolaou method for vaginal cytology gives poor results when applied to spermatozoa. The present modified staining technique has proved useful in the analysis of sperm morphology.

Preparation of specimen
The smear should be air-dried and then fixed in equal parts of 95% ethanol and ether for 5-1 5 minutes.

Staining procedure
Fixed smears should be stained by the following procedure:

ethanol 80%	10 dips
ethanol 70%	10 dips
ethanol 50%	10 dips
distilled water (deionized, demineralized)	10 dips
Harris's or Mayer's haematoxylin	3 minutes exactly
running water	3-5 minutes
acid ethanol	2 dips
running water	3-5 minutes
distilled water	1 dip
ethanol 50%	10 dips
ethanol 70%	10 dips
ethanol 80%	10 dips
ethanol 90%	10 dips
Orange G6'	2 minutes
ethanol 95%	10 dips
ethanol 95%	10 dips
ethanol 95%	5 dips
ethanol 95%	5 dips
ethanol 95%	5 dips
ethanol 99.5%	2 minutes

APPENDIX VI

DATA COLLECTION PROFORMA

APPENDIX VII

Safety requirements the andrology laboratory (Adapted from WHO manual)

APPENDIX VIII

Graph for accurate assessment of sperm motility (WHO manual)

APPENDIX IX

Graph for accurate assessment of sperm count (WHO manual)

APPENDIX X

Picture from WHO manual demonstrating the abnormal morphological forms of a sperm

CHAPTER FIVE

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