Pregnancy by Gamete Intrafallopian Transfer (GIFT) with Sperm Aspirated from the Vaso-epididymal Junction of Spinal Injured Man: Case Report

D. Macourt, FRCSE, FRCOG, FRACOG,¹ S. Engel, FACRM,² R. F. Jones, FRCSE, FRCS, FRACS, DPRM, FRACP, FACRM,³ M. Zaki, MRCOG, MRACOG⁴

¹Senior Lecturer, University of Sydney, Director, St George Infertility Clinic, Sydney, ²Associate Director, Spinal Injury Unit and Department of Rehabilitation Medicine, Prince Henry Hospital, Sydney, ³Director, Spinal Injury Unit, Prince Henry Hospital, Associate Professor, Rehabilitation Medicine, University of New South Wales Teaching Hospitals, Sydney, ⁴Co-ordinator, St George Infertility Clinic, Sydney, Australia.

Summary

A case is reported of pregnancy resulting from oocytes retrieved by transvaginal ultrasound with sperm obtained by aspiration of the vasa deferentia close to the epididymis of a quadriplegic man and replaced by the gamete intra fallopian transfer technique (GIFT) to the wife.

Key words: Spinal cord injury; Infertility; Pregnancy; In vitro fertilization (IVF); Gamete intra fallopian transfer (GIFT); Vasa deferentia aspiration.

Spinal cord injury frequently results in male infertility. For more than 20 years the Spinal Injury Unit of the Prince Henry Hospital in Sydney, Australia has assisted patients in procuring a family. Initially a programme of adoption was entered upon in conjunction with the Department of Child and Social Welfare, a State instrumentality. As the numbers of children offered for adoption declined, couples were able to participate in a newly developed programme of artificial insemination using donor sperm. The programme was supervised by the authors. Physical characteristics of the donor and prospective father were matched. Seventeen pregnancies with 15 live births have been recorded in our patients.

As sperm storage techniques improved, a number of unsuccessful attempts were made in the early 1980's to collect sperm in the immediate post-injury period on the assumption that the sperm count at this time would still be normal. However, electro-ejaculation was impossible until the return of spinal reflexes when the ejaculate contained reduced numbers of non-motile sperm. Assessment of hormonal levels in acute spinal injured men revealed no significant difference in

the levels of androgenic hormones between complete injuries, incomplete injuries and those with no loss.

The demonstration that ejaculation can be reliably produced by a high amplitude vibrator (Brindley, 1981) and rectal probe electrostimulation (Brindley, 1984; Halstead *et al.*, 1987) significantly improved the chances of spinal cord injured men fathering a child. Unfortunately, vibratory ejaculation is not successful in all men, particularly those without sacral reflexes.

The vibratory technique has been shown to produce sperm capable of fertilizing an ovum and that repeated stimulation improves their quality and quantity (Siosteen *et al.*, 1990).

The successful use of the gamete intra fallopian transfer (GIFT) (Asch *et al.*, 1984) and reports of pregnancy caused by sperm from the proximal head of the epididymis (Silber, 1983) led to our attempt at a combination of the techniques in an incomplete C4 male quadriplegic.

Material and methods

The male subject aged 33 years was rendered quadriplegic in a rugby football match in July of 1981 and on admission to the Spinal Unit of the Prince Henry Hospital was noted to have a complete motor quadriplegia at the C6 level with sparing of pin prick sensation on the scrotum, buttocks and perineum. X-rays revealed a fracture dislocation at the C4/C5 level. By the time of his discharge from hospital 7 months later he was walking with some assistance but there remained severe bilateral weakness of the upper limbs. He had a suprasacral neuropathic bladder. Following his physical rehabilitation he returned home and commenced working as a clerk. He married in January 1987 and sought counselling in respect of his sexual potential and fertility. Efforts at obtaining a pregnancy by normal intercourse were unsuccessful and, whilst penile erections occurred, there was no ejaculation. Electroejaculation was abandoned as this caused him severe discomfort and various vibrators were used to stimulate the penis without success. It was then decided to embark upon retrieval of sperm from the genital tract.

His partner, an able-bodied 28-year-old female, underwent two GIFT treatment cycles. Stimulation of the ovary to produce an increased number of oocytes was carried out using a standard regimen of clomiphene citrate, human menopausal gonadotrophin (HMG) and human chorionic gonadotrophin (HCG). Clomiphene 100 mgs orally was commenced on day 5 of the menstrual cycle and continued daily for a total of 5 days. HMG 150 iu/IMI was commenced on day 6 and continued for 5 days. HCG 5000 iu was given on day 12, approximately 38 hours before egg collection. Follicular response was assessed by daily monitoring of plasma oestradiol commencing on day 3 of the menstrual cycle.

First treatment cycle

Two to three hours prior to oocyte retrieval from the patient, we proceeded with the husband's vaso epididymal aspiration. Several aspirates were received in different volumes and mixed with sterile Earle's culture medium. The volumes of aspirates with culture medium varied from 0.5 to 1.0 ml. Using a compound microscope one drop of mixed aspirate with culture medium was put on a slide with

cover slip. No spermatids were seen in aspirate from the epididymis, however, two aspirates from the vas deferens each showed 4 to 5 motile spermatids per HPF and 9 to 10 non-motile. Those two aspirations had volumes of approximately 0.5 ml. 240 µl of Earle's culture medium containing 10% of heat inactivated maternal serum were added to each of the two aspirates and placed in a 5% CO₂ incubator. Transvaginal ultrasound oocyte retrieval on the patient was then performed and 9 oocytes were retrieved. Three oocytes mixed with the husband's sperm were then transferred via laparoscopic GIFT technique into the right fallopian tube. Two of the remaining oocytes were cultured with the husband's remaining spermatozoa. Eighteen hours post insemination, 2 pro nuclei were seen in both eggs after cumulus removal. Twenty-two hours after insemination these were frozen with 1,2-propanediol as cryoprotectant (Testart method) (Lasalle *et al.*, 1985). The remaining oocytes were donated to an infertile couple. Unfortunately in this first treatment cycle the patient did not conceive.

The patient returned in a subsequent cycle for replacement of the 2 frozen zygotes. No stimulation was used. Daily monitoring of plasma oestradiol, progesterone and LH was carried out from day 12 until the normal ovulation hormone pattern was detected (day 16 of cycle). Thawing of the 2 zygotes was performed. One zygote survived and cleaved to a 5-cell embryo and was transferred by the tubal embryo stage transfer (TEST) method into the right tube. This treatment did not result in a pregnancy.

Second stimulation cycle

The patient underwent a second stimulation cycle using the same regimen of clomid/HMG/HCG. Two hours prior to her scheduled egg retrieval, the husband underwent aspiration from the vas. Aspirates were checked under the microscope to look for the presence of motile sperm. All the extracted samples were pooled into one 5 ml falcon tube and 1.5 ml of Earle's culture medium was added and gently mixed.

The tube was spun for 5 minutes at 1500 rpm to remove any red cells. (This time they were spun prior to GIFT procedure). 0.8 ml of fresh culture medium was layered on top of the sperm cells after removing supernatant and left to rise in CO₂ incubator for at least half an hour. The supernatant was then removed into a clean tube to use for insemination. This produced approximately 84 000 motile sperm for each 40 µl of supernatant.

The patient had 11 eggs retrieved by transvaginal ultrasound. Three oocytes were replaced together with 40 µl of supernatant containing 84 000 motile sperm into her right tube using the GIFT technique (Asch *et al.*, 1984, 1986). The spare 8 oocytes remaining after 5 hours incubation period were inseminated with 84 000 motile sperm per drop of medium. Nineteen and a half hours post insemination 6 oocytes were fertilized as detected by presence of 2 pro nuclei. One of the 8 oocytes demonstrated polyspermia and was discarded. The 6 normally fertilized zygotes were frozen with 1,2-propanediol as protectant (Testart method).

Results

Following the GIFT procedure pregnancy was confirmed and subsequently ultrasound demonstrated 3 ongoing pregnancy sacs. The patient had an uneventful

triplet pregnancy and delivered 3 children at 31·5 weeks after transfer. The triplets weighed 4lb 3oz, 4lb 9·5oz and 3lb 9·5oz respectively and were in good health.

Discussion

The combined technique of aspiration of the vasa deferentia with GIFT offers a new hope for the childless spinal injured man. The triplet pregnancy, resulting in 3 living babies, we believe to be a significant medical achievement. Nevertheless, 3 newborn babies involved many hours of care and could be significant social consequence for a family in which the man is spinally injured. For this reason it is recommended that no more than 2 eggs or embryos are replaced if either of the parents are severely physically impaired.

References

- ASCH RH, ELLSWORTH LR, BALMACEDA JP, WONG PC 1984 Pregnancy after translaparoscopic gamete intra fallopian transfer. *Lancet* 3:1034.
- ASCH RH, ELLSWORTH LR, BALMACEDA JP, WONG PC 1986 Preliminary experiences with gamete intra fallopian transfer (GIFT). *Fertility and sterility* 45:366.
- BRINDLEY GS 1984 The fertility of men with spinal injuries. *Paraplegia* 22:337–340.
- BRINDLEY GS 1981 Reflex ejaculation under vibratory stimulation in paraplegic men. *Paraplegia* 19:299–302.
- HALSTEAD LS 1987 Rectal probe electrostimulation in the treatment of anejaculatory spinal cord injured men. *Paraplegia* 25:120–129.
- LASALLE B, TESTART J, RENARD JP 1985 Human embryo features that influence the success of cryo-preservation with the use of 1,2-propanediol. *Fertility and sterility* 44:645–651.
- SILBER SJ, BALMACEDA J, BORREDO C, ORD T, ASCH R 1988 Pregnancy with sperm aspiration from the proximal head of the epididymis: a new treatment for congenital absence of the vas deferens. *Fertility and sterility* Sep:50(3):525–582.
- SILBER SJ 1988 Pregnancy caused by sperm from vasa efferentia. *Fertility and sterility* 49(2):373–375.
- SIOSTEEN A, FORSSMAN L, STEEN Y, SULLIVAN L, WICKSTROM I 1990 Quality of semen after repeated ejaculation treatment in spinal cord injured man. *Paraplegia* 28:96–104.