Commentary

Sperm retrieval in adolescent males with Klinefelter syndrome: medical and ethical issues

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Many men with conditions linked to azoospermia were formerly considered infertile. However, over the last two decades advances in assisted reproductive technology (ART) have been devised and have permitted these men to become biological fathers. Foremost among these have been testicular sperm extraction (TESE) and a "microdissection" advance in which individual spermatic tubules are punctured and sperm extracted (microTESE). In the former multiple biopsies of the subject's testes are taken and sperm retrieved as available (1). The micro technique originally noted by Schlegel and colleagues considers individual tubules for micropuncture using optical magnification (2,3). Sperm retrieval rates (SRR) are somewhat higher when performing the latter technique on men with non-obstructive azoospermia or men with Klinefelter syndrome (KS) or one of its variants (2,4,5).

Previous investigators have reported that younger age was a factor for successful SRR [this report and (4)], although other investigators [see (6), for review] did not find age to be a significant factor for success of sperm retrieval in men with KS, ascertained through infertility clinics. In theory, the residual spermatogenesis that often exists in KS patients after puberty may potentially deteriorate further with age as a continuation of the process of hyalinization of seminiferous tubules that occurs during pubertal maturation. At that age most tubules degenerate totally and become 'ghost tubules' without Sertoli and germ cells.

These reports were updated during a recent Workshop on Klinefelter Syndrome (Muenster, Germany, March 10–12, 2016). The conclusions of a Roundtable discussion chaired by Professor Eberhard Nieschlag at the end of the conference included:

- TESE-ICSI provides similar results concerning SRR, pregnancy rate, miscarriage rate and children's health. In men with KS as in men with non-obstructive azoospermia with normal karyotype;
- The experience of the surgeon and the biologist is important for the success of TESE. MicroTESE performed by trained surgeons' results on average in higher SRR than open biopsy;
- The age range giving rise to higher chances of sperm retrieval is 15–35 years. Caution should prevail because at the younger age the adolescent boys may not be mature enough or not psychologically prepared to address the fertility issue.

Conversely, some important issues remain to be investigated properly:

- Whether previous testosterone treatment, even when withdrawn for at least 6 months at the time of TESE or microTESE is or is not deleterious for the SRR. This could be investigated, first retrospectively, by noting the modality of the previous testosterone treatment (type, dose and duration), and prospectively by randomizing young patients to different treatment modalities (usual treatment, lower dose treatment leaving the gonadotropin levels within the normal range, or no treatment). Such a study will resolve the question whether it is necessary to perform TESE before initiating testosterone therapy or is it safe to wait until paternity is wished?
- Whether a treatment designed to increase intra-testicular testosterone secretion (hCG, clomiphene,

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aromatase inhibitors) is efficient or not in increasing SRRs. This could be investigated prospectively by randomized double-blind clinical trials versus placebo. Multicenter studies would be useful to obtain enough statistical power, however, an effort of standardizing the practice of TESE-ICSI among the different centers would be necessary;

- Identification of predictive markers of successful TESE would be helpful and should be developed.

The good news from the KS research front has, however, been that pockets of seminiferous tubules with preserved spermatogenesis exist besides Sertoli—cell-only tubules in adulthood, and SRRs as high as 50% have been reported in men that typically are around 30 years or older. A factor that may contribute to the preservation of pockets with spermatogenic tissue may be that the extra X chromosome seems to be lost during germ cell maturation. There is also evidence that many of the preserved Sertoli cells are without an extra X (7-9). Taken together these reports suggest that gonadal micro-mosaicism is common in KS patients with a 'pure' 47, XXY karyotype As a consequence, the children that are conceived by sperm harvested through TESE or Micro-TESE from KS patients are usually euploid.

These positive findings led to the pilot clinical trial reported in *The Journal of Pediatrics* by Nahata and co-workers (10) with the goal of improving sperm retrieval by microTESE in adolescent boys and young adults with KS.

The patients selected were probably more toward the normal male end of the KS spectrum, since none required testosterone therapy as adolescents. This certainly is in keeping with the usual caveat for men undergoing TESE or microTESE of having them discontinue T therapy at least 6 months before the surgical procedure. Their study was done under institutional review board approval and within in a single center with a single urologic surgeon.

Selection bias may also be noted from the "flow" of subjects. 95 adolescents/young adults, ages 12 to 25 years were culled from the hospital medical records system. More than 1/3 could either not be reached or opted out from further contact. Of the remaining subjects almost ½ declined to participate with a common reason stated as "lack of psychological readiness to focus on fertility". All subjects had the 47, XXY karyotype, small testes whether examined manually or by an ultrasound technique, although the two did not correlate. There were indications of testicular failure with raised (mainly) levels of FSH, low or low normal levels of T and virtually unmeasurable levels of inhibin B. Prior to the surgical technique each adolescent and young adult was requested to produce a semen sample. Although 14/15 produced an ejaculate, none had sperm on semen analysis.

It should be noted that there are multiple reports of neurocognitive dysfunction in those with KS, especially as they refer to executive function. It is these data that may help to inform how "young" the subjects might be before offering this invasive technique. In any case it seems pertinent that patients should have fully matured gonads before sperm retrieval is considered.

Unilateral microTESE was chosen as the technique given the unlikely possibility that the testis undergoing this procedure may cease to function; for the second testis would remain intact. In a unique twist the authors obtained neurocognitive data employing a previously validated survey to more effectively determine predictors of sperm retrieval.

Ten of the 15 subjects chose to undergo microTESE Viable sperm were found in 5 (50%) in the fluid obtained by this technique. Interestingly, these results are similar to those that have been reported from studies of fully adult KS patients and the authors also note that their SRR was similar. Should adolescents with KS presently undergo sperm retrieval by microTESE? The present data do not suggest that they are better off by undergoing microTESE during adolescence than later in young adulthood. In addition, a number of outstanding questions remain to be addressed, including the suitability of the thawed sperm after very long-term (decades) of freezing. The available techniques, including a reproductive hormone profile, testicular size or any other clinical parameter are not able to identify a sub-set of adolescents/young adults with KS who would have a higher retrieval rate of viable sperm for cryopreservation. Others have noted that neither the number of CAG repeats in the androgen receptor nor parent of origin of the extra X chromosome are useful in predicting the likelihood of successful sperm extraction (11,12).

Nahata *et al*. recognized a number of limitations of their pilot series: small, and likely skewed sample size, that the issue of discussion fertility was premature for a sizeable number of families, that one testis was sampled as a caution factor and these young men never had been treated with testosterone which may denote a "milder" end of the spectrum of adolescents/young adults with KS (10).

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Footnote

Conflict of Interest: The authors have no conflicts of interest to declare.

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