

International Federation of Fertility Societies

Global Standards of Infertility Care

Standard 17

Investigation and Management of Non-Obstructive Azoospermia

Recommendations for Practice

Name	Non-Obstructive Azoospermia
Version Number	17.0/IFFS / Standards
Author	Standards and Practice Committee – main author Ossie Petrucco
Date of first release	June 2014
Date approved by IFFS	June 2014
Date of Review	October 2017

Introduction

The goal of IFFS Practice Standards is to provide policy and decision-makers and the clinical and scientific community with a set of recommendations that can be used as a basis for developing or revising institutional or national guidelines on selected practice recommendations for infertility practice.

The document addresses minimal standards of practice but does not provide rigid guidelines but rather gives recommendations that provide the basis for rationalizing the provision of infertility services in view of the most up-to-date information available.

Because country situations and programme environments vary so greatly, it is inappropriate to set firm international guidelines on infertility practice. However, it is expected that institutional and national programmes will use these practice recommendations for updating or developing their own infertility guidelines in the light of their national health policies, needs, priorities and resources. The intent is to help improve access to, quality of, and safety of infertility and assisted conception services. These improvements must be made within the context of users' informed choice and medical safety. Adaptation is not always an easy task and is best done by those well-acquainted with prevailing health conditions, behaviours, and cultures.

Azoospermia may be due to pre-testicular, testicular (non-obstructive) and post-testicular causes (*see diagram*). Management of obstructive azoospermia is uncontroversial. This practice recommendation is confined to the diagnosis and management of non-obstructive azoospermia (NOA).

Rationale

Azoospermia affects up to 10% of infertility cases (De Croo et al 2000) and current knowledge and the advance of assisted conception techniques has opened up possibilities for treatment with the man's own sperm. In cases of NOA the possible aetiologies necessitate a sophisticated multidisciplinary approach including endocrinology, genetics, pathology and reproductive medicine. Assisted conception maybe possible but requires specialised knowledge and expertise.

Clinical Assessment of Testicular Function

History should include causes of impaired spermatogenesis and clinical examination should document the following:

- Secondary sex characteristics, gynaecomastia
- Testes size (Prader Orchidometer)
- Palpation epididymis and scrotal contents
- Presence vas on each side
- Abnormalities such as hypospadias, varicocele, testicular tumours, accessory gland enlargements
- Height and weight (BMI)

Detailed discussion of the clinical evaluation of the azoospermic patient is discussed in an ASRM Practice Committee Technical Bulletin (2008).

Investigations

- Hormonal - FSH, LH, testosterone, prolactin, oestradiol, thyroid function.
- Karyotype and Y-Chromosome microdeletion.
- Testicular / prostate-ultrasound.

This assessment plan should distinguish between gonadotrophin deficiency, genital tract obstruction and primary seminiferous tubule failure causing testicular or NOA which affects approximately 1% of male population.

a. Endocrine

- FSH and LH levels may be elevated but overall not predictive for sperm production in the testes, thus an elevated FSH level is not a contraindication to attempt sperm retrieval.
- Testosterone (T) (and SHBG if T low) and oestradiol (E2). Low serum levels of T are indicative of likely low levels of intratesticular testosterone. Spermatogenesis is likely to be affected particularly if E2 levels are increased giving rise to an abnormal E2/T ratio.
- Thyroid function and prolactin. May be predictive if abnormal.

b. Genetic

The frequency of chromosome abnormalities increases as the number of spermatozoa in the ejaculate decreases. An incidence of up to 10% is found in NOA cases.

Autosomal abnormalities predominate with five times incidence from normal (1.3% vs 0.25%) with Robertsonian translocations being 8.5 times incidence.

Sex chromosome abnormalities are highest being 27 times more frequent (3.8% vs 0.14%) (Van Assche et al 1996). Chromosomal aneuploidies result in 47 XXY, Klinefelter syndrome; XX male syndrome; mixed gonadal dysgenesis 46XY or 45, X/46, XY.

Chromosomal translocations (reciprocal or Robertsonian results from material of chromosome, 13, 14, 15, 21, 22 being involved in up to 15 different translocations).

Chromosomal inversions often involve chromosome 9. Structural X or Y chromosome abnormalities include translocation of part of X or Y to autosomes or other X or Y sex chromosomes.

The azoospermia factor focus (AZF) is present in band 9 11.23 of the Y chromosome.

Abnormalities AZFa, AZFb, AZFc, AZFd are recognised and regulate different stages of spermatogenesis from Sertoli cell only syndrome, maturation arrest at the spermatid stage or severe hypo spermatogenesis. Men with AZFa and AZFb microdeletions have complete absence of germ cells or maturation arrest at the spermatocyte stage and thus cannot be offered fertility options using their gametes.

AZFc microdeletions are the most common finding and produce variable effects with up to 2/3 having some sperm in the ejaculate. Men with this defect from whom suitable sperm may be collected for ICSI fertilisation will have male progeny who will inherit the same abnormality.

Spermatogenesis is non-homogeneous in the testis however up to 90% have potential for sperm retrieval.

AZFd cases are associated with normal or mildly reduced sperm counts with possible abnormal morphology. Inversions balanced translocations and duplication of chromosomes have variable effect on spermatogenesis from severe impairment to near normality.

Aneuploidic chromosome abnormalities entail potential serious consequences for offspring, and therefore genetic counselling is essential for individuals with these abnormalities (Pauer et al 1997).

Klinefelter syndrome is the most common genetic form of male hypogonadism resulting in testosterone deficiency, small testes, infertility, decreased facial or pubic hair, gynaecomastia, decreased libido/potency, normal to moderately reduced Leydig cell function, azoospermia and increased secretion of FSH.

c. Scrotal Examination

Even markedly reduced testicular size has not been found to be a contraindication for sperm retrieval. Klinefelter individuals if non-mosaic have a substantial chance of sperm recovery despite small size testes.

d. Testicular Histopathology

Progressive development of germ cells in the basement membrane and interstitial cells of the seminiferous epithelium changes spermatogonia (mitosis) to spermatocytes (meiosis) to spermatids (spermiogenesis) and finally spermatozoa (spermiation).

Histopathology of the seminiferous epithelium has been classified by de Kretzer as follows:

- Normal - obstruction
- Hypospermatogenesis - all cell types present in reduced numbers; mild, moderate, severe.
- Germ cell aplasia or Sertoli cell only syndrome
- Germ cell arrest - at spermatogonia, spermatocyte, spermatid stage
- Miscellaneous -hyalinisation, Leydig cell atrophy or hyperplasia, inflammation, carcinoma (de Kretzer).

Testicular biopsy with fixation of tissue in Bouins solution and histological examination of tissue is the most successful predictor of being able to extract sperm suitable for fertilisation. It must be emphasised that a diagnosis of Sertoli cell only on biopsy does not exclude the possibility of successful sperm extraction.

e. Techniques for Testicular Biopsy

There may be only limited sites of spermatogenesis with no geographic identifiable area within the testis. Different techniques are possible including testicular sperm extraction (TESE), open biopsy and multiple fine needle aspirations (TESE). The chance of finding sites of spermatogenesis is (10-15%) higher with open biopsy than fine needle aspiration however there is a low but increased risk of haematoma formation under the tunica albuginea, parenchymal fibrosis, inflammation and permanent devascularisation which may cause further testicular damage (Schlegel and Su 1997). Re-exploration of the testis should be delayed for six months to allow the testis to recover.

Testicular biopsy will also diagnose the unlikely possibility of testicular intratubal germ cell neoplasia (Ca in situ).

TESE can be performed prior to or at the same time as an IVF cycle for the female partner.

f. Imaging

Ultrasound examination of the prostate and testes should be performed for any abnormality found on examination. Because of an increased risk of cancer in menorrhagia with infertility and poor semen quality (Moller and Skakkebaek 1999) and the observed high prevalence of testicular cancer in azoospermic men without spermatogenesis (Mancini et al 2007) a case can be made for routine testicular ultrasound scan examination in these individuals.

Management

a. Pre Treatment

Individuals considered suitable for sperm extraction during an invitro fertilization cycle should be considered for medical pre-treatment to optimise successful sperm retrieval. Klinefelter and other individuals with elevated E2 and low T levels with abnormal E2/T ratios ($T/E < 10$), should be considered for testicular Leydig cell suppression of testosterone aromatisation to E2. This can be achieved by using an Aromatase inhibitor such as Anastrozole for three months following cessation of T supplementation if this was being used. Improvement of semen parameters in the ejaculate and increase in serum T levels have been observed in oligospermic men following this treatment. Azoospermic men usually remain azoospermic however an improved sperm retrieval rate has been observed by Schlegel (2012). In Klinefelter syndrome an increase in serum T levels is also a prognostic finding.

The day before a planned sperm retrieval procedure a semen analysis should be performed to exclude the presence of suitable spermatozoa.

b. Sperm Extraction / Micro TESE

Particularly when there is failure of finding live sperm on TESA open biopsy consideration should be given to Micro TESE technique:

- Magnification – operating microscope to view tissue
- Equatorial incision tunica albuginea
- Observation and avoidance blood vessels under surface testis
- Dissection thin septa parallel to blood vessels using constant irrigation
- Avoid dissection at edge testis
- Maintenance blood supply
- Fool for longer dilated and opaque seminiferous tubules
- Homogeneous thin tubules usually have no sperm
- A traumatic closure

The friable tissue samples are critically examined in the theatre by an experienced embryologist using a multiphoton microscope to observe spermatozoa in a tissue spread. The seminiferous tubules are broken up using a 24 gauge angiocatheter.

If tissues are all homogeneous / multiple tissue no sperm are found random biopsies are processed by collagenase digestion in the laboratory before further observation for sperm.

The experience at Cornell (Rosenwaks & Schlegel) involving 1300 individuals with NOA 5.0% of whom had prior failed procedures was as follows:

- Sperm retrieval - 55%
- Fertilization - 51%
- Clinical pregnancy - 47%

These results were independent of age, FSH / LH levels or testicular volume.

Results according to aetiology:

- Cryptorchidism 74% sperm retrieval
- Klinefelter's 68% sperm retrieval
- Post chemotherapy 43% sperm retrieval

or limited radiotherapy (best results following platinum therapy than following alkylating agents).

- Maturation arrest – worst prognosis group.

Micro TESE exploration causes least postoperative complications with minimal tissue removal.

Recommendations for Practice

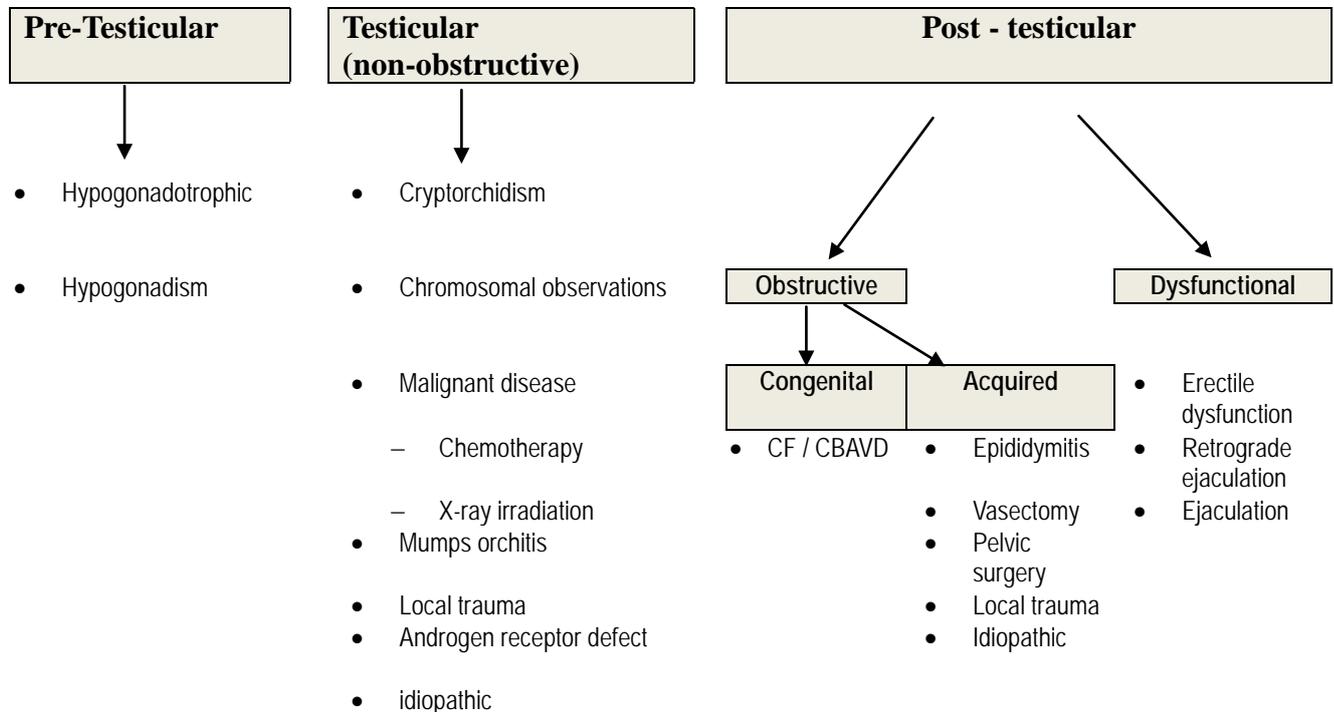
1. A diagnosis of testicular non-obstructive azoospermia (NOA) requires detailed history, examination and specific investigation to differentiate from pre-testicular and post-testicular azoospermia cases.
2. Identification of certain Y chromosome microdeletions is essential to identify non treatable individuals.
3. Genetic counselling is required when autosomal abnormalities are present.
4. Individuals with oligo / azoospermia should have testicular ultrasound performed to exclude precancerous / carcinoma.
5. An abnormal serum testosterone / oestradiol ratio may indicate the need for treatment with Aromatase inhibitors before sperm retrieval is carried out.
6. Histopathology diagnosis of testicular biopsy is the best predictor of successful outcome.
7. The most successful and least traumatic technique for sperm harvesting has been microsurgical TESE however this requires highly specialised surgical and laboratory techniques.
8. Successful outcomes have resulted for all aetiological categories irrespective of age, testicular volume and serum FSH levels.

References:

- Practice Committee of the American Society for Reproductive Medicine. Evaluation of the azoospermic male. *Fertil Steril* 90: (2008) S74-7.
- Craft I, Tsirigotis M, Courtauld E, Farrer-Brown G Testicular needle aspiration as an alternative to biopsy for the assessment of spermatogenesis. *Hum Reprod*, 12 (1997) 1483 – 7.
- Van Assche E, Bonduelle M, Tournaye H, Joris H, Verheyen G, Devroey P, Van Steirteghem A, Liebaers I Cytogenetics of infertile men. *Hum Reprod*, 11 Suppl 4 (1996).
- Pauer HU, Hinney B, Michelmann HW, Krasemann EW, Zoll B, Engel W Reference of genetic counselling in couples prior to intracytoplasmic sperm injection. *Hum Reprod* 12 (1997) 1909 – 12.
- de Kretzer DM, Holstein AF. Testicular biopsy and abnormal sperm cells. In: Hafeez ESE Editor: *The Human semen and fertility regulation in men*. Missouri: Mosby Co. St Louis. 1976: pp332-343
- Skakkebaek NE. Testicular dysgenesis syndrome: new epidemiological evidence. *Int J Androl* 27, (2004) 89 – 191.
- Mancini M, Carmignani L, Gazzano G, Sagone P, Gadda F, Bosari S, Rocco F, Colpi GM. High prevalence of testicular cancer in azoospermic men without spermatogenesis. *Hum Reprod*, 22.4 (2007) 1042 – 1046.
- Schlegel PN, Su LM Physiological consequences of testicular sperm extraction. *Hum Reprod*. 12, (1997) 1688 – 92.
- Schlegel PN Aromatase inhibitors for male infertility. *Fertil and Steril*. 2012; 98: 1359-1362.
- Schlegel PN Testicular sperm extraction: microdissection improves sperm yield with minimal tissue excision. *Hum Reprod*. 14 (1999) 131 – 135.

DIAGRAM

Aetiology Azoospermia



MICROTESE Results (NOA):

TYPE	NO.	SPERM RECOVERY:
Hypospermatogenesis	26	100%
Maturation arrest	16	50%
Sertoli cell only	108	28%
TOTAL	150	(62) 41.3%

In this series 129 individuals had XY chromosome with 40% successful aspiration and 21 were now mosaic Klinefelter with 52.4% successful result.