

# A human model showing the ability of testis XX cells to masculinise into Sertoli cells and success of microTESE surgery in paediatric azoospermia

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## Case History

A 14 year old male was found to have a 46,XX/46,XY karyotype following presentation with a euthyroid goitre, history of vertebral crush fracture and parental concern regarding short stature.

He was born at term with normal birth weight and male genitalia, and underwent spontaneous puberty at the age of 13. On review at age 14, he had 10mL testes and was Tanner stage 3 puberty.

## Diagnosis Of Tetragametic Chimerism

**Chromosomal microarray** revealed a diagnosis of 46,XX/46,XY tetragametic chimerism through the detection of 4 different SNP profiles on chromosomal microarray. That is, the karyotype was made from 4 separate gametes fusing in the one individual.

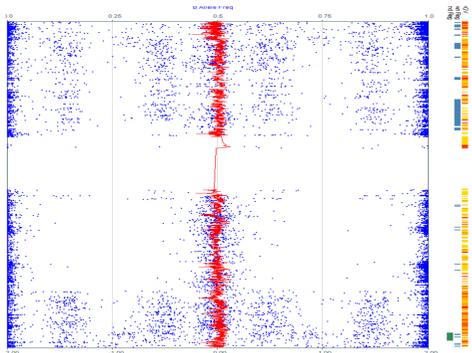


Figure 1: Four distinct SNP profiles are visible on chromosomal microarray.

The fraction of 46,XX and 46,XY cells in different somatic compartments was analysed as below.

Blood (banded karyotype): 60% XX 40% XY

Buccal (microarray): 70% XX 30% XY

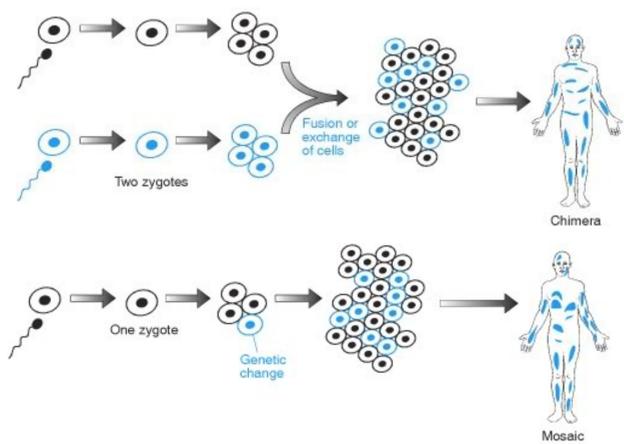


Figure 2: Chimerism involves fusion of 2 separate embryos, as opposed to mosaicism where genetically distinct cell lines originate from mutations during the development of one primary embryo.

## Clinical Progress

The patient was counselled regarding risk of testicular failure, and pubertal progress was monitored closely. He showed signs of testicular failure from the age of 15, with testes reducing in size from 10mL to 6mL and a rising serum FSH.

Age	FSH (IU/L)	LH (IU/L)	Testosterone (nmol/L)
15 yrs 6 mo	18.5	8.4	12.2
16 yrs	27.6	9.8	11.4

Table 1: Biochemical evidence of progressive testicular failure.

Fertility counselling occurred and ejaculate was collected for semen analysis and planned storage. He was found to have azoospermia (sperm count <1 10<sup>6</sup>/mL).

## Advanced Fertility Preservation

Given the degree of initial testis development and the genetically normal XY cell line component, it was felt that there was good potential for some viable sperm to be present. The patient and his family wished to maximise his fertility options and save any viable sperm from the declining testes.

Advanced fertility preservation options included testicular sperm aspiration, conventional biopsy, or the newly developed microsurgical testicular sperm extraction (microTESE) surgery.

MicroTESE is the new gold-standard method in adults for surgical sperm retrieval in non-obstructive azoospermia. The operating microscope is used to observe heterogeneity amongst seminiferous tubules.

Sperm is then extracted from the areas most likely to contain active spermatogenesis. MicroTESE is 1.5x more effective than block biopsy and 3x more effective than testicular sperm aspiration.

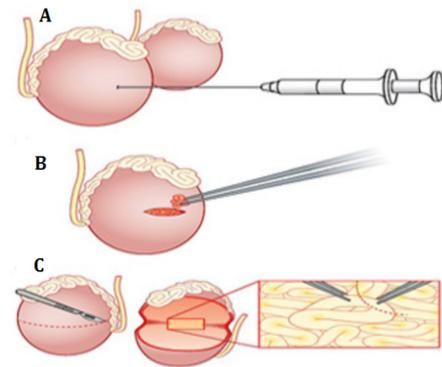


Figure 3: **A)** Testicular sperm aspiration can be performed under local anaesthetic but has an unreliable rate of accuracy with sperm retrieval 6-70%. **B)** Conventional biopsy can be done with either single or multiple block biopsies taken. **C)** Microsurgical testicular sperm extraction (microTESE).

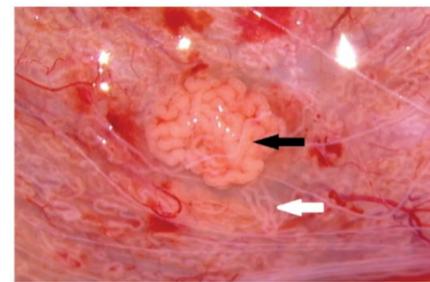


Figure 4: Seminiferous tubules with enlarged diameters (black arrow) are likely to contain active spermatogenesis compared to the thin tubules (white arrow).

## MicroTESE and Biopsy Results

**MicroTESE** was performed successfully, resulting in 13 straws (0.5mL each) of "viable appearing" sperm with 2 motile sperm per drop. The viability of sperm collected remains the same once frozen for at least 30 years, although the freezing process halves the motility of the sperm.

**Serial longitudinal biopsy** was also collected to assess for possible ovotestis or dysgenic testicular tissue. Ten specimens were collected from both the right and left testes. Histopathology showed testicular parenchyma in all specimens, without any evidence of ovarian tissue or regions of undifferentiated stroma (typically seen in the dysgenic testis).

**Fluorescence in Situ Hybridisation (FISH)** was performed to analyse the testis for XX and XY cell components. Unexpectedly, Sertoli cells which were not from the XY cell line but from the XX cell line were detected in structurally normal spermatic cords.

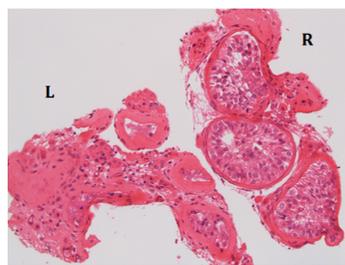


Figure 5: Histopathology of regions of maximal testicular atrophy (L) and minimal testicular atrophy (R). Thickened basement membrane and obliterated lumen occur in the atrophied specimen.

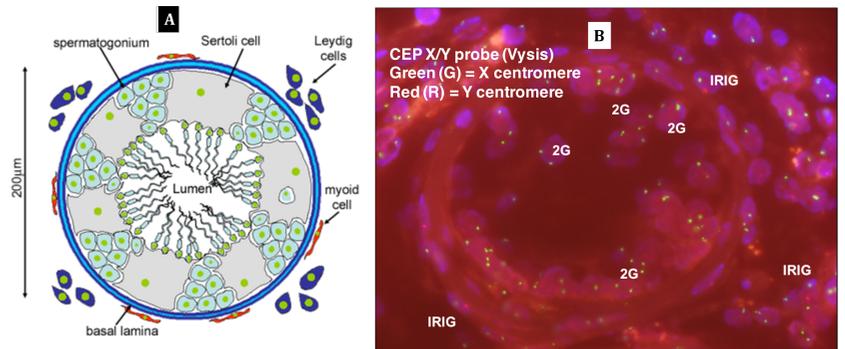


Figure 6: **A.** The seminiferous tubule. Sertoli cells are located inside the basal lamina and are the sex determining cells of the male gonad. **B.** FISH showing XX and XY signals. There were 62% XX signals and 38% XY signals. The 2G signals represent XX cells and compose the Sertoli cell component of the testis.

## Conclusions

This is the first description of normal complement XX cells transforming into Sertoli cells (male determining cells) within the human testis. These cells would have become masculinised following transcription factor and/or hormonal signalling from the external testicular milieu around them. It is known that XY testis cells in animal models can secrete masculinizing growth factors (such as fgf9 and pgd2).

This is also the first reported use of microTESE surgery in a paediatric patient with a disorder of sex development (DSD) for fertility preservation. It is widely recognized that greater effort needs to be made towards fertility preservation in youth with DSD and we feel that the next revolution in DSD care will be in fertility management.

Further analysis of this unique testicular tissue may shed fascinating light on human testis development. Immunohistochemistry of male and female protein expression in the XX testis cells, such as SOX9 and FOXL2 would outline the function of the masculinised cells. Single cell RNA sequencing of a human XX Sertoli cell would outline mechanisms involved for XX cells to become "male" in the human testis.