

THE WORK-UP OF AZOOSPERMIC MALES

Participants:

Dr. Keith Jarvi, Toronto

Dr. Kirk Lo, Toronto

Dr Anthony Fischer

Dr. John Grantmyre, Halifax

Dr. Armand Zini, Montreal

Dr Victor Chow, Vancouver

Dr. Victor Mak, Toronto

Consensus document on the investigation and treatment of azoospermic males.

A committee was established at the request of the CUA to determine guidelines for the investigation and management of azoospermia. Members of the committee, all of whom have special expertise in the investigation and management of male infertility, were chosen from different communities across Canada. The members represent different practices in different communities.

Introduction and Background:

Infertility or subfertility affects 15% of couples in Canada, with a male factor contributing to the fertility problem in close to 50% of these couples. Of the men presenting for fertility investigation, up to 20% are found to be azoospermic. These men can be categorized as having either

- 1) pre-testicular azoospermia (2% of men with azoospermia: due to a hypothalamic or pituitary abnormality: diagnosed with hypo-gonadotropic-hypogonadism),
- 2) testicular failure or non-obstructive azoospermia (49- 93%: while the term testicular failure would seem to indicate a complete absence of spermatogenesis, actually men with testicular failure have either reduced spermatogenesis (hypo-spermatogenesis), maturation arrest at either an early or late stage of spermatogenesis or a complete failure of spermatogenesis noted with Sertoli-cell only syndrome)¹⁻⁵,
- 3) Post-testicular obstruction or retrograde ejaculation (7-51%: normal spermatogenesis but obstructive azoospermia or retrograde ejaculation)¹⁻⁵.

A further group of men have a failure to ejaculate. These may be men with spinal cord injury, psychogenic failure to ejaculate or neurological damage (sympathetic nerve damage from a retroperitoneal lymph node dissection for example).

In order to understand the management of azoospermia it is important to also understand the assisted reproductive technologies (eg.in-vitro fertilization). Since the 1970s, breakthroughs in the assisted reproductive technologies have allowed us to offer potentially successful treatments for up to 98% of couples with male factor infertility⁶. These significant advances had little to do with techniques to improve the sperm quality but relied on the “assisted reproductive technologies (ARTs)”. These programs used techniques to increase the number of mature eggs produced by the women by manipulating the hormonal environment in the women using exogenous hormones (ovulation induction) then either used;

- 1) timed insemination (timed to optimize the pregnancy rates: either through intercourse or intra-uterine insemination of the partners washed sperm) or
- 2) in-vitro fertilization (oocytes are retrieved from the ovaries then are either incubated with the sperm in a dish (IVF) or
- 3) intra-cytoplasmic sperm injection: ICSI, injecting the sperm directly into the cytoplasm of the oocyte

All of the above techniques are widely used to treat couples with male factor infertility. In the USA in 2003, 122,872 IVF/ICSI cycles were performed and over 35,785 IVF babies were delivered⁶. In the USA in 2003, it was estimated that >1.0% of all newborns are IVF or ICSI babies. In Denmark, 5% of newborns are IVF/ICSI babies.

Using ICSI, it is now possible to produce a pregnancy with any live sperm (moving or not), from either the semen or any site within the male reproductive tract. Even men with azoospermia, can now be offered sperm retrieval with ICSI. Sperm could be retrieved from any site in the reproductive tract and used for ICSI. These are the men who previously had very limited chances to ever have biologically related children. Pregnancy rates of close to 50% per cycle of ICSI (women under 35 years old) are expected, with the pregnancy rates independent of the site of the origin of the sperm⁶.

History and Initial investigations for men with azoospermia

After at least 2 semen analyses have confirmed azoospermia the men should be investigated with a history, physical examination and laboratory and imaging studies.

The history should include information about the

- 1) infertility history: duration of infertility, whether the infertility is primary or secondary, any treatments to date, libido and sexual activity,
- 2) the general health of the men, with particular emphasis on the presence of diabetes, respiratory issues,
- 3) history of proven or suspected genito-urinary infections
- 4) exposure to agents which might have an adverse impact on spermatogenesis, including but not limited to:
 - a. medical agents like hormone/steroid therapy, antibiotics (sulphasalazine), alpha-blockers, 5 alpha-reductase inhibitors, chemotherapeutic agents,

- b. environmental factors like pesticides, excessive heat on the testicles
- c. recreational drugs (marijuana, excessive alcohol)
- 5) surgery of the reproductive tract (hydrocelectomies, varicocelectomies etc)
- 6) history of any genetic abnormalities in the patient or his family.

If the man has had exposure to any of the above agents they should be discontinued and the semen retested in 3-6 months. If the man has had a recent serious medical illness or injury or he has evidence of a recent reproductive tract infection, semen testing should be repeated at least 3 months following recovery from the illness.

Physical examination should include a thorough general examination with particular attention paid to the scrotal exam (size and consistency of the testis, presence and grade of varicoceles and palpable vas deferens).

The initial testing will depend on these findings.

Reduced Semen volume:

If the semen volume is reduced (<1.5 mL) and documented on repeat testing, careful questioning should elicit whether this is an artifact (missed the container, difficulty providing specimen etc.) or truly a low semen volume.

Low semen volume could be due to

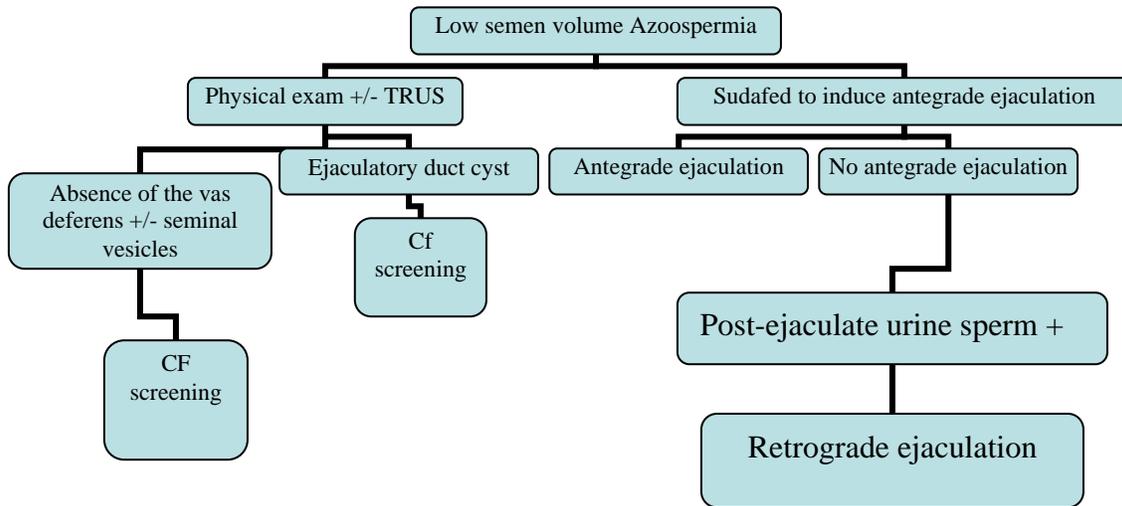
- 1) absence/abnormalities of the vas deferens/seminal vesicles,
- 2) retrograde ejaculation,
- 3) failure of emission.

Testing the post-ejaculate urine should help determine if there is retrograde ejaculation. Occasionally, an alpha agonist (use Sudafed or other just before the semen testing) will convert retrograde into ante-grade ejaculation. Diabetic men often have retrograde ejaculation or failure of emission.

Physical examination will help determine if the vas deferens is present in the scrotum and a TRUS will determine if the seminal vesicles and vas deferens close to the prostate are normal. If absence of the vas deferens and/or the seminal vesicle is identified, the man has approximately an 80% chance to carry a genetic alteration associated with cystic fibrosis⁷. Cystic fibrosis testing should be performed on all men with absence of the vas deferens/seminal vesicles (Grade of recommendation: Grade A).

Obstruction of the ejaculatory duct is detected by TRUS and is usually accompanied by dilation of the seminal vesicles (typically >1.5 cm). Vasography is not required and should be discouraged for men with an ejaculatory duct obstruction (Level of evidence 3, Grade C Recommendation). If an ejaculatory duct obstruction is identified, the man has approximately a 25% chance to carry a genetic alteration associated with cystic fibrosis⁷. Cystic fibrosis testing should be performed on all men with ejaculatory duct cysts.

Algorithm for the Investigation of Azoospermic Men with Low Semen Volume.



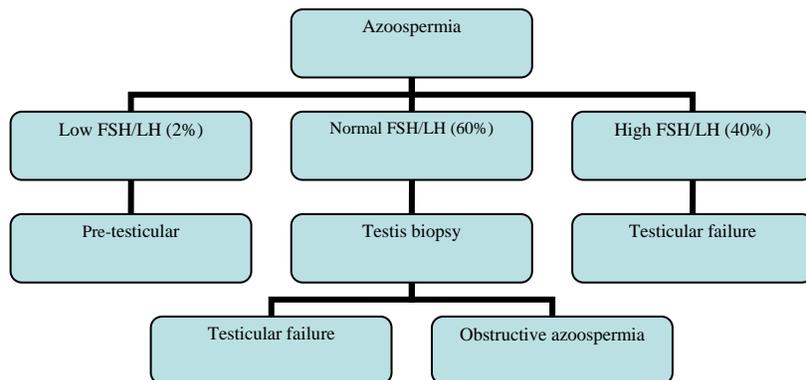
Differentiating the causes of normal volume azoospermia

As mentioned above the categories of the etiology of azoospermia are

- 1) pre-testicular azoospermia (2%: hypothalamic or pituitary etiology),
- 2) testicular failure or non-obstructive azoospermia (49- 93%),
- 3) Post-testicular obstruction (7-51%: normal spermatogenesis but obstructive azoospermia).

The category of azoospermia can often be determined by the LH and FSH levels. The diagnosis of pre-testicular azoospermia is relatively uncomplicated: LH and FSH levels will be low and the Testosterone levels will be either low or normal. Men with elevated FSH and LH and small testis bilaterally have non-obstructive azoospermia. However, men with normal levels of FSH and LH could have either non-obstructive or obstructive azoospermia⁸. Unfortunately, there is no non-invasive method to differentiate obstructive from non-obstructive azoospermia in this group of men. A testicular biopsy is usually required to provide a definitive diagnosis.

Algorithm for differentiating the causes of normal semen volume azoospermia



There have been several recent publications about the use of biomarkers in the semen and serum to differentiate obstructive from non-obstructive azoospermia⁸⁻²⁰. A number of authors report on the use of inhibin B serum levels to determine testicular function^{8, 11-14, 16, 17, 21}. While inhibin B levels are generally lower in those men with more severe testicular dysfunction and is undetectable in those with a Sertoli cell only pattern on testis biopsy, inhibin B levels in men with maturation arrest or hypospermatogenesis patterns on testis biopsies may be identical to those found in men with full spermatogenesis. At present, serum inhibin B levels do not provide significant clinical benefit: with high FSH the inhibin B levels are generally low (both indicating testicular failure), while with normal FSH the inhibin B levels are generally normal (both indicating either obstructive or non-obstructive azoospermia) (Level of evidence: 3, Grade C Recommendation).

At present, for the majority of men, there are no non-invasive methods to differentiate obstructive from non-obstructive azoospermia. As noted above, approximately 60% of men with azoospermia will require a testicular biopsy to provide a definitive diagnosis.

Failure to ejaculate

In men with a clear neurological cause (spinal cord injury, retroperitoneal lymph node surgery etc.), no further investigations are required prior to treatment. Men with idiopathic failure to ejaculate (particularly those with a failure to orgasm) should be seen by a sex therapist.

Genetic investigations for men with azoospermia

All men with hypo-gonadotropic hypo-gonadism should be referred for genetics counseling as almost all of the congenital abnormalities of the hypothalamus are due to a genetic alteration.

All men with absence (absence of the vas deferens) or obstruction (epididymal or ejaculatory duct) of the reproductive tract ductal structures are at an elevated risk to carry a genetic alteration associated with cystic fibrosis. We recommend that not only the man but his partner should be offered cystic fibrosis testing in this situation. If a genetic alteration is identified, then genetic counseling is suggested (Level of evidence: 2, Grade of Recommendation B).

All men with testicular failure should be offered karyotype and Y-micro-deletion testing then referred for genetics counseling if an abnormality is identified²²(Level of evidence: 1, Grade of Recommendation A).

Common Genetic Abnormalities found in Different Categories of Azoospermia

	Cystic fibrosis	Karyotype	Y-microdeletion
Absence or obstruction of the vas deferens, epididymis or ejaculatory ducts	25-80%		
Testicular failure		14%	1-30%

Management options for men with Azoospermia

Couples have many ways to achieve their goal of completing their family. The options of adoption, donor sperm and child-free living should always be discussed with the couple. The treatment options discussed below are those which allow a couple to have children biologically related to the man.

These options depend on the diagnosis:

Hypogonadotropic-hypogonadism or pre-testicular azoospermia; This is best treated with the use of FSH/LH or GnRH analogues to stimulate spermatogenesis. In over 90% of the cases, spermatogenesis is induced and the men have ejaculated sperm. However, therapy may take > 6 months to be effective.

Retrograde ejaculation:

Use of Sudafed or a similar alpha agonist may convert retrograde ejaculation into antegrade ejaculation. If this is not successful, it is often possible to retrieve sperm from the bladder (either using a post-ejaculatory voided or catheterized urine specimen). This sperm could then be used for one of the ARTs. To optimize the sperm quality, it is often necessary to ask the men to alkalinize (pH of 6.5 – 8) the urine using standard medications.

Obstructive azoospermia: managed with either

- 1) sperm retrieved from the reproductive tract (close to 100% chance of finding sperm) then the sperm is used in an ICSI program. The type of sperm retrieval

- used could be a percutaneous or an open microscopic aspiration of sperm from the epididymis or a percutaneous or open biopsy of the testis. Any of the types of retrievals listed above are acceptable.
- 2) Bypass/repair of the obstructed area of the reproductive tract is possible in less than half of the men with obstructive azoospermia²². The most common area of obstruction is within the epididymis. With the present microsurgical techniques, centres with expertise in performing vaso-epididymostomies report over 85% patency of the anastomosis (sperm in the ejaculate is the measure of patency) with over a 50% spontaneous pregnancy rate. However, this is surgery requiring micro-surgical expertise and experience and should only be performed in centres with this kind of expertise. We recommend that all men be offered the option to cryo-bank sperm retrieved during the course of the operation in case the surgery is not successful (Level of evidence: 3, Grade of Recommendation C).
 - 3) Men with an ejaculatory duct obstruction may be candidates for a TUR ejaculatory duct. This is best performed using a TRUS guidance to allow the TUR to precisely unroof the ejaculatory duct cyst. It is important to warn the men of the potential complications associated with a TURP.

Non-obstructive azoospermia: testicular sperm extraction may be used to identify sperm (reported success up to 75%, mean 52%) which could then be processed for use in an ICSI program²²⁻²⁹. At present, the optimum way to identify these pockets of sperm is to perform an extensive, surgical dissection of the seminiferous tubules (a testicular sperm extraction) (Level of evidence: 2, Grade of Recommendation B). Large sections of the seminiferous tubules of the testis are examined with an operating microscope. Those tubules which are larger in size are more likely to have spermatogenesis than smaller diameter tubules. The advantage of this technique over the regular random biopsy method is the ability to identify areas of the seminiferous tubules which are more likely to contain sperm before the tissue is removed from the testicle. Using this technique the chance of finding sperm is higher than the older technique of taking random testicular biopsies alone (in one series 63% compared to 45%) and while the procedure is laborious (surgical time may exceed 3 hours) the damage to the testicle is minimal due to the minimal amount of testis tissue eventually taken²⁸. ICSI pregnancy rates using sperm from a testicular sperm extraction program are reported to be between 19-50%^{22, 25-27, 30}. The testicular sperm extraction procedure should be offered to all men with non-obstructive azoospermia but should only be undertaken in a centre where ICSI is available.

Failure to ejaculate:

Men with a neurological cause for a failure to ejaculate should be offered either vibro – stimulation or electro-ejaculation. Both of these procedures may cause autonomic dysreflexia in men with high spinal cord injuries. The semen specimen may be used for one of the ARTs. It is common that multiple (2-3) procedures several weeks apart may be needed to optimize the semen quality. Occasionally these men may also have a concomitant obstruction in the epididymis, so occasionally sperm aspiration is required.

When should the azospermic man have a testis biopsy?

As mentioned above, close to 60% of the men would need a testicular biopsy to document whether the azoospermia is post-testicular (normal spermatogenesis) or testicular (testicular failure with either Sertoli Cell Only syndrome, Maturation arrest pattern or hypospermatogenesis). However, a testis biopsy should only be offered to men in whom this diagnosis would alter management. As an example, we would discourage a man from having a testis biopsy if the couple is not interested in any of the potential management options that follow (e.g. things like sperm aspiration + ICSI, vaso-epididymostomy).

If the couple is interested in considering the other fertility treatments mentioned above, then the biopsy could be performed either

- 1) as a diagnostic procedure alone (either a percutaneous or an open biopsy are acceptable methods of testicular biopsies). The biopsy results then guide the next treatments.
- 2) as the initial part of the larger fertility treatment. Once the biopsy results are available as a quick section, the surgery would then proceed with either a reconstruction and/or a sperm retrieval (if active spermatogenesis is detected) or a testicular sperm extraction (if a pattern of testicular failure is detected).

A bilateral diagnostic testicular biopsy is generally not required. If there is a discrepancy in testicular size, the larger of the two testes should be biopsied.

What is the role of varicocelectomy in men with azoospermia?

This remains controversial. There is some evidence that a small percentage of men with azoospermia due to testicular failure may benefit from treatment of a clinical varicocele³¹. It is considered reasonable to offer men with clinical varicoceles and testicular failure a varicocele repair, but it is important to warn the men that there is a low probability that this will result in any improvement in his semen parameters (Level of evidence: 4, Grade of Recommendation D).

What is the role of hormone therapy for men with azoospermia?

Apart from the management of men with hypo-gonadotropic hypo-gonadism, the use of hormones to treat men with azoospermia should be discouraged. The use of androgens is contra-indicated (Level of evidence: 1, Grade of Recommendation A).

REFERENCES

1. Fogle, R. H., Steiner, A. Z., Marshall, F. E. & Sokol, R. Z. Etiology of azoospermia in a large nonreferral inner-city population. *Fertil Steril* 86, 197-9 (2006).
2. Jarow, J. P., Espeland, M. A. & Lipshultz, L. I. Evaluation of the azoospermic patient. *J Urol* 142, 62-5 (1989).
3. Fedder, J., Cruger, D., Oestergaard, B. & Petersen, G. B. Etiology of azoospermia in 100 consecutive nonvasectomized men. *Fertil Steril* 82, 1463-5 (2004).
4. Wang, C., So, S. Y., Wong, K. K., So, W. W. & Chan, S. Y. Chronic sinopulmonary disease in Chinese patients with obstructive azoospermia. *J Androl* 8, 225-9 (1987).
5. Matsumiya, K. et al. Clinical study of azoospermia. *Int J Androl* 17, 140-2 (1994).
6. Services, U. D. o. H. a. H. (ed. US Department of Health and Human Services, C. f. D. C. a. P.) (2005).
7. Mak, V. & Jarvi, K. A. The genetics of male infertility. *J Urol* 156, 1245-56; discussion 1256-7 (1996).
8. von Eckardstein, S. et al. Serum inhibin B in combination with serum follicle-stimulating hormone (FSH) is a more sensitive marker than serum FSH alone for impaired spermatogenesis in men, but cannot predict the presence of sperm in testicular tissue samples. *J Clin Endocrinol Metab* 84, 2496-501 (1999).
9. Chen, D. Y., Wang, J. J., Huang, Y. F. & Zhou, K. Y. Relationship between lipocalin-type prostaglandin D synthase and alpha-glucosidase in azoospermia seminal plasma. *Clin Chim Acta* 354, 69-76 (2005).
10. Mitchell, V., Brabant, S., Koscinski, I., Hermand, E. & Rigot, J. M. [Proacrosin, an acrosomal marker for the detection of spermatogenic cells in ejaculates from azoospermic men]. *Gynecol Obstet Fertil* 32, 779-84 (2004).
11. Bordallo, M. A. et al. Decreased serum inhibin B/FSH ratio as a marker of Sertoli cell function in male survivors after chemotherapy in childhood and adolescence. *J Pediatr Endocrinol Metab* 17, 879-87 (2004).
12. Frydelund-Larsen, L. et al. Inhibin B: a marker for the functional state of the seminiferous epithelium in patients with azoospermia factor C microdeletions. *J Clin Endocrinol Metab* 87, 5618-24 (2002).
13. Hipler, U. C., Hochheim, B., Knoll, B., Tittelbach, J. & Schreiber, G. Serum inhibin B as a marker for spermatogenesis. *Arch Androl* 46, 217-22 (2001).
14. Brugo-Olmedo, S. et al. Serum inhibin B may be a reliable marker of the presence of testicular spermatozoa in patients with nonobstructive azoospermia. *Fertil Steril* 76, 1124-9 (2001).
15. Zopfggen, A. et al. Relationship between semen quality and the seminal plasma components carnitine, alpha-glucosidase, fructose, citrate and granulocyte elastase in infertile men compared with a normal population. *Hum Reprod* 15, 840-5 (2000).
16. Foresta, C. et al. Inhibin B levels in azoospermic subjects with cytologically characterized testicular pathology. *Clin Endocrinol (Oxf)* 50, 695-701 (1999).

17. Foppiani, L. et al. Inhibin B is a more sensitive marker of spermatogenetic damage than FSH in the irradiated non-human primate model. *J Endocrinol* 162, 393-400 (1999).
18. Diamandis, E. P. et al. Seminal plasma biochemical markers and their association with semen analysis findings. *Urology* 53, 596-603 (1999).
19. Fujisawa, M. et al. Telomerase activity in the testis of infertile patients with selected causes. *Hum Reprod* 13, 1476-9 (1998).
20. Kret, B., Milad, M. & Jeyendran, R. S. New discriminatory level for glucosidase activity to diagnose epididymal obstruction or dysfunction. *Arch Androl* 35, 29-33 (1995).
21. Hu, Y. A. & Huang, Y. F. [A serum marker of spermatogenesis--inhibin B]. *Zhonghua Nan Ke Xue* 8, 57-60 (2002).
22. Schlegel, P. N. Causes of azoospermia and their management. *Reprod Fertil Dev* 16, 561-72 (2004).
23. Kim, E. D., Gilbaugh, J. H., 3rd, Patel, V. R., Turek, P. J. & Lipshultz, L. I. Testis biopsies frequently demonstrate sperm in men with azoospermia and significantly elevated follicle-stimulating hormone levels. *J Urol* 157, 144-6 (1997).
24. Gil Salom, M. [Spermatic recovery techniques for intracytoplasmic spermatozoid injection (ICSI) in male infertility]. *Arch Esp Urol* 57, 1035-46 (2004).
25. Schiff, J. D. et al. Success of testicular sperm extraction [corrected] and intracytoplasmic sperm injection in men with Klinefelter syndrome. *J Clin Endocrinol Metab* 90, 6263-7 (2005).
26. Raman, J. D. & Schlegel, P. N. Testicular sperm extraction with intracytoplasmic sperm injection is successful for the treatment of nonobstructive azoospermia associated with cryptorchidism. *J Urol* 170, 1287-90 (2003).
27. Chan, P. T., Palermo, G. D., Veeck, L. L., Rosenwaks, Z. & Schlegel, P. N. Testicular sperm extraction combined with intracytoplasmic sperm injection in the treatment of men with persistent azoospermia postchemotherapy. *Cancer* 92, 1632-7 (2001).
28. Schlegel, P. N. Testicular sperm extraction: microdissection improves sperm yield with minimal tissue excision. *Hum Reprod* 14, 131-5 (1999).
29. Schlegel, P. N. et al. Testicular sperm extraction with intracytoplasmic sperm injection for nonobstructive azoospermia. *Urology* 49, 435-40 (1997).
30. Colpi, G. M. et al. Sperm retrieval for intra-cytoplasmic sperm injection in non-obstructive azoospermia. *Minerva Urol Nefrol* 57, 99-107 (2005).
31. Schlegel, P. N. & Kaufmann, J. Role of varicocelectomy in men with nonobstructive azoospermia. *Fertil Steril* 81, 1585-8 (2004).