Cryopreservation of testicular tissue – A preliminary report

Management of male infertility was considered to have a poor outcome until the advent of Assisted Reproductive Technology (ART) and Intra Cytoplasmic Sperm Injection (ICSI) procedures. These new-techniques have opened up new vistas in the treatment of this difficult problem. Now, most cases of male infertility, except where there is total atrophy of testes, can be taken up for ART/ICSI procedures, with the hope of achieving parenthood.

Cases of obstructive and non-obstructive azoospermia are now more confidently treated. Epididymal spermatozoa are known to be successfully cryopreserved and reused[1,2].

Even cases of maturational arrest are successfully tackled with ICSI procedures. Herein, surgical extraction of the spermatid at one or different sites of testes (TESE/SPERT) (sperm extraction from testes) is mandatory. In the event of failure of one attempt at ART/ICSI, it was necessary to repeat the surgical exercise of spermatozoa extraction from the testes. Cryopreservation of testicular extracted spermatozoa for ICSI procedures has been well documented[3].

Since the Sertoli cells are nurturing cells for immature sperm precursors and the maturing spermatozoa in the seminiferous tubules, we decided to apply the same concept in preserving the spermatozoa in their 'natural milieu' while trying to cryopreserve them. Hence, during the last six months, at the time of scrotal exploration, we decided to try cryopreservation of testicular tissue samples and to compare the outcome with Testicular Tissue Extracted Spermatozoa (TESE/SPERT) suspensions.

Four patients of azoospermia at the time of their scrotal exploration, were taken up for the trials after informed consent. Two had obstructive azoospermia, one had bilateral vasal aplasia and the other one had non-obstructive azoospermia.

Under general anaesthesia, scrototomy was done and testicular tissue bits were taken. One bit was used to make a per operative assessment for evidence of spermatozoa–mature/motile or immotile. If spermatozoa are present, further 2–3 mm size bit of testicular tissue was taken and immediately transferred into a sterile vial containing 0.4 ml of culture medium (Earle's balanced salt solution). Twice the amount of cryoprotectant medium (MGM) was added and the temperature was maintained at –4°C. All samples were labelled with name, age, date, side of testes. Along with these tissue bits, two more vials containing the TESE samples in EBSS medium were also frozen at –70°C, from the same patients.

The first batch (Table 1) consisted of samples which were taken out 10 days later for post-thaw recovery of spermatozoa. In the second batch (Table 2), the samples were taken out at 6 weeks after freezing for recovery of spermatozoa. After thawing at room temperature, the tissue samples were gently teased into the culture medium and examined under 400 magnification microscope. Staining was done with eosin and nigrosin.

In both the testicular tissue samples and the TESE samples, the number of live versus dead spermatozoa, their morphology and motility were assessed ten days after freeze in the 1st batch and 6 weeks after freeze in the second batch.

In the 1st batch, motile spermatozoa could be recovered in both TESE and

Table 1. Batch I (10 days freeze)

	Before freeze		After freeze	
No.	Rt	Lt	Rt	Lt
1a)	TESE/SPERT 12–14 Motile Sp/HPF	6–8 Motile Sp/HPF 50% normal	1–2 Motile Sp/HPF	Occassional Motile Sp
b)	Testicular tissue	–	8–10 Sluggish Motile Sp/HPF	3–4 Motile Sp/HPF
2a)	SPERT/TESE 10–12 Sluggishly motile Sp/HPF		1–2 Sluggish Motile Sp/HPF	
b)	MESA 40 Mill 15% active 70% normal	–	Used for FSP	–
c)	Test tissue	–	3–4 Motile Sp/HPF	–

Table 2. Batch II (6 weeks freeze)

	Before freeze		After freeze	
No.	Rt	Lt	Rt	Lt
1a)	SPERT/TESE 1–2 Motile Sp/HPF	Occasional motile Sp/HPF	None motile No live spermatozoa	None motile No live spermatozoa
b)	Testicular tissue	–	1–2 live Sp/HPF	None motile and live
2)	SPERT/TESE 1–2 motile Sp/HPF Testicular tissue	Occasional motile Sp/HPF	None motile No live spermatozoa 4–6 motile Sp/HPF 50% normal	None motile No live spermatozoa 1–2 live spermatozoa Sp/HPF 50% normal

tissue samples – more in number from the tissue samples. In the second batch, motile and live spermatozoa could be retrieved from the frozen tissue samples but none from the TESE/SPERT group.

The technique of cryopreservation of testicular extracted spermatozoa (TESE/SPERT) and their repeated use after thawing has been well established. Joseph Romero *et al.*[3] report two successful ICSI attempts from Spain in 1996, using cryopreserved testicular extracted spermatozoa. Recently, Salz Brunn *et al.*[4] from Hamburg, Germany reported pre-ICSI cryopreservation of testicular tissue bits at their Andrology department and storing at ICSI/IVF centres for recovery of spermatozoa after ovum pick up for administrative convenience.

We, herein, propose our hypothesis that the natural milieu of seminiferous tubules with their supportive Sertoli cells would prove to be a better transport base for spermatozoa while being cryopreserved. In our preliminary study, we observed better post thaw recovery rate from tissue bits rather than from the extracts.

This is our preliminary pilot study to assess the length of cryopreservability of testicular tissue for recurrent ICSI and other ART procedures, if desired at longer intervals. This will enable the couples to avoid unnecessary, recurrent testicular biopsies with subsequent gross adhesions.

This needs further studies of more samples, for more extended periods of time to substantiate our concept which will be very valuable in all infertility centres which practise ICSI/ART procedures.

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Some botanical curiosities

This report draws attention to three botanical accounts in the Andaman and Nicobar islands.

Malformation in coconut trees (*Cocos nucifera* Linn.)

Cocos nucifera Linn., a stately palm generally grows to a height of 25–30 m when fully mature and bears a crown of large pinnate leaves, and being a monoecious palm the relatively few female flowers are born at the base of the panicle while the numerous male flowers are on the top of the panicle. It is known that the deficiency of boron, one of the seven essential elements, causes 'hen and chick' disorder in coconut due to which the female flowers (buttons) in the spathe and spadix fail to develop into coconuts (drupes). Sometimes one or two in the whole spathe and spadix develop fully.

During a recent exploration and survey tour to the Little Andaman island in the Union Territory of A & N Islands, we happened to locate a couple of coconut trees with a marked difference that the trunk which is not so stout but straight or gently curved rising from a swollen base is almost entirely (starting three meters above the base) covered by numerous bunches of aborted panicles and thereby, the whole tree mimicks the Red Oil Palm trees (which are incidentally cultivated in the nearby plantations) except that these coconut trees

Figure 1. Malformation in coconut trees (*Cocos nucifera* Linn.).