CASE REPORT

Triplet pregnancy achieved through intracytoplasmic sperm injection with spermatozoa obtained by prostatic massage of a paraplegic patient

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Spinal cord-injured men with ejaculation disorders can have children thanks to assisted reproduction techniques. Spermatozoa from these patients are usually obtained through vibratory stimulation, electroejaculation or by puncturing the seminal duct or the testicle. We present the first published case, as far as we are aware, of spermatozoa obtained through prostatic massage of a paraplegic patient. Penile vibratory stimulation was unsuccessful in this patient. In-vitro fertilization (IVF) with intracytoplasmic sperm injection (ICSI) with spermatozoa obtained through electroejaculation was performed at another centre but pregnancy was not achieved. Through prostatic massage, we obtained a total semen volume of 6 ml containing a total count of 12.32 × 10⁶ spermatozoa (6.24 × 10⁶ with tails), 8% of which had motility (graded + and + + ); and 16% of which had normal morphology. The spermatozoa obtained were then used to perform IVF with ICSI and a triplet pregnancy was achieved. Prostatic massage appears to be an easy, non-traumatic and risk-free method to obtain spermatozoa from paraplegic patients.

Key words: ICSI/paraplegic/pregnancy/prostatic massage/spinal cord-injured

Introduction

Spinal cord-injured (SCI) men frequently have the further complication of ejaculation disorders because only between 3 and 15% of them maintain the ability to ejaculate (Chung et al., 1995), thus making the remainder infertile. They are often young men who, with improvements in rehabilitation and quality of life, want to have children. It is possible to obtain spermatozoa from these patients, through electrovibration (Brindley, 1984), electroejaculation (Thomas et al., 1975), intrathecal injection of neostigmine (Guttmann and Walsh, 1971), subcutaneous injection of physostigmine (Rawicki and Hill, 1991), aspiration of spermatozoa from the vas deferens (Bustillo and Rajfer, 1986) or by testicular sperm extraction (TESE). All of these techniques, apart from electrovibration, carry a certain degree of risk because they are aggressive and/or invasive. We present the case report of spermatozoa obtained through the non-traumatic method of prostatic massage in an SCI patient.

Case report

A 27-year-old SCI patient who had been married for 3 years visited our centre because of primary infertility. He was paraplegic [complete transverse medullar syndrome below the 12th thoracic (T) neurological segment] as a result of a fracture of T11–T12 caused by a traffic accident 12 years earlier. He was treated in the acute phase in a hospital in Denmark, where arthrodesis was performed with 9th thoracic–2nd lumbar 2 osteosynthesis without complications.

Neurological exploration after the spinal cord lesion that caused the paraplegia had been stable and was as follows: hypoesthesia below T12 and anaesthesia below L3, non-existent tendon reflex in lower extremities, and equivocal plantar reflex. Urologically, he had a neuropathic bladder, and the urodynamic study showed a hypoactive–hypo-sensitive detrusor with negative reflexes, micturition by pressure with good flow and no post-urinary residue.

His libido was intact, but he did not have intercourse because he could not sustain an erection. Nor was penile vibratory stimulation successful. In order to obtain an erection he had used intracavernous injection of papaverine (the last time had been 2 years earlier) with unsatisfactory result. Sometimes, after micturition or when getting onto the examination couch from his wheelchair, a few drops of sticky, yellowish secretion came out. The physical examination was normal from the andrological perspective: testes of 15–20 ml (measured with a Prader orchidometer); normal epididymis, vasa deferentia, penis and prostate. There was no venous return in the left spermatic cord. After urination, a drop of secretion was obtained which revealed 141 immotile and dead spermatozoa (stained with eosin–nigrosine) on microscopic examination (×400) in 13 fields.

A fertility examination of the patient’s 30-year-old wife revealed no relevant abnormalities.

The patient had undergone electroejaculation on five occasions, under general anaesthesia. He provided a report on semen quality obtained on two previous electroejaculations: (i) a volume of 1.5 ml was recovered, sperm count of 51.2 × 10⁶/ml; 2.6% motile and 10% normal morphology; and (ii) volume 2 ml, sperm count of 49.9 × 10⁶/ml; 1.3% motile but sperm morphology was not available.
In-vitro fertilization (IVF) with intra-cytoplasmic sperm injection (ICSI) was also performed at another centre: eight oocytes were obtained, five in the metaphase II stage (MII); five embryos cleaved, three of which were transferred. Pregnancy was not achieved.

**Prostatic massage and the related IVF–embryo transfer cycle**

**Ovulation**

The patient’s partner was stimulated using gonadotrophin-releasing hormone analogues (GnRHa; Procrin; Abbot, Madrid, Spain) and recombinant follicle stimulating hormone (rFSH; Gonal-F®, Serono, Barcelona, Spain). Ovarian response was monitored by means of a series of intravaginal echographic examinations and by measuring the plasma concentrations of 17-β-oestradiol. Ultrasound-guided transvaginal follicular puncture was performed under sedation with propofol. From the 27 aspirated follicles, 19 oocytes were obtained – 16 MII, two MI and one abnormal.

**Collection of spermatozoa**

The patient was placed in the prone position on the examination couch, resting only on his arms and feet. The knee–chest position was not possible, as the patient’s knee joints were ankylosed. Two Falcon (ref. 2001), tubes were prepared each with 2.5 ml of HEPES buffered HTF (human tubal fluid) supplemented with 0.3% human serum albumin (HSA). The prostatic–vesicular and ampullae deferentiae massage was performed from lateral to mid-line. The hypotonic anal sphincter facilitated the massage and no discomfort was caused to the patient. An abundant secretion was easily collected from the glans. The total volume secreted was 6 ml containing a total number of 12.32 x 10^6 spermatozoa (6.24 x 10^6 with tails) and 8% with motility (8% graded + and ++; WHO, 1992); and 16% with normal morphology (Papanicolaou stain; WHO, 1992).

**Sperm preparation**

Spermatozoa were prepared for ICSI using PureSperm (Scandinavian IVF Science®, Gothenburg, Sweden) 70% centrifuged at 270 g for 20 min and 0.162500 x 10^6 spermatozoa were obtained, 20% of which were motile (11% graded + + and +++; 9% graded +). ICSI was performed using a Nikon® microscope, micromanipulators TM5 and ZM3 (Narishige Co. Ltd, Tokyo, Japan), home-made holding micropipettes and injecting micropipettes (Cook Australia, Queensland, Australia). The spermatozoa were obtained from a drop of 5% polyvinyl pyrrolidone in HTF.

**Microinjection**

Eighteen oocytes were microinjected at the 3 o’clock position with the polar body placed at 12 o’clock. Eighteen hours after microinjection there were 12 zygotes with two pronuclei (PN), one with 1 PN, three with 0 PN and two oocytes had degenerated. Embryos were individually cultured in vitro in HTF + 0.3% HSA drops under mineral oil and standard conditions until day 3.

**Embryo transfer**

Immediately before embryo transfer, assisted hatching was performed with Tyrode’s acid solution, as is usual in all the embryo transfers in our laboratory. Because most of the oocytes had excessive granularity concentrated in the central part of the cytoplasm, we decided to transfer four embryos. Three of them derived from oocytes with excessive granularity. The four embryos (7–9 cells) were transferred after informing the couple about the risks of multiple pregnancy and receiving their consent. The woman was subjected to immunodepressive treatment with methylprednisolone (16 mg per day/4 days) starting from the day before embryo transfer. Thirteen days after embryo transfer plasma concentrations of β-human chorionic gonadotrophin compatible with pregnancy were detected. The echogram showed three fetal sacs with positive heart beat. At the 32nd week of pregnancy, three healthy babies, two males and a female, were born by Caesarean section. They weighed 1800, 1600 and 1200 g respectively.

**Discussion**

Patients with ejaculatory disorders due to spinal-cord lesions currently have a good chance of having children of their own, by means of artificial insemination, IVF or ICSI. To choose which is the best method to obtain spermatozoa from these patients, the following points must be taken into consideration.

**Efficacy in obtaining spermatozoa**

Vibratory stimulation is effective in 82.8% of patients with high neuronal lesions, above T10, and intact lower spinal reflex (Dahlberg et al., 1995). The efficacy of electroejaculation is 83% (Dahlberg et al., 1995), and it is indicated when vibratory stimulation has failed or the neuronal lesion is low on the spinal cord. In SCI men, the efficacy of sperm extraction from the vasa deferentia, epididymis or testicles is expected to be 100%. In the case presented here, vibratory stimulation was not effective, whereas prostatic massage was as effective as electroejaculation, thus making testicular sperm extraction (TESE) unnecessary. We were previously unaware of the efficacy of prostatic massage in obtaining spermatozoa from these patients.

**Simplicity, safety and cost**

Vibratory stimulation can be performed at home (Beretta et al., 1989) but may cause hypertension and autonomic dysreflexia (Steinberger et al., 1990). If retrograde ejaculation occurs (65% of patients; Ohl et al., 1996), collection of spermatozoa from urinary alkalinization is required. Both electroejaculation and collection of spermatozoa via the seminal ducts or from the testes seem to be highly efficient protocols but, with or without general anaesthesia, are expensive and uncomfortable techniques. We consider prostatic massage as an ideal technique because of its simplicity and cheapness.

**Quality of the semen obtained**

Depending on the quality of the semen obtained, it is possible to perform artificial insemination or IVF-ICSI. Due to the poor quality of their semen sample, most SCI patients have to undergo ICSI cycles (Hultling et al., 1997; Quinn et al., 1998), although repeating electroejaculation appears to improve semen quality (Ohl et al., 1996) but leads to increasing patient
annoyance. Electroejaculation often provides many more spermatozoa than surgical sperm retrieval, thus permitting semen cryostorage and multiple ICSI attempts. With the prostatic massage we were able to obtain as many spermatozoa as with electroejaculation and also to freeze them.

In conclusion, we consider prostatic massage to be useful for obtaining spermatozoa from SCI patients and even from patients with ejaculatory disorders of other aetiologies, although the efficacy of this technique can only be validated through experience with a large number of patients. The simplicity and safety of prostatic massage and the efficacy shown in this case encourages us to explore this option.

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References

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