Successful pregnancy after round spermatid microinjection

Sir,

Spermatozoa may not be available at biopsy in some azoospermic patients with various pathological conditions such as Sertoli cell only syndrome, maturation arrest, post-cryptorchidsm tubular atrophy, post-chemotherapy testicular atrophy, post-mumps orchitis and Kleinfelter syndrome.[1] It has been demonstrated in animal studies that, fertilization and pregnancy can be achieved using round spermatids and even secondary spermatocytes. The implementation of those technologies in humans is a very novel concept. The incidence of fertilization with spermatid microinjection is persistently low. Some studies yielded no success with this type of technology. The outcome seems to be particularly poor in patients who have never had more mature forms such as elongated spermatids or spermatozoa at any stage in their previous history.[2] To date, of the 8 reported round spermatid injection (ROSI) pregnancies, 5 had been from testicular sample round spermatids that were microinjected into oocytes.[3] We describe the 6th pregnancy to a father with idiopathic non-obstructive azoospermia.

A 43-year-old man and his 26-year-old spouse were referred to our in-vitro fertilization (IVF) program in March 2000 after 2 years of primary subfertility. Clinical examination was unremarkable. Testicular volume was estimated to be 4.5ml on the right side and 0.5 ml on the left. Four semen analyses with an average volume of 2.2 $\pm$ 0.8 ml revealed no spermatozoa. Centrifugation showed no spermatozoa or spermatogenic cells in the pellet. Blood analysis demonstrated an elevated concentration of follicle stimulating hormone (FSH) of 24.5 IU/L, luteinizing hormone (LH) of 12.6 IU/L and low testosterone of 6.5 nmol/L (normal range in males 10.3-30.9 nmol/L) and normal prolactin 310 pmol/L. The patient declined chromosome analysis. Histopathology report on a previously performed testicular biopsy was unavailable.

The wife had regular ovulatory cycle. Pituitary desensitization was induced with 3.6 mg of goserelin (Zoladex®, Zeneca, UK) starting on day 21 of the cycle. After 2 weeks, ovarian suppression was assessed. Ovarian stimulation was started with 225 IU of human menopausal gonadotrophin (HMG) (Humegon; Oregon, Holland). Follicular development was monitored. Human menopausal gonadotrophin was increased to 375 IU (Pergonal® 500; Serono, Rome, Italy) on day 16 due to slow progress. Four days later, follicle maturity was ascertained (3 leading follicles, mean diameter of $\geq$ 21 mm; E2 level > 200 pg/ml per mature follicle, 10,000 IU of HMG (Pregnyl; Organon, Holland) was administered.

On the morning of the operation, testicular biopsy was performed. The samples were collected in a pre-incubated sperm preparation medium (SPM), teased in a Petri dish under the microscope with the use of tuberculin syringes then washed with 2 ml SPM. Prepared testicular samples were gently layered on top of a gradient Percoll column prepared by obtaining isotonic 100% Percoll (Sigma Chemical Co, USA) through the addition of 9 parts of Percoll to one part of Earl's balanced salt solution 10x (Imperial, UK). The 100% Percoll was diluted again to obtain dilution increments of 5% from 30 to 100% in a 10 ml test tube washed with 100% SPM. One ml of each concentration was gently stratified starting with 100% using a Pasteur pipette followed by centrifugation for 25 minutes at 800g at room temperature. These fractions were mixed with Earl's medium and the cell concentration was examined. The various spermatids were developmentally classified. After careful search, only Sa1 spermatids were found.

Transvaginal ultrasound guided oocyte retrieval was performed 35 hours after the hCG injection. Twelve oocytes were collected; 11 methaphase II (MII) oocytes were used for ROSI and Sa1 spermatids. Three of these oocytes were injured and 3 fertilized. At 48 hours, one embryo was at the 4-blastomere stage; the 2nd was at the 2-blastomere stage. Both had minimal fragmentation. The 3rd was a syngamy stage zygote. All 3 were replaced 48 hours after oocyte retrieval. The 2 embryos were judged to be of grade III morphology (anucleate fragments occupying between 20 and 50% of the volume of the embryo). The luteal phase was supplemented with 400 mg progesterone suppositories daily (Cyclogest, Cox Pharmaceuticals, UK). Pregnancy was achieved and the first B-hCG concentration was 636 mlU/ml 16 days after the embryo transfer. A singleton pregnancy with visible heart pulsation could be seen at 3 weeks post embryo transfer. Repeat scans were consistent with her dates. The patient delivered a healthy male infant spontaneously at 39 weeks of gestation.

At our center, about 60% of patients with non-obstructive azoospermia due to germinal failure exhibit a tiny number of spermatozoa (or elongated spermatids) at testicular biopsy, and this tiny number is sufficient for successful intracytoplasmic sperm injection (ICSI). However, no sperm are recoverable in at least 40% of these cases. Round spermatid nucleus injection (ROSNI) and ROSI have been practiced as a possible solution in azoospermia patients when no fully formed spermatozoa or

elongated spermatids are recoverable in the testis. With this type of technology, we are faced with the difficulty in identifying immature germ cells in unstained fresh samples. Round cells are abundant in morselated testicular tissue of almost all azoospermia men, but difficulties arise in distinguishing under Hoffman or Normarski optics whether these round cells are haploid round spermatids, diploid spermatocytes or spermatogonia, or even somatic cells like Sertoli cells nuclei or Leydig cells. Various attempts have been made in order to identify specific subpopulations of immature germ cells for clinical purposes. Percoll gradient has allowed other authors to collect spermatids identified by staining and by evaluating haploidy using fluorescence in-situ hybridization.[4] More recently, a purified population of immature germ cells was obtained from the testicular tissue of azoospermic men employing germ cell separation using the modified discontinuous Percoll gradient technique. Fractions from 30-45% contained the highest concentration of immature germ cells and lowest leukocyte contamination.

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Etiology of end-stage renal disease in Najran, Kingdom of Saudi Arabia

With the availability of hemodialysis, patients with end-stage renal disease (ESRD) are living longer than ever. Many of the patients are enjoying, if not normal, a reasonable quality of health. In view of the high prevalence of diabetes[1] and hypertension[2] in the Saudi population, the magnitude of ESRD might reach epidemic proportions in the future. In order to ascertain the pattern of ESRD, we undertook a study of these patients that will serve as the baseline for a future reference. It will also help to complete the national level data in this country. To the best of our knowledge, there has been no such study so far from Najran, the southwestern province of the Kingdom of Saudi Arabia. This study was conducted on the registered patients of the Artificial Kidney Unit (AKU) of Najran General Hospital in the month of Rabbi-II 1421 (June 2000). Patients from all over the region are referred here. All patients with renal failure are followed here and undergo maintenance hemodialysis when needed. Non-Saudi patients are not dialyzed except by special permission or in case of an emergency. Data was collected under the following headings: name, age, sex, nationality and the underlying cause of chronic renal failure. We studied a total of 67 patients, currently registered in our unit undergoing regular hemodialysis. This total comprised of 37 males and 30 females (male-female ratio of 1.2:1). There were 29 Saudi males and 24 Saudi females ranging from 13 to 66 years (mean 39 ± 12.8 years in males and mean 38.51 ± 11 years in females). There were 7 patients less than 19 years, 10 patients between 20 to 29 years, 16 patients between 30 to 39 years, 8 patients between 40 to 49 years, 13 patients between 50 to 59 years and 13 patients more than 60 years. **Table 1** shows the underlying cause of ESRD in these patients. In our study there were mainly Saudi patients (53 out of 67). Out of the non-Saudi patients, the majority were Yemenis (12 out of 14). Yemen is the neighboring country and the population does not differ in culture,

Table 1 - Underlying cases of ESRD in Najran, Kingdom of Saudi Arabia.

Disease entity	Males	Females	Total (%)
Diabetic nephropathy	10	3	13 (19.40)
Glomerulonephritis	2	1	3 (4.49)
Pyelo-nephritis	2	5	7 (10.44)
Polycystic kidney disease	2	2	4 (5.90)
Collagen vascular disease	nil	3	3 (4.49)
Obstructive uropathy	2	1	3 (4.49)
Alports syndrome	4	none	4 (5.90)
Not established	15	15	30 (44.77)
Total	**37**	**30**	**67 (100)**