CUA Guideline: THE WORK-UP AND MANAGEMENT OF AZOOSPERMIC MALES

Consensus document on the investigation and treatment of azoospermic males.

A committee was established at the request of the CUA to develop 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.

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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);
   a. due to a hypothalamic or pituitary abnormality (diagnosed with hypogonadotropic-hypogonadism)

2) testicular failure or non-obstructive azoospermia (NOA: 49-93% of men with azoospermia); or
   a. while the term testicular failure would seem to indicate a complete absence of spermatogenesis, men with testicular failure actually have either reduced spermatogenesis, maturation arrest or a complete failure of spermatogenesis noted with Sertoli-cell only syndrome\(^1\-^5\)

3) post-testicular causes for azoospermia (7-51% of men with azoospermia).
   a. obstruction or ejaculatory dysfunction
   b. normal spermatogenesis but obstructive azoospermia (OA) or ejaculatory dysfunction\(^1\-^5\)
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, for example, a retroperitoneal lymph node dissection).

In order to understand the management of azoospermia it is important to also understand the role of assisted reproductive technologies (ARTs) (for example, in-vitro fertilization) in the treatment of azoospermia. Since the 1970s, breakthroughs in the ARTs have allowed us to offer potentially successful treatments for up to 98% of couples with male factor infertility\[^6\]. These significant advances had little to do with techniques to improve the sperm quality but relied on the use of ARTs to “treat” the male infertility. 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 used either:

1. timed insemination;
   a. timed to optimize the pregnancy rates
   b. either through intercourse or intra-uterine insemination of the partners washed sperm
2. in-vitro fertilization (IVF); or
   oocytes are retrieved from the ovaries then are either incubated with the sperm in a dish
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 2012, over 165,000 IVF/ICSI cycles were performed (https://www.sartcorsonline.com). Worldwide the numbers of IVF/ICSI cycles was high: in 2013, the International Committee for Monitoring Assisted reproductive Technology reported that in 2004 there were 954,743 IVF or ICSI cycles worldwide with 237,809 babies born\[^7\].

Using ICSI, it is now possible to produce a pregnancy with any live sperm (motile 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 of age) are expected, with the pregnancy rates independent of the site of the origin of the sperm\[^6\].

**History, Physical Exam and Initial Investigations for Men with Azoospermia:**

After at least two semen analyses have confirmed azoospermia, the men should be investigated with a thorough history and physical examination. Most men will also require laboratory and imaging studies.

The **history** should include information about:

1. the infertility history, including:
   a. duration of infertility;
   b. whether the infertility is primary or secondary;
   c. any treatments to date;
d. libido;
e. sexual function; and
f. sexual activity.

2) the general health of the man, with particular emphasis on the presence of:
   a. diabetes;
   b. respiratory issues; and
   c. recent illnesses.

3) any proven or suspected genito-urinary infections, testicular infections or inflammation including:
   a. sexually transmitted infections;
   b. epididymo-orchitis; and
   c. mumps orchitis.

4) any surgery of the reproductive tract, including:
   a. testis cancer;
   b. undescended testis;
   c. hydrocelectomies;
   d. spermatocelectomies;
   e. varicocelectomies; and
   f. vasectomies.

5) exposure to medications and therapies which might have an adverse impact on spermatogenesis, including but not limited to:
   a. hormone/steroid therapy;
   b. antibiotics (sulphasalazine);
   c. alpha-blockers;
   d. 5 alpha-reductase inhibitors;
   e. chemotherapeutic agents;
   f. radiation;
   g. finasteride; and
   h. narcotics.

6) environmental exposures, such as:
   a. pesticides, or
   b. excessive heat on the testicles

7) any recreational drugs, such as:
   a. marijuana; and
   b. excessive alcohol.

8) history of any genetic abnormalities in the patient or his family.

If the man has had exposure to any of the above listed gonadotoxic 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 focus on the:

1) state of virilization;
2) scrotal exam, including:
   a. size and consistency of the testis;
b. presence and grade of varicoceles; and
c. palpable vas deferens.

3) abdominal examination with particular focus on the:
   a. presence of scars in the inguinal area indicative of previous inguinal surgery or
treatment of undescended testis.

The initial investigations will depend on the information obtained from the history and physical
examination, in addition to the results of the semen analyses.

Azoospermia with 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.\textsuperscript{10}

Since most of the semen comes from the seminal vesicles and prostate (>90%), low semen
volume means that either:
   1) the seminal vesicles are abnormal or obstructed;
   2) the ejaculatory ducts are obstructed; or
   3) there is an ejaculatory dysfunction (either failure of emission or retrograde ejaculation).

Physical examination will help determine if the vas deferens is present in the scrotum. Absence
of the vas deferens in the scrotum is usually associated with absence of the seminal vesicles and
is a cause for low semen volume and azoospermia.

The first laboratory test is to determine if there is retrograde ejaculation by testing the post-
ejaculate urine for the presence of sperm. The presence of any sperm in the urine post ejaculation
in men with azoospermia is diagnostic of retrograde ejaculation. Since retrograde ejaculation
may be due to a failure of the bladder neck to close with orgasm, use of an alpha agonist
(pseudoephedrine or other alpha agonist) before ejaculation may close the bladder neck and
convert retrograde into ante-grade ejaculation. Diabetic men often have retrograde ejaculation or
failure of emission.\textsuperscript{11}

If there is no evidence of retrograde ejaculation, diagnostic imaging of the reproductive tract is
usually required to identify reproductive tract obstruction or abnormalities. A transrectal
ultrasound (TRUS) will determine if the seminal vesicles and vas deferens close to the prostate
are normal. Obstruction of the ejaculatory duct is usually detected by a TRUS and is usually
accompanied by dilation of the seminal vesicles (typically >1.5 cm wide).\textsuperscript{12} If absence of the vas
dererens and/or the seminal vesicle is identified, the man has approximately an 80% chance of
carrying a genetic alteration associated with cystic fibrosis.\textsuperscript{13} Cystic fibrosis testing should be
performed on all men with absence of the vas deferens/seminal vesicles (Grade of
recommendation: Grade A). Men with congenital bilateral absence of the vas deferens (CBAVD)
typically have normal spermatogenesis and a diagnostic biopsy is usually not required to
diagnose active spermatogenesis. An abdominal ultrasound to assess the kidneys is indicated in
men with CBAVD who are not carriers of cystic fibrosis mutations, since these men have a
higher chance of having absence of one of their kidneys.\textsuperscript{12,14} See Figure 1.
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 of carrying a genetic alteration associated with cystic fibrosis. Cystic fibrosis testing should be performed on all men with ejaculatory duct cysts.

Figure 1. Algorithm for the Investigation of Low Volume Azoospermia

**Normal Semen Volume Azoospermia**

As stated above, the categories of azoospermia are:
1) pre-testicular azoospermia;
2) testicular failure or non-obstructive azoospermia; and
3) post-testicular obstruction.

The category of azoospermia may often be determined by the luteinizing hormone (LH) and follicular stimulating hormone (FSH) levels without need for a testicular biopsy.

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 azoospermia. 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. See Figure 2.
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 hypogonadism should be referred for genetics counseling as almost all of the congenital abnormalities of the hypothalamus are due to a genetic alteration.\(^\text{13}\)

All men with absence (absence of the vas deferens) or obstruction (epididymal or ejaculatory duct) of the reproductive tract ductal structures have an increased risk of carrying a genetic alteration associated with cystic fibrosis.\(^\text{13,16}\) We recommend that not only the man but also his partner be offered cystic fibrosis testing in this situation.\(^\text{17}\)

If a genetic alteration is identified, then genetic counseling is suggested (Level of evidence: 2, Grade of Recommendation B). Men with obstructive azoospermia do not require any other routine genetic testing.\(^\text{13}\)

All men with testicular failure should be offered karyotype and Y-micro-deletion testing, then referred for genetics counseling if an abnormality is identified (Level of evidence: 1, Grade of Recommendation A). Men with NOA do not require CF testing.\(^\text{18}\) See Table 1.
### Table 1: Common Genetic Abnormalities found in Different Categories of Azoospermia

<table>
<thead>
<tr>
<th></th>
<th>Cystic fibrosis</th>
<th>Karyotype</th>
<th>Y-microdeletion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absence or obstruction of the vas deferens, epididymis or ejaculatory ducts</td>
<td>25-80%</td>
<td>14%</td>
<td>1-30%</td>
</tr>
<tr>
<td>Testicular failure</td>
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<td></td>
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</tbody>
</table>

**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 gonadotropin-releasing hormone (GnRH) analogues to stimulate spermatogenesis\(^\text{19}\). In over 90% of the cases, spermatogenesis is induced and the men have ejaculated sperm. However, therapy may take more than six months to be effective.

**Retrograde ejaculation:** Use of pseudoephedrine (use 60 mg prior to ejaculation) 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 catherized urine specimen). This sperm could then be used for one of the ARTs.

**Obstructive azoospermia:** This is managed with either:

1) sperm retrieved from the reproductive tract (close to 100% chance of finding sperm) to be used in an ICSI program. The method of sperm retrieval used may 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 retrieval methods listed above are acceptable\(^\text{16}\);

2) bypass/repair of the obstructed area of the reproductive tract. This is a realistic therapy for most men with obstructive azoospermia\(^\text{16}\). 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\(^\text{20}\). 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); or

3) transurethral resection (TUR) of the ejaculatory duct. Men with an ejaculatory duct obstruction may be candidates for a TUR ejaculatory duct\(^\text{21}\). This is best performed using
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 TUR of the prostate.

**Non-obstructive azoospermia:** Testicular sperm extraction (TESE) may be used to identify sperm (reported success up to 75%, mean 52%) which could then be processed for use in an ICSI program\(^{16,22-28}\). 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 taken\(^{27}\). ICSI pregnancy rates using sperm from a testicular sperm extraction program are reported to be between 19-50%\(^{16,22,25,26,29}\). The testicular sperm extraction procedure should be offered to all men with non-obstructive azoospermia but should only be undertaken in a centre with expertise in micro-TESE and where an ICSI laboratory with expertise in handling these samples is available.

**Failure to ejaculate:** Men with a neurological cause for a failure to ejaculate should be offered either vibro-stimulation or electro-ejaculation\(^{30}\). 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 of the epididymis, so occasionally sperm aspiration is required.

**Which azoospermic men might need a diagnostic testis biopsy?**

As mentioned above, men having normal FSH and LH levels could have either OA or NOA. Currently, the only way to make this diagnosis is by using a testicular biopsy. However, a testis biopsy should only be offered to men in whom this diagnosis would alter management. For example, we would discourage a man from having a testis biopsy if he and his partner are not interested in any of the potential management options that follow, such as sperm aspiration plus ICSI or 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) or as a combination of a diagnostic and a therapeutic biopsy (some of the tissue is cryo-preserved for later use). The biopsy results then guide the next treatments; or
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 a reconstruction and/or sperm retrieval (if active spermatogenesis is detected) or a testicular sperm extraction (if a pattern of testicular failure is detected). This should only be performed in centres with the expertise to perform the needed microsurgery and with a laboratory with the capacity to cryopreserve sperm.

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 varicocele. Schlegel et al reported that close to 20% of the men with azoospermia had sperm in the ejaculation following a varicocele repair. It is considered reasonable to offer men with clinical varicoceles and testicular failure a varicocele repair, but it is important to warn the man that there is a low probability that this will result in any improvement in his semen parameters and the vast majority of men will still need to use ICSI to help conceive (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 hypogonadism, the use of hormones to treat men with azoospermia remains controversial.

Based on a multi-centre study, Hussein et al reported that in men with NOA given clomiphene and/or human chorionic gonadotropin (HCG) to increase FSH to a target level of 1.5 fold times the initial FSH levels and a target serum testosterone of 600-800 ng/dl, the yield of sperm on a micro-TESE was 57% compared to 33.6% in the control group.

Further support for the concept that hormonal therapies might benefit men with NOA came from Shiraishi et al who studied the effects of HCG 5000U three times/wk with the addition of FSH for men found to have declining FSH on men who had no sperm found on an initial micro-TESE surgery. In total, 6/28 men who received the hormone therapy had sperm retrieved on a subsequent micro-TESE, while 0/20 of the men not receiving hormones had sperm found on a second micro-TESE.

Conversely, Reifsnyder et al reported that men with NOA having initially lower serum testosterone levels (<300ng/dl) who took hormone therapies with increases in serum testosterone levels, had no different results of the testicular sperm extraction (sperm retrieval or pregnancy rates) than men who had higher testosterone levels initially.

We cannot presently recommend the use of any hormone therapy for men with NOA.

The use of androgens is contraindicated in men with azoospermia (Level of evidence: 1, Grade of Recommendation A).
REFERENCES

15. 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, 2496-2501 (1999).