OBSTRUCTIONS OF THE MALE REPRODUCTIVE TRACT:
DIAGNOSIS AND MANAGEMENT
The research described in this thesis was performed at the department of Andrology, Urology and Clinical Genetics of the Erasmus University Hospital Rotterdam, The Netherlands. Financial support for the studies presented was given by the "Stichting Urologisch Wetenschappelijk Onderzoek" (SUWO) and the department of Clinical Genetics, Erasmus University Rotterdam.

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Lay-out: Bas Doble


No part of this thesis may be reproduced or transmitted in any form, by any means, or stored in any information or retrieval system, without written permission of the author and, when appropriate, of the publishers of the publications.
“Cheshire Puss”, Alice began, “Would you tell me, please, which way I ought to go from here?”

“That depends a good deal on where you want to get to,” said the Cat.

“I don’t much care where-” said Alice.

“Then it doesn’t matter which way you go,” said the Cat.

“-So long as I get somewhere,” Alice added as an explanation.

“Oh, you’re sure to do that,” said the cat, “if you only walk long enough.”

From: “Alice Adventures in Wonderland”
by Lewis Caroll, 1865.

Voor mijn moeder,
Voor Laya en de kinderen
OBSTRUCTIONS OF THE MALE REPRODUCTIVE TRACT:
DIAGNOSIS AND MANAGEMENT

Diagnostiek en behandeling van obstructies van de tractus genitalis van de man.

Proefschrift
ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam
op gezag van de Rector Magnificus
Prof.dr.ir. J.H. van Bemmel
en volgens besluit van het College voor Promoties.

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- General discussion
- Summary
- Samenvatting
- Curriculum vitae
- List of publications
- Dankwoord
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ART</td>
<td>Artificial reproductive techniques</td>
</tr>
<tr>
<td>AZF</td>
<td>Azoospermia factor region of the Y chromosome</td>
</tr>
<tr>
<td>CBAVD</td>
<td>Congenital bilateral absence of the vas deferens</td>
</tr>
<tr>
<td>CF</td>
<td>Cystic fibrosis</td>
</tr>
<tr>
<td>CFTR</td>
<td>Cystic fibrosis transmembrane conductance regulator</td>
</tr>
<tr>
<td>DAZ</td>
<td>Deleted in azoospermia gene</td>
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<tr>
<td>EDO</td>
<td>Ejaculatory duct obstruction</td>
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<tr>
<td>FISH</td>
<td>Fluorescence in situ hybridization</td>
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<tr>
<td>FSH</td>
<td>Follicle stimulating hormone</td>
</tr>
<tr>
<td>GnRH</td>
<td>Gonadotrophin releasing hormone</td>
</tr>
<tr>
<td>HPA</td>
<td>Hypothalamus-pituitary axis</td>
</tr>
<tr>
<td>ICM</td>
<td>Interstitial current measurement</td>
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<tr>
<td>ICSI</td>
<td>Intracytoplasmic sperm injection</td>
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<tr>
<td>IVF</td>
<td><em>In vitro</em> fertilization</td>
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<tr>
<td>LH</td>
<td>Luteinizing hormone</td>
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<tr>
<td>MAGI</td>
<td>Male accessory gland infection</td>
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<tr>
<td>MESA</td>
<td>Microsurgical epididymal sperm aspiration</td>
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<tr>
<td>OAT</td>
<td>Oligo-asthenoteratozoospermia</td>
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<tr>
<td>PESA</td>
<td>Percutaneous epididymal sperm aspiration</td>
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<tr>
<td>PRL</td>
<td>Prolactin</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
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<tr>
<td>SCI</td>
<td>Spinal cord injury</td>
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<td>TESE</td>
<td>Testicular sperm extraction</td>
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<tr>
<td>T-stretch</td>
<td>Polypyrimidine (T) stretch of intron 8 of the CF-gene</td>
</tr>
<tr>
<td>TRUS</td>
<td>Transrectal ultrasound</td>
</tr>
<tr>
<td>TUR</td>
<td>Transurethral resection</td>
</tr>
<tr>
<td>TURED</td>
<td>Transurethral resection of the ejaculatory ducts</td>
</tr>
<tr>
<td>VES</td>
<td>Vasoperidymostomy</td>
</tr>
<tr>
<td>VVS</td>
<td>Vasovasostomy</td>
</tr>
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</table>
SCOPE OF THIS THESIS.

In this thesis several aspects of diagnosis and management of obstructive male infertility are discussed. The introduction gives a general overview of both male infertility and azoospermia. Diagnostic, genetic and therapeutic aspects of male infertility according to the current literature are discussed. The chapter on diagnosis of obstructions of the genital tract describes a study on the incidence and management of subtotal obstructions in men with severe oligozoospermia. Furthermore, genetic risk factors are investigated in two studies: in men with congenital bilateral absence of the vas deferens the relationship with cystic fibrosis is investigated and in men with either azoospermia or severe oligozoospermia cytogenetic abnormalities, Y chromosome deletions and cystic fibrosis gene mutations are determined. Practical advises for genetic investigations and genetic counselling are given. The chapter on management of male reproductive tract obstructions describes the results of transurethral resection of cystic lesions in the prostatic gland, causing ejaculatory duct obstruction. In a review on microsurgical treatment of the male genital tract, different operative techniques, outcome and prognostic factors are discussed extensively, including our own results. Finally, the technique of epididymal sperm extraction and intracytoplasmic sperm injection are presented together with the results of these combined treatments for men with irreparable obstructions of the seminal path. The scope of this thesis is to give an overview of recent developments in the diagnosis and treatment of obstructive male infertility, and to discuss the genetic risk factors associated with these forms of male infertility.
DIAGNOSTIC WORK-UP OF SEVERE OLIGOZOOSPERMIA

SEVERE OLIGOZOOSPERMIA: < 1 MILLION SPERMATOZOA/ML

PHYSICAL EXAMINATION
SCROTAL ULTRASOUND
FSH

NORMAL
TESTICULAR Biopsy

LOW SEMINAL VOLUME
TRANSRECTAL ULTRASOUND

SCROTAL EXPLORATION
VASOGRAPHY
VASO-EPIDIDYMOSTOMY
MESA/CRYOPRESERVATION

EJACULATORY DUCT OBSTRUCTION
TRANSURETHRAL RESECTION
SPERM ASPIRATION FROM THE SEMINAL VESICLES

ABNORMAL
GENETIC SCREENING

GENETIC COUNSELING
ICS
INTRODUCTION
FACTORS INFLUENCING MALE INFERTILITY.
Department of Urology¹, Andrology², Endocrinology and Reproduction², Erasmus University Rotterdam, The Netherlands.

Introduction

Infertility is defined as lack of conception after at least 12 months of unprotected intercourse (1). However, in many centres infertility is only considered after 24 months of non-conception. Data on the incidence of fertility problems are scarce. The proportion of couples seeking medical treatment is estimated at 4% - 17%. The increasing awareness of infertility and the current availability of the many techniques of assisted fertilization has changed the patterns of referral. Couples tend to ask medical advice after a shorter period of infertility and demand artificial reproductive techniques at an earlier stage.

Infertility affects both men and women. Male causes for infertility are found in 50% of involuntarily childless couples. In many couples, however, a male and a female factor coincide. In case of a single factor the fertile partner may compensate for the less fertile partner. Infertility than usually becomes manifest if both partners are subfertile, meaning reduced fertility.

About 10% of the infertile couples are in the group of unexplained infertility. Unexplained infertility is a term applied to a couple who have been trying to achieve pregnancy and whose standard investigations are normal. Finally, 3% of the couples will remain childless, probably representing true biological infertility (2).

It has to be emphasized that to categorize infertility, both partners should be investigated simultaneously. In order to evaluate the infertile couple, it is important to get information about the duration of infertility, previous pregnancies and the age of the female partner. When the duration of infertility exceeds four years of unprotected intercourse, the conception rate per month is only 1.5%. At present, many women postpone their pregnancy until they have finished their education and have started a professional career. However, the fertility of a woman of 35 years is only 50% of the fertility potential of a woman aged 25 years; by the age of 38 years this has reduced to only 25%, and over the age of 40 years it is less than 5%. Female age is the most important single variable influencing outcome in assisted reproduction (3). Therefore, in the diagnosis and management of male infertility it is essential to consider the fertility chances of the female partner, since this might determine the final outcome.

In this review several factors influencing male infertility are discussed in detail, with a special focus on new insight in the aetiology of idiopathic male infertility, like environmental toxins, lifestyle factors and genetic abnormalities.

Male infertility.

Reduced male fertility can be the result of congenital and acquired urogenital abnormalities, infections of the genital tract, increased scrotal temperature (varicocele), endocrine disturbances,
genetic abnormalities and immunological factors (2). No causal factor is found in 40-50% of cases (idiopathic male infertility). Many of these men present with no previous history associated with fertility problems and have normal findings on physical examination and endocrine laboratory testing. Semen analysis reveals a decreased number of spermatozoa (oligozoospermia), decreased motility (asthenozoospermia) and many abnormal forms on morphological examination (teratozoospermia). Usually these abnormalities come together and are described as the OAT-syndrome (oligo-astheno-teratozoospermia). Table 1 summarizes the main aetiological causes of male subfertility.

<table>
<thead>
<tr>
<th>Aetiology and distribution(%) of male infertility among 7057 men (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No demonstrable cause</td>
</tr>
<tr>
<td>Sexual factors</td>
</tr>
<tr>
<td>Urogenital infection</td>
</tr>
<tr>
<td>Congenital anomalies</td>
</tr>
<tr>
<td>Acquired factors</td>
</tr>
<tr>
<td>Varicocele</td>
</tr>
<tr>
<td>Endocrine disturbances</td>
</tr>
<tr>
<td>Immunological factors</td>
</tr>
<tr>
<td>Idiopathic abnormal semen (OAT syndrome)</td>
</tr>
<tr>
<td>Other abnormalities</td>
</tr>
</tbody>
</table>

The unexplained forms of male subfertility may be caused by several factors like chronic stress factors, endocrine disruption due to environmental pollution, increased scrotal temperature, reactive oxygen species, and genetic abnormalities. These factors will be discussed in more detail.

**Stress, prolactin and reproduction**

It has been observed that strenuous exercise may lead to impairment of reproductive functions. Menarche can be postponed by several years. Female marathon runners may develop oligo- and even amenorrhoea. Also, the pulsatility of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) is impaired during stress. Although data on exercise in men is less abundant, it has also been shown that over-training leads to a decrease of serum testosterone concentration and a decrease of sperm concentration that may last several months. Interestingly, an increase of
both serum prolactin (PRL) and cortisol has been found during this type of stress; both PRL and adrenal corticosteroids are considered as stress factors (4).

Hyperprolactinaemia in men and women due to prolactin-secreting pituitary adenomas is associated with loss of libido, potency and menstrual cycle disturbances, respectively. However, the development of these clinical manifestations, apparently due to hypogonadotrophic-hypogonadism during hyperprolactinaemia, might also be explained by the size of the tumour and destruction of normal pituitary tissue, rather than by PRL itself.

In rats, hyperprolactinaemia leads to suppression of gonadotrophin-releasing hormone (GnRH)-release from the hypothalamus, suppression of serum gonadotrophins and gonadal steroids (5). It has been shown both in vivo and in vitro that PRL is able to activate the hypothalamus-pituitary-adrenal (HPA) axis, primarily by stimulation of corticotrophin-releasing factor (CRF). Subsequently, CRF is able to stimulate production of pro-opiomelanocortin (POMC) and flendorphin, which have been shown to be potent suppressors of GnRH-release. This mechanism has also been shown during stress (6).

In conclusion, the suppressing effects of PRL and stress on gonadal function in rats are at least partly due to the activation of the HPA-axis. Evidence for this mechanism in men is still fragmentary.

**Endocrine disruption**

In 1992 Carlsen et al. published a meta-analysis of semen quality over the period 1938-1990 and found a significant decrease of sperm-concentration and semen-volume, together with an increase of cryptorchidism, hypospadias and testicular cancer (7). This study prompted several investigators to evaluate their data on semen-quality. The studies of Auger et al. (8) and Irvine et al. (9) provided evidence for a decline in sperm concentration and total number of motile sperm. Other studies argue that there has not been a decrease in semen quality over the past 20-50 years (10,11). However, the reported decrease in sperm-concentration may seem difficult to reconcile with the absence of any detectable decrease in male fertility.

Another remarkable observation is the increased incidence of testicular cancer (12). The epidemiological data indicative of an increasing incidence of cryptorchidism (13) are unequivocal. Birth data from several reports have indicated a substantial increase in prevalence of hypospadias (14). The contradictory observations on sperm-quality and the unequivocal data on testicular cancer, cryptorchidism and hypospadias lead to the conclusion that the available data are sufficient to raise concerns, especially since it has been questioned whether environmental toxins, often estrogenic in effect, may affect male reproductive health.
Figure 1. Central effects of prolactin on the hypothalamic-pituitary-gonadal axis. (PRL, prolactin. CRF, corticotrophin-releasing factor. POMC, pro-opiomelanocortin. GnRH, gonadotrophin-releasing hormone. ACTH, adrenocorticotropic hormone. LH, luteinizing hormone. ARC, arcuate nucleus. MPOA, medial preoptic area. PVN, paraventricular nucleus) Short arrows indicate changes in levels.
It has been hypothesised that these male reproductive disorders might be explained by over-
exposure to estrogens or estrogenic chemicals in foetal life (15). However, high doses of diethyl-
stilbestrol (DES) did not lead to impairment of fertility in men who had been exposed to DES in
utero (16), although congenital malformations of the genitalia were reported three times as often
by the DES-exposed men as by the sons of the women in the placebo group. Another observation
that challenges this hypothesis is the persistent difference in the incidence rate of testicular
cancer in the Scandinavian countries in men exposed in utero in the mid-1960s to p,p'-DDE, the
main DDT-metabolite, an organochlorine pesticide with known estrogenic and androgenic
effects (17).

The major routes of exposure to environmental chemicals are thought to be dietary,
environmental from pollution of air and water, domestic and occupational. Nevertheless, any
proof that exposure to estrogenic compounds may lead to deterioration of reproductive function
only comes from wild life.

**Scrotal temperature.**

In humans both testes are positioned outside the abdomen to induce a temperature that is 2-3º C
below the body temperature. Spermatogenesis and epididymal function are optimal at this
temperature. Scrotal cooling is at its best in a standing position and during walking. Increased
scrotal temperature impairs sperm production and epididymal function, resulting in low sperm
numbers and poor motility (18). The function of the epididymis is storage and maturation of
spermatozoa and passage through the organ is essential for normal fertilization. It has been
shown in animals that the speed of transportation through the epididymis is increased in case of
high scrotal temperature. Conditions like a non-scrotal position of the testes (retractile testis,
cryptorchidism), varicocele and fever influence spermatogenesis by increasing the gonadal
temperature. Also, hot baths, frequent sauna visits, isolating underwear and occupational
exposure to strong heat sources can induce alterations in spermatogenesis and increase the inci-
dence of male subfertility. Measuring scrotal temperature in men with varicocele before and after
ligation of the spermatic vein supported the view that varicocele-related damage to the testis
results from a lack of adequate cooling (19).

After many years of study, some insight is finally being gained into the mechanism(s), which
may cause the destruction at body temperature. In mice, germ cells show a stress response at an
appreciably lower temperature than somatic cells. Heat shock factor 1, involved in the regulation
of heat shock proteins, is activated in spermatocytes at a threshold temperature of 35º C instead
of 42º C, as in somatic cells.
Increased scrotal temperature is usually a preventable cause of male infertility: varicocele treatment and simple avoidance of exogenous heat exposure can improve semen quality substantially.

**Reactive oxygen species.**

In cells reactive oxygen species (ROS) are generated during oxidation of organic substances. Most cells have the ability to protect themselves against damage by the enzymes superoxide dismutase, catalase and glutathione peroxidase and by antioxidants like taurine, ascorbic acid and tocopherol. The deleterious effect of ROS on semen quality has been documented by numerous investigators and was recently reviewed (20).

There are several reasons why spermatozoa are more vulnerable to ROS than other cells. First, the sperm membrane contains a high level of polyunsaturated fatty acids (PUFAs), which are extremely susceptible to peroxidation. Peroxidative damage may result in a loss of membrane functions and may lead to a reduction of fertilizing ability, motility and viability. Second, in contrast to other cells, spermatozoa have a very limited system to repair damaged structures as a consequence of their small amount of cytoplasm and an inactive, highly condensed chromatin. Third, spermatozoa are equipped with a poor defense system against ROS; while catalase is fully absent, glutathione peroxidase and superoxide dismutase are present in relatively low amounts.

The possible sources of ROS in semen are spermatozoa and leucocytes. Under physiological conditions ROS are mediators of normal sperm function, like capacitation, hyperactivation and the acrosome reaction. High levels of ROS can produce extensive protein damage, cytoskeletal modifications and functional impairment, like reduced motility and sperm-oocyte fusion (21). At the moment it is not clear where in the male or female reproductive tract most damage to sperm is induced by ROS. Our knowledge about ROS-induced damage is mainly based upon studies carried out with ejaculated specimen. These investigations have demonstrated that ROS is generated by leucocytes and by spermatozoa themselves and that in general spermatozoa from oligozoospermic samples generate more ROS than those from normospermic samples.

In spinal cord injured (SCI) patients ROS levels are even higher than in oligozoospermic men and seem to be associated with urogenital infection and the presence of polymorphonuclear neutrophils. Sperm motility is usually very low in ejaculated sperm from SCI patients.

Reduction of oxidative stress can be accomplished by antibiotic treatment, but in patients with asymptomatic leucocytospermia this has usually little effect on the sperm quality. Other options for treatment can be antioxidant supplementation like alpha-tocopherol (Vitamin E) and ascorbic
acid (Vitamin C). Separation of the good spermatozoa from inferior ones and from leucocytes by centrifugation over density gradients and swim-up procedures may help to prevent ROS-induced damage in the ejaculate.

**Genetic abnormalities.**

Although chromosomally derived infertility has long been recognized, the development of intracytoplasmic sperm injection (ICSI) as a method to overcome severe spermatogenic defects has prompted many investigators to further explore the genetic basis of male infertility. In a recent study we confirmed that male infertility is associated with several genetic abnormalities such as constitutive chromosome abnormalities, submicroscopic deletions of the Y chromosome and mutations in the cystic fibrosis gene (22).

The chance of having a chromosomal defect increases with the severity of the spermatogenic defect (23). In a recent study the frequency of karyotypic abnormalities in 1007 males with infertility was found to be as high as 6.5%, of which 63% were sex chromosome aneuploidies (24). From fluorescence *in situ* hybridization (FISH) labelling of sperm chromosomes it was concluded that infertile men carry a high number of abnormal spermatozoa. In case of OAT almost 20% of the spermatozoa were found to be aneuploid. Many of these spermatozoa are the result of meiotic pairing disruption. It has been suggested that these pairing abnormalities in infertile men could lead to meiotic arrest in some cells causing oligozoospermia and aneuploidy in other cells capable of completing spermatogenesis (25).

DNA-analysis of the Y chromosome revealed deletions in a region of the long arm, also known as the iA zoospermia Factor" (AZF) region, in 5-15% of infertile men with oligozoospermia and azoospermia (26). These *de novo* deletions are predominantly found in men with severe oligozoospermia and non-obstructive azoospermia, with normal physical findings and normal levels of gonadotrophins. Histological studies of testicular biopsies from men carrying a AZF deletion exhibit- dependent upon the site of the deletion- a wide spectrum of spermatogenic defects from complete absence of germ cells (Sertoli-cell-only syndrome) to maturation arrest with occasional production of mature, condensed spermatids.

Men with defects of the genital ductal system, like congenital bilateral absence of the vas deferens (CBAVD), were found to carry different mutations of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene (27). CBAVD has recently been described as a genital form of cystic fibrosis (CF). Cystic fibrosis has a wide range of clinical manifestations with pancreatic and pulmonary insufficiency on the severe end of the phenotypic spectrum and infertility due to congenital anomalies of the genital tract on the other end.
It is estimated that 20% of idiopathic male infertility can be explained by genetic abnormalities. The use of ICSI in these men may lead to offspring with an increased risk of an unbalanced chromosome complement, male infertility due to Y-linked transmission of an AZF deletion and a form of cystic fibrosis in case of CF-related genital abnormalities. Unbalanced chromosome complement will lead to either an increased risk for miscarriage or, in case of ongoing pregnancy, to multiple congenital abnormalities and mental retardation. Sex-chromosomal aneuploidy and Y-linked transmission of an AZF deletion will lead to a new generation of infertile men, whose ability to reproduce will be completely dependent on assisted reproductive techniques. The development of microsurgical epididymal sperm aspiration (MESA) and ICSI has greatly improved the chances for CBAVD males to father their own children. However, in these couples there will be an increased risk of having children with CF or CBAVD, particularly when the female also carries a CFTR gene mutation. We conclude that all men with idiopathic oligozoospermia or azoospermia should be offered genetic testing and counselling before assisted reproduction is considered.

Conclusions.
Unexplained male infertility can be the results of environmental toxins causing endocrine disruption, lifestyle factors like stress, immunological factors and genetic abnormalities. The need for further research on the subject of pathophysiology of idiopathic male infertility has increased due to the recent advancements of artificial reproductive techniques such as ICSI.

References:
2. WHO manual for the standardized investigation and diagnosis of the infertile couple. Cambridge University Press, 1993


25. Martin R. The risk of chromosomal abnormalities following ICSI. *Hum Reprod* 1996;11:924-925


CLINICAL ASPECTS OF AZOOSPERMIA.


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Introduction
There has recently been renewed interest in azoospermia, mainly because of new therapeutic options for obstructive azoospermia and some cases of non-obstructive azoospermia.

Intracytoplasmic sperm injection (ICSI), a new In Vitro Fertilization (IVF) technique, has proved to be successful in cases of severe oligospermia, where conventional IVF failed to achieve fertilization (1). Injection of a single sperm directly into the oolemma of the oocyte gives a 60-80% chance of fertilization and a 25% chance of an ongoing pregnancy (Figure 1). More recently this technique has also been applied successfully with epididymal and testicular sperm.

Microsurgical epididymal sperm aspiration (MESA,2) and testicular sperm extraction (TESE,3) are new techniques by which viable spermatozoa can be aspirated for IVF-ICSI procedures. These techniques offer a chance of biological parenthood to couples for whom pregnancy used to be impossible. These techniques also raise questions about the safety of using immature spermatozoa for micromanipulation. Several congenital diseases and genetic disorders, leading to ductal obstruction or testicular failure can be transferred to a subsequent generation by these artificial reproduction techniques (ART). Our knowledge of the genetics and pathophysiology of azoospermia is still limited and more research on this issue is urgently requested. Currently the technical advances are ahead of the basic understanding of the mechanisms that lead to ductal obstruction or testicular failure. Our knowledge of the genetics and pathophysiology of azoospermia is limited and more research on this issue is urgently required.

Classification.
Approximately 85 percent of the couples achieve their desired pregnancy within one year of unprotected intercourse. In the remaining couples another 30-50% pregnancies occur in the following year. Ultimately, 4% of the couples remain childless. Azoospermia and anovulation are common causes in these cases.

Male factors account for at least 50% of all cases of infertility; azoospermia is found in 20%. A classification of azoospermia can be based on obstructive and non-obstructive azoospermia.

Non-obstructive azoospermia can be subdivided in several etiological categories. Table 1 summarizes the most common causes of testicular failure. For the definitive diagnosis of testicular failure a testicular biopsy is needed. This procedure is usually performed only in cases where physical examination and follicle-stimulating hormone (FSH) are normal, when ductal obstruction is suspected and testicular failure has to be ruled out. Since FSH feedback is determined by the function of the sertoli cells, maturation arrest and even some form of germinal aplasia (Sertoli cell only syndrome) can be present with normal FSH levels (4). Testicular failure
is found in a third of the cases of azoospermia with normal testicular size and normal FSH. Simply counting the number of mature spermatids gives the most reliable quantification of the testicular function.

Common causes of testicular insufficiency are cryptorchidism, testicular torsion, viral (mumps) orchitis, drug-induced testicular failure (cytotoxic therapy), irradiation, endocrinopathy and genetic causes, such as the Klinefelter’s syndrome. In at least half of all cases no identifiable cause of testicular failure is found (idiopathic forms).

Since mild forms of maturation arrest can be caused by obstruction and only a few mature spermatozoa are needed for fertilisation with ICSI-MESA and ICSI-TESE procedures, an accurate diagnosis is mandatory in cases of azoospermia with normal FSH levels (5). It should be remembered that maturation arrest and even germinal aplasia can be found next to normal spermatogenesis in the same testis.

Table 1. A classification of non-obstructive azoospermia on the basis of the results of testicular biopsy

<table>
<thead>
<tr>
<th>1 Maturation arrest.</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Idiopathic</td>
</tr>
<tr>
<td>- Cryptorchidism</td>
</tr>
<tr>
<td>- Viral orchitis (mumps)</td>
</tr>
<tr>
<td>- Drugs, cytotoxic therapy</td>
</tr>
<tr>
<td>- Irradiation</td>
</tr>
<tr>
<td>- Systemic illness</td>
</tr>
<tr>
<td>- Environmental toxins</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2 Germinal aplasia (Sertoli cell only syndrome)</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Idiopathic</td>
</tr>
<tr>
<td>- Drugs</td>
</tr>
<tr>
<td>- Irradiation</td>
</tr>
<tr>
<td>- Klinefelter’s syndrome with mosaicism</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>3 Seminiferous tubular sclerosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Klinefelter’s syndrome</td>
</tr>
<tr>
<td>- Other genetic abnormalities</td>
</tr>
<tr>
<td>- Vascular injury, testicular torsion</td>
</tr>
</tbody>
</table>
Figure 1. Intracytoplasmic sperm injection. A single sperm is injected directly into the oolemma (cytoplasm) of a 2PN oocyte.
Table 2. A classification of obstructive azoospermia on basis of ductal obstruction due to congenital and acquired causes

| 1 Epididymal obstruction |  
|--------------------------|--------------------------|
| Congenital forms | - Capital block/ Young’s syndrome |
| Acquired forms | - Post-infective (epididymitis) |
|  | - Post-surgical (epididymal cysts) |

| 2 Vas deferens obstruction |  
|---------------------------|--------------------------|
| Congenital forms | - Congenital absence of the vas deferens |
| Acquired forms | - Post-vasectomy |
|  | - Post-surgical (hernia, scrotal surgery) |

| 3 Ejaculatory duct obstruction |  
|-----------------------------|--------------------------|
| Congenital forms | - Prostatic cysts (Mullerian cysts) |
| Acquired forms | - Post-surgical (bladder neck surgery) |
|  | - Post-infective |

Obstructive azoospermia can be divided in congenital forms and acquired forms of ductal obstruction (Table 2). Obstruction is found in 9% of males with infertility and is frequently caused by epididymal blockage (4.4%), secondary to epididymal infection (6). Congenital blockage of the ductal system occurs less frequently: absence of the vas deferens is found in 2% of infertile males and can be genetically associated with cystic fibrosis (7). Caput epididymal blocks, as in the Young’s syndrome, are rare (8).

**Aetiological factors.**

In at least 50% of the cases the cause of the testicular failure is not identified. Although varicoceles are present in 20-30% of the males with fertility problems, a causal relation with azoospermia is unproven. Varicoceles are usually associated with oligospermia and some form of maturation arrest is found on testicular biopsy. Cryptorchidism accounts for 4.4% of the cases of azoospermia (9). Progressive testicular failure is found in untreated cryptorchidism due to the increased temperature of the extrascrotal position. Furthermore, epididymal abnormalities are present in 40% of patients (10). On testicular biopsy defective spermatogenesis can be found, frequently also in the contralateral testis. It is suggested
that a congenital dystrophy causes the maldescent as well as the impaired spermatogenesis and the increased chance of developing a testicular tumour.

Testicular torsion usually occurs unilaterally. It may affect fertility by an immunological response on both testes. This will lead to subfertility, though azoospermia is rare in these cases. Viral orchitis is found to be an cause of testicular failure in less than 5% of infertile cases. Mumps orchitis is rare in pre-pubertal boys, but is a common complication after puberty, occurring in 37% of cases. The mumps virus directly affects the testis, causing an inflammatory reaction with cell death and tissue necrosis due to raised intratesticular pressure. Testicular biopsy shows mononuclear infiltration and atrophy of the seminiferous tubules. Severe bilateral orchitis may lead to hypergonadotropic hypogonadism.

Several drugs and industrial agents are associated with testicular failure. Cytotoxic therapy for cancer and autoimmune diseases has been reported to cause azoospermia in up to 68% (11). The use of chemotherapy has increased over the last decade, since leukaemias, Hodgkin’s disease, testicular cancers and other malignancies can be treated successfully. This will lead to an increase of the number of infertile men in the future. The effect on spermatogenesis depends on the duration and severity of the specific agents used. Alkylating drugs and combination chemotherapy regimens in high doses will cause permanent testicular failure in more than half of the patients. It should be noted that pre-treatment semen analysis is abnormal in 60% of cases of testicular cancer. Furthermore, retroperitoneal lymph node dissection in testicular cancer may affect seminal emission, causing anejaculation or retrograde ejaculation due to hypogastric nerve damage.

Several drugs may influence spermatogenesis: cyclosporin, sulphasalazine, anabolic steroids, nitrofurantoin, cimetidine, spironolactone, colchicine and allopurinol are known for their gonadotoxic effects. Usually, the effects are reversible after discontinuing medication. The drugs may interfere with the hypothalamic-pituitary-gonadal axis (anabolic steroids, cimetididne, cyclosporin), impair testosterone biosynthesis (spironolactone, sulphasalazine) or have a direct gonadotoxic effect (nitrofurantoin). Sulphasalazine, frequently used as treatment for inflammatory bowel disease, causes abnormal semen parameters in 86% of patients (10).

Exposure to chemical agents and environmental toxins may cause permanent damage to the testis. Dibromochloropropane (DBCP), a widely used pesticide, has been reported to cause azoospermia in 25% of banana workers (12). Several industrial metals, especially lead are associated with decreased fertility (13). More recently, evidence for decreasing quality of semen, due to environmental factors has been associated with fetal exposure to exogenous oestrogens (14). Also, a two-fold increase in genito-urinary malformation (hypospadias,
cryptorchidism, testicular cancer) is reported, possibly related to exposure to oestrogens and oestrogenic chemicals like dioxin. In a meta-analysis of the literature of the past 50 years, Carlsen et al. (15) found a reduction of semen volume and sperm concentration of 50%. In animal studies, a dose-dependent reduction of testicular size and number of Sertoli cells have been observed after exposure to oestrogenic chemicals (16).

Irradiation of the lower abdomen may be responsible for azoospermia. Fast proliferating germ cells are highly radiosensitive. Azoospermia usually results from dosages greater than 50 rad. Recovery may take several years. Pregnancies following radiotherapy or chemotherapy have not shown increased incidence of congenital anomalies in the offspring (17).

Chronic diseases like renal insufficiency and liver cirrhosis are associated with testicular failure. The mechanism is unknown, but hormonal impairment is likely. Several congenital diseases can cause infertility: cystic fibrosis is associated with azoospermia, due to bilateral absence of the vas deferens. Myotonic dystrophy causes tubular atrophy, not affecting Leydig cell function. Von Hippel-Lindau disease is associated with cystic degeneration of the epididymis. Young’s syndrome is characterised by chronic sinopulmonary infections and obstructed azoospermia by epididymal blockage. The immotile cilia or Kartagener’s syndrome is associated with a history of respiratory infections, situs inversus and defective spermatogenesis.

Although much attention is given in the literature to endocrine disorders, they account for less than 5% of cases of male infertility (18). Pituitary disease is the most common cause and should be suspected in cases of hypogonadism, which is often first diagnosed after puberty. Gonadotrophic deficiency associated with normal pituitary function is found in the Kallmann’s syndrome, an infrequent but treatable cause of hypogonadotropic hypogonadism. The syndrome is sometimes associated with multiple congenital anomalies, such as midline defects (cleft palate), deafness, cryptorchidism and renal pathology. Puberty is delayed because of gonadotrophin-releasing hormone (GnRH) deficiency. Other congenital endocrine abnormalities are extremely rare (Prader- Labhart-Willy syndrome, Laurence-Moon-Biedl syndrome, Pasqualini syndrome).

Acquired endocrine disorders mainly concern hyperprolactinaemia, due to pituitary tumors. Adrenal and thyroid abnormalities may occasionally cause testicular failure with maturation arrest.

Obstructive azoospermia can be classified into congenital and acquired forms of ductal obstruction. Congenital forms mainly concern bilateral absence of the vas deferens, capital blockage of the epididymis (Young’s syndrome) and Mullerian duct cysts in the central zone of the prostate. Surprisingly, congenital ductal obstruction is often associated with pulmonary illness, such as cystic fibrosis (absence of the vas deferens) and chronic sinopulmonary infections.
(Young's syndrome, immotile cilia syndrome). Obstruction in these cases occurs from epidi-dymal blockages by inspissated secretions, due to defective electrolytes conductance \( (6,8) \).

Spermatogenesis is usually normal. Congenital absence of the vas deferens can occur unilateral, bilateral and can be partial or complete; the anomaly is found in approximately one of thousand males. Congenital bilateral absence of the vas deferens (CBAVD) is strongly associated with cystic fibrosis. Young’s syndrome, a combination of obstructive azoospermia and chronic sinopulmonary infections, occurs in 3% of the infertile males. Men with this syndrome have mildly impaired respiratory function. Spermatogenesis appears normal, but sperms derived from the caput epididymis have been shown to have a low fertilising capacity, because of their short passage through the epididymis.

Distal ductal obstruction, caused by Mullerian duct cysts are characterized by painful ejaculation with low volume of ejaculate. Oligospermia or azoospermia is usually present. The diagnosis is made by transrectal ultrasound of the prostate and the vesicula seminalis: a midline cyst is seen in the central zone of the prostate, together with a dilatation of the ejaculatory ducts and vesicula seminalis \( (19,20) \). The congenital forms of ejaculatory duct obstruction are sometimes associated with abnormalities and obstructions in the more proximal parts of the ductal system.

Acquired ductal obstructions account for most of the cases of obstructive azoospermia. Infections of the ductal system and surgery of the scrotum and the groin are the most important causes of this type of obstructions. Ductal Infections used to be caused by ascending urinary tract infections, gonorrhoea and tuberculosis. Recently, \textit{Chlamydia trachomatis} has been recognised as an infective agent, causing multiple blocks of the ductal system. The blocks are asymmetrical and most often seen in the corpus and the tail of the epididymis. A history of epididymitis or urethritis is found in many cases. Coexisting vasal blocks are common (Figure 2).

Post-surgical causes of ductal obstruction are nowadays mainly the result of previous vasectomy procedures. Vasectomy reversal is currently performed in 1-2% of the cases and has been shown to be successful in terms of pregnancies in 50-70% of patients. Surgical damage near the vas deferens may cause innervation problems, leading to a functional obstruction. This is most likely to occur in the most proximal part of the vas deferens. Ductal obstructions can also occur from epididymal cysts extirpation, iatrogenic vasal ligation (hernia repair, orchidopexy) and previous bladder neck surgery.
Figure 2. Epididymal obstruction by a caudal epididymal blockage. Vasography should be performed to rule out associated vassal blockage.
Genetic aspects of azoospermia.

It has been recognised for many years that chromosomal abnormalities occur more often in males with azoospermia. Also, several congenital abnormalities can be associated with testicular dysfunction. As mentioned before, pulmonary dysfunction is observed in cases of congenital ductal obstruction.

Chromosomal abnormalities are estimated to be present in 15% of the azoospermic patients (21,22,23). Numerical chromosomal abnormalities are most often associated with Klinefelter's syndrome (47,XXY), resulting from a meiotic non-disjunction of the gametes. The syndrome occurs in 0.2% of the males, but is more prominent in cases of azoospermia (2.7%). A state of hypergonadotrophic hypogonadism is found and azoospermia is usually present, although cases of mosaicism (46)(Y/47xxY) may appear to be subfertile. The syndrome is sometimes associated with mental retardation or various psychiatric disorders. FSH levels are high and on testicular biopsy tubular sclerosis is found. Testosterone levels are low in 40% of the cases and testosterone should be substituted.

Structural chromosomal abnormalities mainly concern translocations and deletions of the Y chromosome and are found in approximately 10% of the azoospermic males (22). Deletions of the long-arm of the Y chromosome are held responsible for some cases of unexplained azoospermia. Microdeletions can only be found by fluorescence in situ hybridisation (FISH) techniques, using Y chromosome specific DNA fragments as probes for detection (21).

Several congenital syndrome's present with testicular failure: the Prader-Willi syndrome is associated with abnormalities of chromosome 15 and demonstrate a state of hypogonadism due to a lack of GnRH. Down syndrome is associated with maturation arrest to germinal aplasia.

Testicular dysfunction is seen in 80% of the cases of myotonic dystrophy. In Von Hippel-Lindau disease cystic degeneration of the epididymis can be found to cause obstruction.

Congenital bilateral absence of the vas deferens (CBAVD) is found in 1-2% of infertile males and is found in almost all male cystic fibrosis patients. A genetic relationship with cystic fibrosis (CF) has recently been suggested (25.26). The CF gene, the cystic fibrosis transmembrane regulator (CFTR) gene, is localised on the long arm of chromosome 7 and encodes for a large protein (CFTR, 250,000 base pairs), which is situated in the cell membrane of epithelial tissue. CFTR is responsible for the electrolyte transport through the cell membrane and protein dysfunction may cause deficient chloride excretion. This will result in a thick mucus with obstruction of epithelial glands of the pancreas (pancreatic insufficiency with malabsorption) and recurrent pulmonary infections due to highly viscous mucus. Since the discovery of the CFTR gene mild forms of CF have been found with a deficient, but not absent chloride excretion and
are associated with a better life expectancy than the severe cases of CF (26). The disease is inherited in an autosomal recessive manner; two $CFTR$ gene mutations are required for the development of a CF phenotype. One gene is inherited from the mother, one from the father. The carrier risk for CF shows significant racial variance. In most western countries the carrier risk for (one) $CFTR$ gene mutation is $1:25$. $CFTR$ gene mutations are found in 87% of CF patients and in 78% of CBAVD patients. The delta F508 mutation is most prominent in CF, but already more than 500 different mutations have been found, most of them appearing as a point mutation, found in a single patient. The risk of inheritance of CF or CBAVD is mainly determined by the partner’s genetic investigation: if she carries a $CFTR$ gene mutation ($1:25$) there is a 50 percent chance of a form of CF (severe, mild, CBAVD) in the offspring.

**Treatment of azoospermia.**

Obstructive azoospermia, caused by epididymal and vasal blocks and prostatic cysts can be successfully treated by (micro)surgery. Vasectomy reversal is a common microsurgical procedure and gives patency in 80-90% and pregnancies in more than half of the cases (27,28). The interval between the vasectomy and the vaso-vasostomy is of major prognostic significance: after an interval of more than 10 years pregnancies occur in less than 20%. Fibrosis of the epididymis, due to a prolonged obstruction, is the main reason for failed vasectomy reversal. Antisperm antibodies occur in most cases after vasectomy and may play an important role in persistent infertility, despite successful reversal. We have performed over 260 microsurgical vasectomy reversal procedures: patency was obtained in 86% and pregnancies in 51%. Second reversal after failed first procedure showed pregnancy in only 27% of the cases (unpublished results).

Epididymal blocks can be treated by vaso-epididymostomy in cases of normal testicular function and patent vas deferens. Testicular biopsy and vasography should be performed prior to surgery. In one-third of the scrotal explorations an empty epididymis is found, despite normal testicular biopsy. Multiple blocks, caused by a *Chlamydia trachomatis* infection are difficult to treat. Patency is reached in 36-78% of cases and pregnancies are reported in 21-56% after vaso-tubulostomy (29). Pregnancies after microsurgery for capital blocks occur in less than 30%, because of poor fertilisation capacity of the spermatozoa after only short passage through the epididymis (6).
Azoospermia

FSH

Normal Elevated

Testicular biopsy Testicular failure /
no treatment options

Normal Defective spermatogenesis /
TESE/ICSI

Semen volume/pH

Low Normal

TRUS CBAVD Scrotal exploration

MESA/ICSI

Vasography

Ejaculatory Duct obstruction Vasal Block(s) Epididymal Block(s)

TUR MESA/ICSI MESA/ICSI

Vaso-vasostomy MESA/ICSI Vaso-epididymostomy MESA/ICSI

CBAVD = Congenital Bilateral Absence of the Vas Deferens
ICSI = Intracytoplasmic sperm injection
MESA = Microsurgical epididymal sperm aspiration
TESE = Testicular sperm extraction
TRUS = Transrectal ultrasound
TUR = Transurethral resection

Figure 3. Algorithm of the diagnostic work-up of azoospermia
Figure 4. Transrectal ultrasound images, showing a midline prostatic cyst in the central zone of the prostate (ejaculatory duct cyst) and dilated seminal vesicles.
Distal ductal obstructions are caused by prostatic cysts or post-inflammatory obstruction of the ejaculatory ducts. A low-volume ejaculate is usually found. The diagnose is made by transrectal ultrasound, showing dilatation of the vesicula seminalis and ejaculatory ducts. Trans-urethral loop resection with unroofing of the cyst and opening of the ejaculatory ducts can be successful in restoring fertility in selective cases (19,20).

The congenital absence of the vas deferens cannot be treated by microsurgery. Pregnancy can only be reached by aspiration of viable sperms from the epididymis (MESA) for IVF-ICSI procedures. The results of MESA-IVF procedures, however, are disappointing. Ongoing pregnancies are reported in less than 10 percent. Decreased sperm motility and fertilisation capacity is the main reason for these poor results (5). Intracytoplasmic sperm injection, however, has been shown to give high number of fertilisation rates with ejaculated, epididymal, frozen-thawed and testicular sperms (1,3,5). Ongoing pregnancy is observed in 20-30% per cycle. The implantation rate after ICSI is 40-50%, but pregnancy loss occurs in at least 26%.

Non-obstruction is found in 80% of cases of azoospermia and used to be a non-treatable condition. Limited results are available from ICSI procedures with testicular biopsy sperms. They appear to fertilize normal in 84% and (early) pregnancies are obtained in almost 40% (3). In the near future some cases of non-obstructive azoospermia will appear to be treatable by TESE-ICSI procedures.

In the series of the Free University of Brussels, 130 children born after ICSI were compared to the follow-up of children born after IVF. No significant differences in minor and major malformations were found. Most of the children were born after ICSI procedures with ejaculated sperms, the data of the offspring from azoospermic patients are not yet available. Numerical chromosomal abnormalities were found in 1.3% (0.5% in fertile couples) and major malformations were found in 3.5% (23). A longer follow-up of the children is, however, required to establish the true incidence of complications, since several abnormalities may appear later in life (CF, inborn errors of metabolism, pulmonary dysfunction, infertility). Genetic screening is advised by pre-implantation diagnosis or pre-natal screening. A close cooperation with a genetic institute is therefore mandatory for safe ICSI procedures.

References.


DIAGNOSIS
SUBTOTAL OBSTRUCTION OF THE MALE REPRODUCTIVE TRACT.
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Submitted to The Journal of Urology
ABSTRACT.

Bilateral obstruction of the male reproductive tract is suspected in men with azoospermia, normal testicular volume and normal FSH. A testicular biopsy is required to differentiate between obstruction and a testicular insufficiency. Unilateral or subtotal bilateral obstructions and epididymal dysfunction may cause severe oligozoospermia in men with a normal intra-testicular spermatogenesis. However, information on spermatogenesis in oligozoospermic men is lacking, since testicular biopsy is not routinely performed.

Men with a sperm concentration of $< 1 \times 10^6$ spermatozoa/ml were investigated for possible partial obstruction, by performing a testicular biopsy under local anaesthesia. Spermatogenesis was determined by the Johnsen scoring method. A testicular biopsy was performed in 78 men with severe oligozoospermia. The medical history showed male accessory gland infection in 12.8%, previous hernia repair in 14.1%, and a history of cryptorchidism in 12.8%. A normal or slightly disturbed spermatogenesis (Johnsen score $>8$) was present in 39/78 (50%) men. Hernia repair occurred more often in men with a normal spermatogenesis. A varicocele was predominantly seen in men with a disturbed spermatogenesis. FSH was significantly lower ($p<0.0001$) in men with normal spermatogenesis. Microsurgical repair of the seminal tract was performed in 9 men and resulted in significantly improved semen quality in 4 and spontaneous pregnancy in 2 couples. Alternatively, intracytoplasmic sperm injection (ICSI) was performed in 24 couples and resulted in 4 (16.6%) ongoing pregnancies.

Subtotal obstruction of the male reproductive tract is a frequent cause of severe oligozoospermia in men with a normal testicular volume and a normal FSH. In other cases an epididymal dysfunction might explain the oligozoospermia in men with a normal testicular biopsy score. Microsurgical repair and epididymal sperm aspiration for cryopreservation should be considered in men with severe oligozoospermia and normal spermatogenesis.

KEY WORDS: SUBTOTAL OBSTRUCTION - MALE GENITAL TRACT - MALE INFERTILITY - OLIGOZOOOSPERMIA.
INTRODUCTION

Partial obstruction of the male reproductive tract is defined as the presence of oligozoospermia with normal or nearly normal spermatogenesis (1). This diagnosis can be suspected in men with severe oligozoospermia, normal physical findings and normal serum follicle-stimulating hormone (FSH). Indicative for a subtotal obstruction is a history of inguinal or scrotal surgery and recurrent genital infections. Other features that suggest obstruction are low seminal volume, epididymal congestion, enlarged seminal vesicles and cystic lesions of the epididymis and prostate.

For the definite diagnosis of obstruction a testicular biopsy is required, confirming the presence of mature spermatids and spermatozoa in the seminiferous tubules (2,3). Because of the invasive nature of this procedure, the limited results of surgical treatment of obstructions of the seminal path and alternative options like assisted reproductive treatments, testicular biopsies are not routinely performed in men with oligozoospermia.

However, in selective cases the state of spermatogenesis could be assessed, both for estimation of genetic risks factors specifically related to obstructive male infertility (4) and to determine the possibility of performing a microsurgical vasoepididymostomy together with an epididymal sperm aspiration and cryopreservation.

In men with progressive deterioration of the semen quality and in men with a low semen volume an obstruction of the ejaculatory ducts can be found on transrectal ultrasound of the prostate (5). Both surgical incision of cystic lesions in the prostate and aspiration of spermatozoa from the seminal vesicles are treatment options that may require information on the state of the spermatogenesis in advance, since obstructions of the epididymis frequently coincide with obstructions of the ejaculatory ducts (6).

To determine the true incidence of subtotal obstruction of the male reproductive tract in oligozoospermia and to offer the option of surgical exploration and microsurgical reconstruction we have performed a testicular biopsy in 78 patients with < 1 million spermatozoa/ml and analysed the results of the testicular histology, in relation to the medical history, the physical findings and the FSH. Also, the results of microsurgical repair of the seminal tract in men with normal spermatogenesis and the results of ICSI were determined in these patients.

PATIENTS AND METHODS

All men participating in the study were informed about the scientific goals and therapeutic options related to the performance of the testicular biopsy. Also, alternative treatment modalities like ICSI and donor insemination were discussed and offered, where appropriate.
The andrological work-up was performed according to the WHO manual for the investigation and diagnosis of the infertile couple (7). Semen analysis was performed twice according to WHO laboratory manual for the examination of human semen (8). FSH was determined at intake. In men with a consistently low seminal volume or a history of male accessory gland infection transrectal ultrasound investigation of the prostate and the seminal vesicles was performed. Scrotal ultrasound was performed routinely to detect intrascrotal abnormalities that go undetected during physical examination (9).

A bilateral excisional testicular biopsy was performed under local anaesthesia. The Johnsen score was determined of the left and right testis (3). In men with a normal biopsy score, scrotal exploration and microsurgical repair was discussed. In men with an obstruction of the ejaculatory ducts a transurethral incision of the ducts was offered. Alternatively, couples could choose for ICSI if motile sperm were present in the ejaculate. The results of these treatments were evaluated by patient charts readings and questionnaires.

Statistical analysis of the data was performed using the non-parametric Student's test, the Wilcoxon Rank test and Spearman's correlation procedure, where appropriate. Two-sided p-values less than 0.05 were considered significant.

RESULTS
In 78 men with less than 1 million spermatozoa/ml in both analyses a testicular biopsy was performed. Table 1 shows the results of the clinical and ultrasound investigation. A history of inguinal or scrotal surgery was present in 21 patients (26.9%), including 10 men with a history of cryptorchidism and orchidopexia (12.8%).

| TABLE 1: Male infertility associated factors in 78 men with severe oligozoospermia. |
|--------------------------------------|----------------|----------------|
| Childhood hernia repair             | 8              | 3              |
| Cryptorchidism/orchidopexia         | 5              | 5              |
| Male accessory gland infection      | 5              | 5              |
| Malignancy                          | 3              | 4              |
| Chronic disease                     | 0              | 3              |
| Varicocele                           | 1              | 8              |
| Total                                | 22             | 28             |
| Total (%)                            | 50/78 (64.1%)  |                |
Male accessory gland infection was present in 10 men (12.8%), a previous testicular tumour in 4 (5.1%). The mean infertility duration was 2.9 years. Transrectal ultrasound was performed in 10 patients and showed abnormal in 6: 4 patients showed a cystic lesion in the prostate midline, 2 showed a dilatation of the seminal vesicles. Scrotal ultrasound was performed 66 men and showed abnormalities in 10 men, including 9 cases of varicocele and 1 testicular tumour. In men with a normal spermatogenesis only 1 varicocele was detected by ultrasound versus 8 varicoceles in men with impaired spermatogenesis.

A normal Johnsen score (>9) was found in 14 men, a slightly disturbed spermatogenesis (Johnsen score 8-9) in another 25 patients. Thus, a total of 39/78 (50%) patients showed a complete or nearly complete spermatogenesis. FSH was normal in 57 men of which 38 (66.6%) had a normal spermatogenesis. FSH was significantly different in men with an intact spermatogenesis as compared to men with impaired spermatogenesis (P<0.0001, Wilcoxon Rank test). Testicular volume was significantly lower in men with an abnormal spermatogenesis (mean 13.6 cc) as compared to the testis volume of men with normal spermatogenesis (mean 16.7 cc, p = 0.01, Student's test).

Trans-urethral roof resection of the midline prostatic cyst was performed in 2 patients and resulted in improved semen quality in 1 man, but not in a spontaneous pregnancy. A microsurgical repair of the seminal tract (vasoepididymostomy) was performed in 9 men and resulted in significantly improved semen quality in 4 and spontaneous pregnancy in 2 couples. Alternatively, intracytoplasmic sperm injection (ICSI) was performed in 24 couples and resulted in 4 (16.6%) ongoing pregnancies.

Table 2. Results of treatment in 78 men with severe oligozoospermia.

<table>
<thead>
<tr>
<th></th>
<th>Vaso-epididymostomy</th>
<th>ICSI</th>
<th>Midline prostatic cyst</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>9</td>
<td>24</td>
<td>2</td>
</tr>
<tr>
<td>Obstruction</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No obstruction</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improved sperm quality</td>
<td>4</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>2</td>
<td>4 (16%)</td>
<td>0</td>
</tr>
</tbody>
</table>
DISCUSSION

Obstructions of the male reproductive tract are usually associated with azoospermia, normal physical findings and normal FSH. A testicular biopsy is required to confirm the diagnosis of obstruction before scrotal exploration is performed. Although obstructions in primary infertile men are commonly present at the epididymal level, other sides of obstruction are the ejaculatory ducts and the vas deferens. In 20% of men with a suspected obstruction no spermatozoa are found in the epididymis during scrotal exploration, indicating that there is an intratubular obstruction. Berardinucci et al. reported that anatomical abnormalities are often found in men with suspected epididymal obstruction (6). In a series of 147 men evaluation of the reproductive tract resulted in 52.7% epididymal obstructions and 47.3% other anatomical abnormalities, like vasal obstruction and aplasia, epididymal atresia and intra-testicular obstructions. Jarvi et al. detected abnormalities of the ejaculatory ducts in 33% of men with suspected epididymal obstruction (10). These abnormalities were associated with a poor outcome for vasoepididymostomy.

Different classification systems for testicular biopsies have been proposed to objectively quantify the presence of a ductal obstruction (2, 3, 11). The mean number of mature spermatids per tubule correlated closely to the diagnosis of obstruction. In men with severe oligozoospermia Silber et al. found that the presence of more than 20 mature spermatids per tubule indicated partial obstruction of the epididymis.

Unilateral or partial obstruction of the male genital tract may be the result of childhood hernia repair: Matsuda et al. investigated subfertile men with a history of hernia repair and found a unilateral obstruction to be present in 26.7% (12). A vasovasostomy procedure was performed in 10 men and resulted in improvement of the semen quality in 5 cases and in pregnancy in 2 patients. Obstructive oligozoospermia was present in 61% of 60 patients, investigated by testicular biopsy (1). Normal spermatogenesis was found in 70.2% of men with a history of cryptorchidism. They concluded that in all men with oligozoospermia it is necessary to exclude a partial obstruction of the seminal path. The most frequent cause for this obstruction was epididymitis, which caused progressive deterioration of the spermiogram.

We examined a subset of infertile men with less than 1 million spermatozoa/ml for possible obstruction of the male genital tract. Normal or nearly normal spermatogenesis appeared to be present in 50% of the patients. A history of inguinal and genital surgery and male accessory gland infection was present in 18/78 (23%) of the men with an obstruction. Furthermore, in 6 men signs of an obstruction of the ejaculatory ducts were found on trans-rectal ultrasound investigations. A normal FSH was found in 57/78 men with severe oligozoospermia and 38
(66.6%) showed to have an intact spermatogenesis. Microsurgical repair of the epididymal obstruction could be performed in 9 men and was successful in 4 (44.4%).

We conclude that obstructions of the male reproductive tract are highly prevalent in men with severe oligozoospermia, normal testis volume and a normal FSH. A testicular biopsy and microsurgical repair of the seminal path should be considered in these men.

REFERENCES

THE COMPLEX RELATIONSHIPS BETWEEN CYSTIC FIBROSIS AND CONGENITAL BILATERAL ABSENCE OF THE VAS DEFERENS: CLINICAL, ELECTROPHYSIOLOGICAL AND GENETIC DATA.

Department of andrology(1), paediatrics(2), pulmonary diseases(3), clinical genetics(4) and urology(5), University Hospital Dijkzigt and Erasmus University, Rotterdam, The Netherlands.

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Abstract
Congenital bilateral absence of the vas deferens (CBAVD) is found in 1-2% of the infertile males and in most male cystic fibrosis (CF) patients. CF and part of the CBAVD cases were found to share the same genetic background.

In this study 21 males with CBAVD had extensive physical and laboratory testing for symptoms of CF. Possible defective cellular chloride transport was measured by Interstitial current measurements (ICM) of rectal suction biopsies. Cystic fibrosis transmembrane conductance regulator (CFTR) gene mutation analysis was performed for 10 common CFTR-mutations.

CF-related symptoms were found in six men. On laboratory testing slightly abnormal liver and pancreatic function were found in seven patients. The sweat was found abnormal in four patients. ICM showed defective chloride excretion in 11 patients. CFTR-gene mutations were found in 66% of the patients: eight were compound heterozygotes; in six only one common mutation could be detected. The 5T allele in one copy of intron 8 was found in four men.

CBAVD appears to be a heterogeneous clinical and genetic condition. A CFTR-gene mutation was found in both copies of the allele or ICM showed defective chloride excretion in 14/21 cases. Genetic counselling is clearly indicated for couples seeking pregnancy through epididymal or testicular sperm aspiration and intracytoplasmic sperm injection.

Key words: Congenital bilateral absence of the vas deferens / Cystic Fibrosis / Clinical Symptoms / CFTR-gene analysis / Intestinal current measurements.

Running Title: Congenital bilateral absence of the vas deferens and cystic fibrosis.
Introduction
Congenital bilateral absence of the vas deferens (CBAVD), due to bilateral regression of the mesonephric duct, occurs in 1-2% of infertile males and in 6% of azoospermic men (Oates and Amos, 1994). Clinical symptoms of CBAVD are bilateral non-palpable vas deferens, absence of the distal part of the epididymis and hypoplasia of the vesicula seminalis. Azoospermia with low semen plasma volume (<1.5 ml) and low pH (<7.5) is consistently found. Testis volume and serum gonadotrophins are usually normal. Testicular biopsy shows normal or slightly defective spermatogenesis. Viable sperms can be harvested from the epididymis by micropuncture (MESA) and pregnancy can be obtained by in vitro fertilisation (IVF) or intracytoplasmic sperm injection (ICSI, Silber et al., 1994).

The aetiology of CBAVD is unknown. Most male cystic fibrosis (CF) patients have CBAVD and it was suggested that CBAVD represents an incomplete form of CF (Holsclaw et al., 1971). Since the identification of the cystic fibrosis transmembrane regulator (CFTR) gene (Riordan et al., 1989), mutations have been found in 62% of cases of CBAVD (Patricio et al., 1993; Mercier et al., 1993; De Brackeleer and Ferec, 1996).

CBAVD patients have been reported to carry two, one or no CFTR-gene mutations, one of them being 'F508, the most frequent CF mutation. Recently the R117H mutation, a rare mutation in CF patients, was found to occur frequently in CBAVD patients (Gervais et al., 1993). Furthermore, the 5T variant of the polypyrimidine stretch in intron 8, which is thought to influence splicing was shown to occur more frequently in CBAVD as compared to controls (Chillon et al., 1995; Chu et al., 1993). In cases where CBAVD is associated with urogenital malformation CFTR-gene mutations appear to be absent, suggesting a different aetiology (Dumur et al., 1996).

The aim of this study was to investigate whether patients with CBAVD have other CF related non-genital manifestations, and if so, how to improve genetic counselling in case of demand for MESA/ICSI treatment.

Materials and methods

Patients
This study was approved by the Hospital Medical Ethical committee and by patients by written informed consent. Twenty-one infertile patients with obstructive azoospermia due to bilateral absence of the scrotal vas deferens were enrolled in the study. The diagnosis was made by physical examination and confirmed by demonstration of azoospermia with low semen plasma volume and low pH.
Figure 1. Interstitial current measurements of a rectal biopsy.

Pos = positive, Neg = negative, I = CF response, II = low residual chloride secretion, III = high residual chloride secretion.
A medical history was obtained focused on symptoms common in CF, such as rhino-sinusitis, nasal polyps, obstructive lung disease, recurrent pulmonary infections, gastro-intestinal malabsorption, fat intolerance, oily stools, cholelithiasis, liver dysfunction and intestinal obstruction. Family history was documented for CF and other genetic abnormalities.

**Physical examination**

Physical examination included urogenital and pulmonary investigation and measurement of weight, height and nutritional state. Pulmonary function tests included forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV₁). A chest X-ray was performed. The Shwachman score was determined for all patients (Shwachman, 1990). Sonography of the kidneys and transrectal ultrasound was performed to detect urogenital malformations.

**Laboratory testing**

Laboratory testing included bacterial cultures in sputum, measurements of gonadotrophins (Luteinizing hormone (LH) and follicle-stimulating hormone (FSH)), liver function, serum glucose level and faecal chymotrypsin. Bilateral Sweat tests were performed and, in the absence of normal values for adults, judged as abnormal if chloride > 50 mmol/l.

**Electrophysiological study**

Interstitial current measurements (ICM) on rectal tissue were performed (Veeze et al., 1994). Rectal suction biopsies were mounted in an Ussing chamber with an exposed area of 1.13 mm². Sodium channels were blocked by adding amiloride (10⁻⁴ mol/l). Endogenous prostaglandin synthesis that is possibly linked to cAMP-mediated chloride secretion was inhibited by adding indomethacin (10⁻⁵ mol/l). Carbachol (10⁻⁴ mol/l) was added for cholinergic activation of chloride secretion.

In healthy controls, a carbachol-provoked change in ICM values was found (Control, Fig 1). The inward current in controls reflects transcellular chloride transport (serosa to mucosa) through the Na-K-Cl co-transporter in the basolateral membrane and the CFTR-chloride channel in the apical membrane. In the majority of CF patients a carbachol induced outward current response (Type I negative, fig 1) occurs. This reversed response probably results from apical potassium secretion that is unmasked in the case of absent or largely reduced chloride secretion.

In a subclass of CF patients, usually after a negative peak response, a separate and small positive peak response was observed, the result of low residual chloride secretion (Type II, fig 1). In another subset of cases a high residual chloride secretion was seen (type III, fig 1), due to chloride excretion by non-CFTR channels. The amount of residual chloride secretion

58
appeared to correlate with preserved pancreatic function and delayed presentation of the
disease, which was not exclusively determined by the CF genotype (Veeze et al., 1994).
ICM is especially indicated in individuals with borderline or high normal sweat test results
and an inconclusive CFTR mutation analysis, who can otherwise not be distinguished from
CF-carriers.
DNA analysis
DNA was isolated from peripheral leucocytes. CFTR mutation analysis was performed for 10
mutations: we analyzed for the mutations R117H, A455E, *F508, 1717-1G->A, G542X,
R553X, R1162X, S1251N, W1282X, and N1303K. The length of the T-stretch in intron 8
was determined (Kiesewetter et al., 1993). Only the allele specific oligonucleotide for the
identification of the 9 T-stretch was changed into: 5'- TGTGTG TTT TTT TTT AAC AG-3',
using a hybridization temperature of 37°C for all allele specific oligonucleotides.

Results
Table I summarizes the abnormal physical and laboratory findings. The history revealed nasal
polyps/rhino-sinusitis (n=3), obstructive lung disease (n=1) and fatty stools (n=2). Two
patients had a positive family history for CF. Pulmonary function was abnormal in 1 case with
a history of pertussis in childhood: on chest X-ray atelectasis and bronchiectasis were found.
The Shwachman score was abnormal in one (80) and borderline (95) in another case.
High gamma GT, not related to alcohol consumption was found in 6 cases. Faecal
chymotrypsin was low in 4 cases, indicating exocrine pancreatic dysfunction. In 4 cases the
sweat test was borderline by using strict criteria.
ICM showed either a typical CF-response (fig 1, type I) (n=4), a low residual chloride
excretion (fig 1, type II)(n=1) or a high residual secretion (fig 1, type III)(n=6). The test was
found inconclusive in 1 case and normal (fig 1, control) in 10 patients.
CFTR-gene analysis showed 1 or 2 mutations in 14/21 cases. In 8 patients two different
mutations (compound heterozygosity) were found, in 6 patients only 1 mutation could be
identified. In 7 cases no common CFTR-gene mutation could be detected: 4/7 of these were
non-Caucasians. A 5T allele in one copy of the CFTR gene was found in 4 cases, 3 times in
combination with a mutation in the other allele.
The *F508 mutation was found in 8 patients, R117H in 6 , A455E in 3 and 1717-1G->A and
R553X both in one. Three partners were found to have a single CFTR-gene mutation (R117H,
<table>
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<th>Case no.</th>
<th>Age (years)</th>
<th>History tests</th>
<th>Laboratory tests</th>
<th>Sweat test</th>
<th>ICM</th>
<th>CFTR mutations</th>
<th>T-stretch intron 8</th>
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<td>ΔF508/R117H</td>
<td>9/7</td>
<td>CF in family</td>
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<td>33</td>
<td>CARA/oily stools</td>
<td>Ggt&gt;, Chymotr. &lt; 23/36 CF response (I)</td>
<td>ΔF508/-</td>
<td>9/7</td>
<td></td>
<td></td>
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<td>4</td>
<td>31</td>
<td>Pelvic re kidney</td>
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<td>7/7</td>
<td></td>
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<tr>
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</table>

NA = no abnormalities, GgT = gamma glutamyl transpeptidase, Chymotr. = chymotrypsin, ICM = interstitial current measurement (see Figure 1), CFTR = cystic fibrosis transmembrane conductance regulator gene, /- none of 10 CF mutations, T-stretch = polypyrimidine (T) stretch intron 8.

Shwachman score was 95 in case 1 and 80 in case 8. Physical examination showed bronchiectasis in case 8 and hypogonadism in case 19.
Discussion

The observation that the vas deferens is absent in almost all male CF patients suggested that CBAVD is a primary genital form of CF (Holsclaw et al., 1971). Since the identification of the CFTR-gene, CBAVD and CF were often found to share the same genetic background (Patricio et al., 1993; Mercier et al., 1993). CFTR, the product of the CFTR-gene, a cell membrane protein of 1480 amino-acids regulates transmembrane chloride transport. Over 750 mutations of the CFTR-gene have been reported, *F508 being the most frequent mutation in CF patients. CF is an autosomal recessive disease; a patient with CF receives two defective alleles. The carrier risk in caucasians is 1:25.

In CF conductive chloride transport is defective in epithelial tissues, resulting in viscous secretions associated with pulmonary infections, malabsorption and intestinal obstruction. The severity of the disease varies widely: homozygosity for the *F508 mutation was found to correlate with pancreatic insufficiency, early manifestations, poor lung function and high mortality (Kerem et al., 1990). Other mutations, like R117H, are associated with a milder form of CF where conductive chloride transport is defective, but not absent (Gervais et al., 1993).

The CFTR-gene mutations occur frequently in CBAVD (Oates and Amos, 1994; Patricio et al., 1993; de Braekeleer et al., 1996), but the molecular basis of CBAVD is not completely understood. Mutations with a low frequency in classic CF, like R117H, were found to occur regularly in CBAVD (Gervais et al., 1993). Homozygosity for *F508 or compound heterozygosity for 2 severe mutations was not found in cases of CBAVD. It has been suggested that CBAVD patients are compound heterozygotes for a severe mutation on one allele in combination with a mild CFTR gene mutation on the other allele. In the majority of cases, however, only one CFTR-gene mutation could be detected in CBAVD. Recently alterations in the non-coding regions of the gene, such as the polypyrimidine stretch in intron 8, in combination with a mutation in the other allele, were found to cause abnormal levels of CFTR-protein (Chu et al., 1993). Impaired CFTR-protein function may cause defective, but not absent chloride excretion resulting in absence of the vas deferens, but not in pulmonary or pancreatic insufficiency (Anguiano et al., 1992). The epididymis may be more susceptible to defective chloride transport, resulting in an early regression of the mesonephric duct. In contrast, only 6% of CFTR-protein function is necessary for normal pancreatic function (Tizzano et al., 1994). Also, the wide variability of symptoms related to various combinations of CFTR-mutations suggests a possible role for unlinked genetic factors in the expression of these mutations.
In this study 21 patients with CBAVD were investigated for non-genital manifestations of CF: in 6 patients mild CF symptoms were present. Slightly abnormal liver and pancreatic function were detected in 7, sweat tests showed high levels of chloride in 4 patients. Electrophysiology of rectal suction biopsies, not previously performed in CBAVD, showed defective chloride excretion in 11 patients. Three of these patients showed very low sweat test results, indicating different tissue expression of impaired CFTR function.

CBAVD appears to be a heterogenous clinical and genetic condition: 2 CFTR gene mutations were detected in 8 patients, 5 of them showing CF characteristics on ICM. In these men the CBAVD might represent a mild form of CF. Of the patients carrying a single CFTR mutation 4 also showed defective chloride excretion on ICM, suggesting mutations going undetected with the current screening technology. So far no convincing evidence has been brought forward that the presence of a single CFTR mutation (i.e. simple heterozygosity) has any phenotypic consequences (Meschede et al., 1997). Therefore, in case of CBAVD and defective chloride excretion further analysis of the CFTR gene is required to detect rare variant mutations.

In most cases of CBAVD residual or normal chloride excretion was found in combination with either an abnormal sweat test or CFTR-gene mutations. Only in 5 cases of CBAVD no abnormalities could be found, 4 of these men being non-caucasians. These results suggest that there is a wide spectrum of phenotypical expressions of cystic fibrosis, with pancreatic and pulmonary insufficiency on the one end and CBAVD on the other end of the spectrum.

Since the introduction of microsurgical epididymal sperm aspiration and intracytoplasmic sperm injection (Silber et al., 1994) infertility due to CBAVD was treated successfully resulting in ongoing pregnancy. Biological parenthood is now a realistic option for males with CBAVD, producing ongoing pregnancies in 20 - 30% of cases (Dohle et al., 1998).

For couples with CBAVD-related infertility CFTR mutation analysis and genetic counselling of the patient and his partner is essential before MESA/ICSI procedures are performed (Pauer et al., 1997). Although the apriori carrier risk for a CFTR-gene mutations is only 3-4%, three partners of the male CBAVD group had a single CFTR-gene mutation. In these cases the risk of offspring with a (mild or severe) form of CF could be 50%.

As there is no straightforward relation between the genotype and the phenotype for most CFTR-gene mutations, genetic counselling in these situations is complex, as no precise predictions on rare compound phenotypes of CF are possible. Considering all the medical and psychological burdens of MESA/ICSI procedures, followed up by chorionic biopsy for early prenatal diagnosis of CF, reproduction becomes complicated for these couples. Pre-implan-
tation screening of embryos would be an alternative technique for prenatal diagnosis, but does not solve all ethical problems. In case of a CFTR mutation in the partner and no detectable mutation in the CBAVD male, a positive ICM test in the patient will indicate rare variant alleles of the CF gene. However, if no CFTR-gene mutations are found in the female partner, the risk of offspring with CF is at the most 1:400. Pre-natal or pre-implantation screening for CF is not possible in these cases.

In conclusion, CBAVD appears to be a heterogeneous condition with respect to CF symptoms, tissue expression of defective chloride excretion and CFTR gene mutation analysis. Only in a small subset of men with CBAVD no abnormalities could be detected.

References


GENETIC RISK FACTORS IN INFERTILE MEN WITH SEVERE OLI GOZOOSPERMIA AND AZOOSPERMIA


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Abstract

Male infertility due to severe oligozoospermia and azoospermia has been associated with a number of genetic risk factors. In this study 150 men from couples who applied for intracytoplasmic sperm injection (ICSI) were investigated for genetic abnormalities, such as constitutive chromosome abnormalities, microdeletions of the Y chromosome (AZF region) and mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. A male infertility associated factor was found in 63/150 (42%) men, including a history of cryptorchidism (13.3%), varicocele (10.0%), male accessory gland infection (5.3%) and congenital vas deferens abnormality (4.0%). Genetic analysis identified 16/150 (10.6%) abnormal karyotypes, 8/150 (5.3%) AZFc deletions and 14/150 (9.3%) CFTR gene mutations. An abnormal karyotype was found both in men with oligozoospermia and azoospermia: 9 men had a sex-chromosomal aneuploidy, 6 translocations were identified and one marker chromosome was found. Six Klinefelter’s patients (47,XXY) showed hypogonadism and azoospermia or occasionally a non-motile spermatozoa in the ejaculate. FSH was high in all Klinefelter patients. In patients with a translocation no specific abnormalities were found on andrological evaluation: all men showed oligozoospermia and a normal or slightly elevated FSH.

Y chromosomal microdeletions were mainly associated with male infertility, due to testicular insufficiency. All deletions identified comprised the AZFc region, containing the Deleted in Azoospermia (DAZ) gene. CFTR gene mutations were commonly seen in men with congenital absence of the vas deferens, but also in 16% of men with azoospermia without any apparent abnormality of the vas deferens.

A genetic abnormality was identified in 36/150 (24%) men with extreme oligozoospermia and azoospermia. Excluding those with a congenital abnormality of the vas deferens (N=6) a genetic risk factor was found in 20/113 (17.6%) men with oligozoospermia and in 11/32 (34%) men with azoospermia. Two men showed to have 2 genetic risk factors: 1 Klinefelter patient also carried a CFTR gene mutation (R117H), another man was found to have both an AZFc deletion and a CFTR gene mutation ("F508). Application of ICSI in these couples can result in offspring with an enhanced risk for unbalanced chromosome complement, male infertility due to the transmission of a Y-chromosomal microdeletion, and cystic fibrosis if both partners are CFTR gene mutation carriers. Genetic testing and counselling is clearly indicated in these couples before ICSI is considered.
Key Words: Chromosomal abnormalities / CFTR gene mutations / ICSI / Male infertility/ Y chromosome microdeletions.

Introduction
Male infertility has been associated with several genetic and non-genetic conditions, like hypogonadotrophic hypogonadism, testicular maldescent, structural abnormalities of the male genital tract, genital infections, previous scrotal or inguinal surgery, varicoceles, chronic illness, medication and exposure to chemicals. In at least 40% of men no cause of the infertility was found (Unexplained infertility, E.S.R.H.E., 1992). Genetic abnormalities were identified in men with unexplained oligozoospermia and azoospermia, including numerical and structural chromosomal abnormalities (Chandley et al., 1998), deletions of the Azoospermia factor region of the Y chromosome (AZF, Vogt et al., 1996, Reijo et al., 1995) and mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, commonly associated with congenital vas deferens abnormalities (Jaffe et al., 1994, Dohle et al., 1999). Most numerical chromosome abnormalities and AZF deletions are de novo events in the parental germ cells. Some abnormalities associated with infertility are inherited, like reciprocal and Robertsonian translocations and CFTR mutations (Mak et al., 1996).

ICSI is the most significant recent development in the treatment of male infertility, enabling couples who were previously deemed infertile to produce offspring, however, with the risk of passing on genetic abnormalities, and possibly decreased fertility. To assess the couple’s risk for transmitting a genetic abnormality we analysed the results of a combined andrological, cytogenetic and molecular genetic screening of 150 men, with oligozoospermia and azoospermia. We discuss the necessity of genetic counselling for these infertile couples.

Patients and methods
For this study men were selected with an infertility duration of at least one year and a semen analysis, showing less than 1 million motile spermatozoa /ml. History taking was focused on urogenital development, chronic illness, pulmonary diseases, growth disturbances, medication, male accessory gland infections (MAGI), previous inguinal and scrotal surgery and occupational exposure to heat or chemicals. All men were investigated for genital malformations and ultrasound investigation of the scrotal content was performed.
Semen analysis was performed twice according to the WHO guidelines for semen analysis (Rowe et al., 1992) with an interval of at least one month. Laboratory investigations included serum gonadotrophins, testosterone, prolactin and sex hormone binding globulin.
Cytogenetic analysis was performed on metaphase spreads of cultured lymphocytes. Fluorescence in situ hybridization (FISH) was applied in case 10 according to Kievits et al. (Kievits et al., 1990) with the probes cp9-23.1 and pDP105, specific for the X and Y chromosome short arm telomere regions, and the testis determining region of the Y chromosome, respectively.

DNA was extracted according to standard procedure and the presence of sub-microscopic deletions of the AZF region on the Y chromosome long arm was analysed using a multiplex PCR for Y-specific markers, including DYS148 and DYS 273 (AZFa), DYS218 and DYS224 (AZFb), SY245 and SY255 (DAZ gene, AZFc region). These STS were chosen based on the laboratory guidelines described by Simoni et al. (Simoni et al., 1999)

Twelve common mutations of the CFTR gene were tested (F508, A445E, G542X, 1717-1G->A, R553X, R117H, R1162X, N1303K, W1282X, 3659delC, E60X, S1251N). The mutations tested comprise about 85% of the mutations identified in the Dutch Caucasian CF population. The length of the T-stretch in intron 8 was tested as previously described (Dohle et al. 1999).

**Results**

Genetic testing was performed on 37 men with azoospermia (24.7%) and 113 with severe oligozoospermia (75.3%). Table 1 shows the results of the andrological evaluation. A testicular tumour was found in 2 cases. In 56.6% of the andrological evaluations no male infertility associated factor was identified.

Table 2 shows 38 genetic abnormalities identified in 36 patients. Cytogenetic analysis showed an abnormal result in 16 (10.6 %) patients. A sex-chromosomal aneuploidy was identified in 9 men. The Klinefelter syndrome (47,XXY) was present in 6 patients, of which 3 with azoospermia and 3 with an occasional non-motile sperm in the ejaculate (cryptozoospermia).

One patient presented a 46,XX karyotype, with Y-short arm DNA sequences present in one of the X chromosomes short arms. FSH was high (>7 IU/ml) in all men with a 47,XXY karyotype and in the 46,XX male. One Klinefelter patient (case nr.5) also carried a CFTR gene mutation (R117H). Six balanced translocations and one marker chromosome were found in men with oligozoospermia and a normal phenotype. FSH was either normal or slightly elevated, oligozoospermia was present in all translocations.

Analysis of the AZF-a,-b and -c regions of the Y chromosome identified 8 (5.3%) men with an AZFc (DAZ) deletion, 5 with severe oligozoospermia and 3 with azoospermia. A normal testicular volume was present in 5 men and FSH was increased in 3. Six men with an Yq11
microdeletion had unexplained male infertility, one man (case nr. 2) also carried a CFTR gene mutation (F508).

CFTR gene mutations were identified in 14/150 (9.3%) patients, associated with congenital abnormalities of the vas deferens in 4 cases. All CBAVD patients showed low volume (<1.0 ml) and low pH (<7.0) semen analysis and azoospermia and had normal abdominal ultrasound investigations. In 2 patients the scrotal vas deferens was present, but on vasography a blind ending was visualised in the distal part of the vas deferens. Both men also had low semen volume, low pH and azoospermia. A single CFTR gene mutation was identified in one. Six (16%) men with azoospermia but without an apparent congenital abnormality of the vas deferens showed a single CFTR mutation. In 5/113 (4.4%) patients with severe oligozoospermia a single CFTR mutation was identified.

A genetic abnormality was present in 36/150 (24.0%) men with severe oligozoospermia and azoospermia. Excluding those with a congenital abnormality of the vas deferens a genetic risk factor was present in 11/32 (34%) of men with azoospermia. In men with extreme oligozoospermia a genetic abnormality was identified in 20/113 (17.6%). Two patients carried two risk factors: 1 Klinefelter patient (47,XXY) also carried a R117H mutation in the CFTR gene, another man was found to have both an AZFc deletion of the Y chromosome and a F508 CFTR gene mutation.

**Discussion**

Genetic counselling in reproductive medicine starts by obtaining an accurate diagnosis. The family history, physical examination (including evaluation of dysmorphology) and various laboratory tests on both partners are generally required (Rowe et al., 1993). A substantial number of infertile men, however, present without a history associated with fertility problems and have normal findings on physical examination and endocrine laboratory testing. Only semen analysis is abnormal, often showing a decreased number of sperm cells (oligozoospermia), decreased sperm motility (asthenozoospermia), and many abnormal forms on morphological examination (teratozoospermia). Combinations of these abnormalities occur frequently and are described as the OAT (oligo-astheno-teratozoospermia) -syndrome. These idiopathic forms of male subfertility may be explained by factors like endocrine disruption due to environmental pollution, reactive oxygen species and genetic abnormalities (E.S.R.H.E., 1992). Although infertility associated with chromosome abnormalities has long been recognised, the introduction of intracytoplasmic sperm injection (ICSI) as a method to overcome severe spermatogenic defects has stimulated many investigators to further explore the genetic basis of male infertility.
In this study we confirmed that male infertility, both with and without a detectable cause, was associated with several genetic risk factors such as constitutional chromosome abnormalities, microdeletions of the Y chromosome and mutations in the CFTR gene.

An abnormal karyotype was found in 10.6% of the men with severe oligozoospermia or azoospermia. In a recent review (van Assche et al., 1996) the frequency of karyotypic abnormalities in 1701 males with infertility was found to be as high as 13.7% in azoospermia and 4.6% in oligozoospermia. Numerical sex chromosomal abnormalities, present in 63% of all cytogenetic abnormalities in infertile males, were commonly found together with azoospermia. Autosomal anomalies, like Robertsonian and reciprocal translocations and inversions were usually present in men with oligozoospermia. In the Klinefelter syndrome the extra X chromosome was associated with germ cell atresia, whereas in the other cytogenetic abnormalities no specific relation to meiotic disturbances was found as these abnormalities also occurred in men with normal spermatogenesis.

In this study DNA-analysis of the AZF-a,-b and -c regions on the Y chromosome showed deletions in 5.3% of the patients. All deletions identified comprised the AZFc region, containing the Deleted in Azoospermia (DAZ) gene (Reijo et al., 1995). The Y chromosomal microdeletions are the most common genetic causes of male infertility due to spermatogenic failure and have been reported in 3-21% of the infertile men (van der Ven et al., 1997). These microdeletions were associated with severe oligozoospermia and non-obstructive azoospermia, normal physical findings and normal concentrations of gonadotrophins. Histological studies of testicular biopsies from men carrying an AZF deletion exhibit a wide spectrum of spermatogenetic defects from complete absence of germ cells (Sertoli-cell-only syndrome) to maturation arrest with occasional production of mature, condensed spermatids (Reijo et al., 1995).

Men with defects of the Wolffian duct, presenting as congenital bilateral absence of the vas deferens, were found to carry different mutations of the cystic fibrosis transmembrane conductance regulator gene (Oates et al., 1994, Jaffe et al., 1994). CBAVD, previously described as a genital form of cystic fibrosis (CF) appears to be a heterogeneous clinical and genetic condition. We have shown that some patients with CBAVD also have non-genital symptoms of CF, like defective cellular chloride excretion and disturbed pancreatic function (Dohle et al., 1999). It has been suggested that CBAVD patients are compound heterozygotes for a severe mutation in one allele in combination with a mild CFTR gene mutation in the other. Alterations in the non-coding regions of the gene, such as the polypyrimidine stretch in intron 8, in combination with a mutation in the other allele, were found to cause abnormal levels of CFTR-protein, due to exon 9 skipping translation (Chu et al., 1993). CFTR intron 8
DNA variants may alter the splicing efficiency of the CFTR mRNA in exon 9 (Kiesewetter et al., 1993), thus causing reduced concentrations of CFTR protein (Chillon et al., 1995). Impaired CFTR-protein function may cause defective, but not absent chloride excretion resulting in absence of the vas deferens, but not in pulmonary or pancreatic insufficiency (Anguiano et al., 1992). Mutations of the CFTR gene are commonly associated with obstructions of the male genital tract (Mak et al., 2000). We detected at least one CFTR mutation in 4/6 men with CBAVD and in 10/144 men without a vas deferens-related problem. This seems to confirm the association of mutations in the CFTR gene with obstruction of the male genital tract rather than with primary testicular failure, although recently some men with CBAVD were found to have a defective spermatogenesis (Meng et al., 2001). However, the rate of mutations detected in this study in the non-obstructed group does seem elevated compared with the carrier risk in the Dutch population of 1:30 (De Vries et al., 1997). This prompted us to initiate an extended study with a larger cohort of patients. Since we tested for mutations that are common among CF patients previously, we are now using extensive screening methods to evaluate the true rate of CFTR mutations/variants in this group of infertile men.

In this study 38 genetic abnormalities were identified in 36 men from a population of 150 men with severe male infertility. The application of ICSI in these couples may lead to offspring with an enhanced risk of unbalanced chromosome complement, male infertility due to transmission of an AZF deletion and cystic fibrosis in case of CF-related genital abnormalities. We conclude that all men with severe oligozoospermia and azoospermia should be offered genetic testing and counselling before assisted reproduction is applied.

References


**Table 1.** Male infertility associated factors in 150 men with oligozoospermia and azoospermia.

<table>
<thead>
<tr>
<th>Male infertility associated factor</th>
<th>N (%)</th>
<th>Genetic abnormality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptorchidism</td>
<td>23 (13.3%)</td>
<td>5 (21.7%)</td>
</tr>
<tr>
<td>Varicocele</td>
<td>15 (10.0%)</td>
<td>3 (20.0%)</td>
</tr>
<tr>
<td>Congenital abnormality of the vas deferens</td>
<td>6 (4.0%)</td>
<td>4 (66.6%)</td>
</tr>
<tr>
<td>Male accessory gland infection</td>
<td>8 (5.3%)</td>
<td>0</td>
</tr>
<tr>
<td>Endocrine abnormality</td>
<td>3 (2%)</td>
<td>0</td>
</tr>
<tr>
<td>Chronic illness</td>
<td>4 (2.6%)</td>
<td>0</td>
</tr>
<tr>
<td>Malignancy</td>
<td>2 (1.3%)</td>
<td>0</td>
</tr>
<tr>
<td>Previous inguinal and scrotal surgery</td>
<td>4 (2.6%)</td>
<td>0</td>
</tr>
<tr>
<td>Unexplained male infertility</td>
<td>85 (56.6%)</td>
<td>27 (31.7%)</td>
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</table>
Table 2: Genetic abnormalities in 36 men with severe oligozoospermia and azoospermia.

Abnormal karyotype

<table>
<thead>
<tr>
<th>Nr</th>
<th>Genetic abnormality</th>
<th>Medical history</th>
<th>Physical examination</th>
<th>Semen analysis</th>
<th>FSH</th>
<th>Remarks</th>
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<td>2</td>
<td>47,XXY</td>
<td>Gynaecomastia</td>
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<td>Cryptozoospermia</td>
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<td></td>
</tr>
<tr>
<td>3</td>
<td>47,XXY</td>
<td>Delayed puberty</td>
<td>Hypogonadism</td>
<td>Cryptozoospermia</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>47,XXY</td>
<td>Cryptorchidism</td>
<td>Varicocele</td>
<td>Azoospermia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>47,XXY</td>
<td>Normal</td>
<td>Hypogonadism</td>
<td>Azoospermia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>47,XXY</td>
<td>Normal</td>
<td>Hypogonadism</td>
<td>Azoospermia</td>
<td>11.0</td>
<td>CFTR: R117H</td>
</tr>
<tr>
<td>7</td>
<td>47,XXY</td>
<td>Normal</td>
<td>Hypogonadism</td>
<td>OAT</td>
<td>12</td>
<td></td>
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<tr>
<td>8</td>
<td>47,XXY</td>
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<td>Hypogonadism</td>
<td>OAT</td>
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<td>9</td>
<td>46.XX ISH(cp9-23.1;+pDp105+)</td>
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<td>Diabetes mellitus</td>
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<td>OAT</td>
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<td>12</td>
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<td>Cryptorchidism</td>
<td>Hypogonadism</td>
<td>OAT</td>
<td>9.6</td>
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<tr>
<td>13</td>
<td>46.X,Y:2)(q11:22;q34)</td>
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<td>OAT</td>
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<td>15</td>
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<td>OAT</td>
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<tr>
<td>16</td>
<td>47.XY,+-Mar(mat=iso(15p))</td>
<td>Urogenital abn.</td>
<td>Varicocele</td>
<td>OAT</td>
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Deletion of the Azoospermia factor (AZF) region

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<tr>
<th>Nr</th>
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<th>Medical history</th>
<th>Physical examination</th>
<th>Semen analysis</th>
<th>FSH</th>
<th>Remarks</th>
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<td>OAT</td>
<td>7.3</td>
<td>CFTR: *F508/-</td>
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<tr>
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<td>Normal</td>
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<td>1.0</td>
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</tr>
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Cystic fibrosis transmembrane conductance regulator (*CFTR*) gene mutations

<table>
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<th><em>CFTR</em> mutation</th>
<th>T-Stretch</th>
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<tr>
<td>1</td>
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<td>7T/-</td>
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<td>*F508/-</td>
<td>5T/9T</td>
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<td>CBAVD</td>
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<td>3</td>
<td>*F508/R117H</td>
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<tr>
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<td>A455E/-</td>
<td>5T/9T</td>
<td>Ejaculatory failure</td>
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<td>E60X/-</td>
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<td>OAT</td>
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<tr>
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<td>Varicocele</td>
<td>OAT</td>
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<td>OAT</td>
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<td>OAT</td>
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<tr>
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<td>7T/7T</td>
<td>Normal</td>
<td>Normal</td>
<td>Azoospermia</td>
<td>3.2</td>
<td></td>
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</tbody>
</table>

CBAVD = congenital bilateral absence of the vas deferens. MAGI = male accessory gland infection.
OAT = oligo-astheno-teratozoospermia. FSH = follicle-stimulating hormone. AZF = azoospermia factor (Yq11)
TRANSURETHRAL DEROOFING OF MIDLINE PROSTATIC CYST FOR SUBFERTILE MEN.


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Department of Urology, Academic Hospital Rotterdam, The Netherlands.

Abstract
We evaluated the efficacy of transurethral deroofing of a midline prostatic cyst in subfertile men with one or more of the following semen abnormalities: decreased ejaculatory volume, decreased sperm motility and oligo- or azoospermia. Results of treatment of a series of 11 subfertile men with a midline prostatic cyst are presented.
Five patients showed an improvement of seminal volume. Only one demonstrated an improvement of sperm count. Sperm motility was not influenced. No relation was found between positive outcome following operation and either size of the cyst or dilatation of the seminal vesicles. Spontaneous pregnancies did not occur after transurthral deroofing of the midline prostatic cyst.
In conclusion, our study suggests a poor efficacy of transurethral deroofing of a midline prostatic cyst in subfertile men with the above mentioned semen abnormalities.

KEY WORDS: MALE SUBFERTILITY – PROSTATIC CYST – TRANSURETHRAL DEROOFING.
INTRODUCTION
In the last decade refinements of assisted reproductive techniques have gained importance in the treatment of male subfertility. Due to the success of assisted male reproductive techniques, irrespective of the aetiology of male subfertility, appropriate evaluation is often bypassed although several causes can be effectively treated. In almost half of the infertile couples, male subfertility, defined as the failure to induce a pregnancy within one year of regular unprotected intercourse (Meuleman, 1998), is present (Mosher and Pratt, 1991). It is therefore of importance to identify those subfertile males for whom treatment options are available before embarking on expensive and potentially hazardous assisted reproductive techniques.

Ejaculatory duct obstruction (EDO) is regarded as one of the treatable causes of male subfertility. EDO is found in 7-14% of the subfertile male population and can be congenital or acquired (Goldwasser et al., 1985; Pryor and Hendry, 1991). Normal hormone status and one or more of the following distinct semen abnormalities such as low or absent fructose, decreased sperm motility, decreased ejaculatory volume and oligo- or azoospermia are suggestive for total or partial EDO. The diagnosis of EDO can be confirmed directly by vasography (Banner and Hassler, 1978) or indirectly by transrectal ultrasonography (TRUS) (Jarrow, 1993). Due to its non-invasive nature TRUS is currently the diagnostic method of choice.

Theoretically, a midline prostatic cyst can be one of the causes of EDO. The cyst, localized in the central zone of the prostate, can compress or displace the ejaculatory ducts to the lateral side (Fisch, 1992). It has been our policy to offer transurethral deroofing of prostatic cyst to subfertile men with one or more of the following semen abnormalities: decreased seminal volume, decreased motility, oligozoospermia or azoospermia, in order to improve semen quantity and quality and pregnancy rate. In this retrospective study we evaluated the efficacy of this treatment.

MATERIAL AND METHODS
Since 1996 11 patients who presented in our departments with subfertility were diagnosed with a median prostatic cyst. Age ranged from 25-39 years, mean 34 years. There were no urological complaints. Infertility was primary in 10 patients and one patient developed azoospermia after fathering one son. At physical examination all patients had normal sized testes (15-25 ml), bilateral palpable vas deferens and absence of varicoceles. Follicle stimulating hormone and testosterone were measured and always normal. Semen analyses was performed at least twice before operation. Patients were asked to abstain from sexual activity.
for 2-3 days before semen collection. Semen evaluation was performed at the university-based andrology laboratory and included ejaculate volume, seminal plasma pH, sperm concentration, percentage of sperm motility and sperm viability. TRUS of the prostate, performed if EDO was suspected, demonstrated the presence of a midline prostatic cyst and, if present, concurrent dilatation of seminal vesicles or ejaculatory duct.

The diagnosis of subfertility based on a median prostatic cyst was based on semen analysis with one or more of the following findings in addition to normal physical examination and normal FSH and testosterone: (i) low ejaculate volume (<2ml) azoospermia; (ii) low or normal ejaculate volume with a sperm concentration <20 million/ml; or (iii) low or normal ejaculate volume with sperm motility < 30% (Kim et al., 1997).

Transurethral incision of the midline cyst was performed under local anaesthesia with the patient in lithotomy position. The roof of the cyst was incised under TRUS guidance and under direct vision through a Collins hook (Karl Storz GmbH and Co., Tuttingen, Germany). Generally, the prostatic floor had to be incised between the bladder neck and the verumontanum that resulted in complete marsupilization of the cyst. Minimal coagulation was used. A Ch 16 transurethral catheter (Bardex I→C>; Bard Benelux n.v., Olen, Belgium) was introduced in the bladder and removed 24 h post-operatively.

Patients were discharged the day after the procedure. There were no complications after transurethral incision of the midline cyst and this procedure was well tolerated by all patients. Semen analyses was obtained 4-12 weeks post-operatively.

RESULTS

Table I summarizes pre- and post-operative seminal parameters of all patients. Pre-operative seminal parameters corresponded to the mean value of at least two semen analysis performed within 6 months before transurethral incision of the midline prostatic cyst. Post-operative seminal parameters corresponded to the mean values of at least two semen analysis performed at the latest follow-up.

Effect of transurethral incision of the midline prostatic cyst on sperm volume

Patients 3, 5, 8, 10 and 11 showed improvement of seminal volume after the procedure (Table I). No relationship was found between the positive effect of the incision and the size of the cyst or dilatation of the seminal vesicles.

Effect of transurethral incision of the midline prostatic cyst on sperm quality

Sperm concentration did not change in eight patients, decreased in patient no. 4 and increased only in patient no 10. (Table I). Additionally, the percentage of motile spermatozoa and the
quality of motility did not change after surgery. A remarkable, but transient improvement in sperm quality was seen in one patient (no. 6). In the first 3 months following the transurethral incision of the cyst, semen analysis showed dramatic sperm improvement, but eventually the patient developed azoospermia again.

**Effect of transurethral incision of the midline prostatic cyst on pregnancy rate**

No spontaneous pregnancies occurred following transurethral incision.

<table>
<thead>
<tr>
<th>Table I. Semen parameters before and after transurethral deroofing of midline prostatic cyst in subfertile men.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient no.</strong></td>
</tr>
<tr>
<td>Pre-op No</td>
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<td>Post-op No</td>
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<tr>
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</tr>
<tr>
<td>Post-op No</td>
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</table>
DISCUSSION

The widespread utilization of TRUS to diagnose prostatic abnormalities and as a diagnostic tool in the evaluation of low ejaculate volume, azoospermia or oligozoospermia in subfertile men has resulted in more frequent detection of midline prostatic cysts (Kim et al., 1997). These cysts can be utricular or Mullerian duct origin (Golubuff et al., 1995). Utricular cysts are of endodermal origin, contain no spermatozoa and are located near the verumontanum whereas Mullerian cysts are of mesodermal origin, contain spermatozoa and are located more posteriorly and nearer to the prostate base. The ejaculatory ducts enter the prostate obliquely posteriorly at its base, course medially and anteriorly through the glandular tissue of the prostate and end in the prostatic urethra at the verumontanum (Globuloff et al., 1995). Globuloff et al. demonstrated that the ejaculatory ducts run in an almost straight course from the prostatic base to the verumontanum (Globuloff et al., 1995) and it is therefore believed that a midline prostatic cyst can be one of the causes of ejaculatory duct obstruction by lateral compression of the ejaculatory ducts (Fisch et al., 1992).

Historically, ejaculatory duct obstruction was diagnosed by vasography (Pryor and Hendry, 1991; Jarrow, 1993). A complete block in the ejaculatory ducts is conclusive for the diagnosis of total obstruction. The main drawback, however, is the invasiveness of the procedure, and the subsequent risk of iatrogenic occlusion. In contrast, TRUS is readily available, inexpensive and non-invasive. The ultrasonographic diagnosis of EDO is based upon dilatation of seminal vesicles or abnormalities such as midline prostatic cysts or calcifications in the region of the ejaculatory ducts. Unfortunately, not all patients with EDO have dilated seminal vesicles and, conversely, not all patients with dilated seminal vesicles have EDO (Littup et al., 1988). Moreover, the functional implication of a midline prostatic cyst or prostatic calcification cannot be determined by TRUS (Jarrow, 1996a). It provides only circumstantial evidence for obstruction. Nevertheless, at present TRUS has replaced vasography as imaging modality for suspected ejaculatory duct obstruction.

Several treatments for EDO caused by midline prostatic cysts, e.g. transurethral resection of the ejaculatory ducts (TURED), transrectal aspiration together with with sclerotherapy, transurethral marsupialization and open surgery of the midline prostatic cysts have been described (Ritchey et al., 1988; Fisch, 1992; Stricker et al., 1993; Jarrow, 1996b). Currently, the standard treatment has become TURED (Fisch, 1992; Mecham et al., 1993). The overall success rate of TURED has been quite good (Jarrow, 1996b). In the literature more than 100 patients have been described as having had this procedure for subfertility with improvement in semen parameters seen in 50%. Spontaneous pregnancies occurred in 25% of
the cases (Jarrow, 1996b; Netto et al., 1988). Hendry and Pryor (1992) performed a transurethral incision of a Mullerian cyst in 21 patients, of which 10 (48%) experienced an undisclosed improvement in semen quality and eight (38%) partners conceived (Hendry and Pryor, 1992). Dik et al. (1996) described in their series of transurethral marsupialization of the medial prostatic cyst in patients with prostatitis-like symptoms, three patients with infertility of whom 2 demonstrated significant improvement of semen quantity and quality (Dik et al., 1996). Moreover, eight of the 10 patients with prostatitis-like symptoms who had preoperatively small volume ejaculate without infertility demonstrated improvement in semen volume after transurethral marsupialization (Dik et al., 1996). These results clearly advocate a functional relationship between midline prostatic cysts and low semen volume. Our poor results of transurethral incision of midline prostatic cyst for infertility are therefore surprising. Only five (46%) patients demonstrated an improvement in seminal volume and in one patient improvement of sperm concentration was seen. Sperm motility was not affected in any patient. In our series spontaneous pregnancies did not occur after transurethral deroofing of the midline prostatic cyst.

The diagnosis of subfertility caused by obstruction due to midline prostatic cyst was based on history, physical examination, semen analyses and TRUS. Since vasography was not used in the diagnostic process, a functional relationship between the midline prostatic cyst and obstruction was not established, which may explain our poor results. Functional investigation, i.e. seminal vesiculography or vasography may still be mandatory to prove the existence of an obstruction and to prove its removal afterwards. Secondly, the midline prostatic cyst was marsupialized and the cyst walls were not resected. The edges may heal together once again thus allowing the obstruction to return. Theoretically, a midline prostatic cyst can cause direct obstruction of the ejaculatory ducts by compression, but secondary fibrosis/stenosis of the ejaculatory ducts might occur on different levels between the ejaculatory ducts orifice in the urethra and the epididymal junction. Although marsupialization of the midline cyst might solve the direct obstructive factor, the secondary obstruction might still be present. Digital massage of the seminal vesicles, possibly prior to the operation and injection with coloured dye, could therefore be helpful to check patency of the ejaculatory ducts following marsupialization of the cyst.

Finally, an improved patient selection might influence pregnancy outcome. Patients with subfertility based only on low semen volume might benefit from improvement of seminal of seminal volume because the concomitant increase in pH of the seminal fluid may protect the spermatozoa against the harmful influence of the acid vaginal mucous.
In conclusion, our study suggests a poor efficacy of transurethral deroofing of midline prostatic cyst alone in subfertile males with low semen volume, oligozoospermia or azoospermia. To improve the results, selective vasography or vesicography prior to an incision to confirm obstruction may be useful despite the potential harmful effects. Moreover, marsupialization of the cyst in combination with a resection of the wall of the cyst might improve the results. Only time will tell if these adjustments will improve the results, in terms of pregnancy rates, of transurethral deroofing of a midline prostatic cyst in subfertile men with low semen volume and/or azoo- or oligozoospermia.

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MICROSURGICAL REPAIR OF THE MALE GENITAL TRACT: REFINEMENTS AND PREDICTORS OF SUCCESS.


European Urology (In press).
ABSTRACT
Microsurgical repair of obstructive male infertility is a challenge for the physician, but successful treatment depends on the experience and skills of the surgeon. Fertility can often be restored, thus avoiding the need for artificial reproductive techniques. The surgical procedures can be combined with sperm aspiration and cryopreservation, to be used for Intracytoplasmic sperm injection (ICSI) in cases of surgical failure. In this review we will discuss the results of microsurgical procedures for obstructive male infertility, with special emphasis on technical refinements and prognostic indicators.

Recently, surgical refinements, such as the invagination technique, have been introduced for the vasoepididymostomy procedure, showing promising first results. This simplified technique enables less experienced microsurgeons to perform this difficult operation successfully. The procedure is indicated for men with primary infertility and epididymal obstruction and for vasectomised men with secondary epididymal obstruction due to leakage of semen from the dilated epididymal tubule with subsequent fibrosis. This can be found in men with a long obstructive interval between vasectomy and reversal.

In men with obstruction of the seminal tract patency in found in 70-90% after microsurgical repair. In primary infertility and epididymal obstruction other anatomical abnormalities of the reproductive tract can be found in nearly half of them, like obstructions of the ejaculatory ducts. In men with extreme oligozoospermia, normal physical examination and normal hormonal evaluation partial obstructions of the seminal tract can be found in 50-60%.

Vasectomy reversal is still the treatment of choice for secondary infertility after previous vasectomy. Although patency remains high, even after a long obstructive interval, the number of spontaneous pregnancies progressively decrease after a period of 10 years, due to loss of epididymal function and sperm motility. The main predictive factors for a successful outcome are the obstructive interval and the age of the female partner. Late stenosis after initial successful operation occurs in 12-18%: repeat reversal procedures show a patency rate of 64-79%, pregnancies are reported in 27-31%.

Microsurgical reconstruction of obstructive male infertility can be a very awarding procedure for both the infertile couple and the physician. Practical teaching courses are very helpful and a learning curve should be taken into account. Urologist with an interest in male infertility should be encouraged to learn microsurgery as a part of their surgical training. The operations are best performed in centres for reproductive medicine, allowing different options to be performed for selective cases.
**INTRODUCTION**

Microsurgery in urology is mainly applied in obstructive male infertility and varicocele repair. Other indications are vascular erectile dysfunction and penile or testicular trauma. Obstructions of the male genital tract represent 5-10% of the causes of male infertility and in 80% of these men surgical repair can be performed [1]. The patient presents with either azoospermia or extreme oligozoospermia, normal testicular volume, and normal serum concentrations of FSH and Inhibin-B, both reliable markers for spermatogenesis. To finalise the diagnosis of a male genital tract obstruction a testicular biopsy may be required in men with primary infertility.

The site of the obstruction is commonly at the epididymal level, causing dilation of the epididymal tubule. Back pressure in the obstructed epididymal tubule may lead to a “blow-out” with subsequent leakage of semen in the connective tissue of the organ, resulting in fibrosis and scarring. Also, a long interval of obstruction will eventually cause loss of epididymal function, poor fertilising capacity of the spermatozoa and the formation of antisperm antibodies. In about 20% of the men with obstructive azoospermia fibrosis and other irreparable conditions of the epididymis are found during scrotal exploration and microsurgery cannot be performed. Furthermore, epididymal obstruction is frequently accompanied by congenital abnormalities of the genital tract and ejaculatory duct obstruction [2].

Epididymal obstruction can be congenital (idiopathic) or acquired, due to epididymal infection or previous inguinal and scrotal surgery. About 10% of the men with obstructive azoospermia present with a primary structural abnormality of the genital tract, like congenital bilateral absence of the vas deferens (CBAVD), a mild form of cystic fibrosis [3]. In these men part of the epididymis and the scrotal vas deferens are absent, due to an early regression of the Wolffian duct. Also, the seminal vesicles are either hypoplastic or absent, resulting in a low volume semen (<1.0ml) and low seminal pH (<7.0).

Obstructions of the ejaculatory ducts are found in men with recurrent prostatitis and with prostatic cysts. On transrectal ultrasound the effects of distal obstructions of the genital tract can be found like dilations of the ejaculatory ducts and the seminal vesicles as well as calcifications in this region [4].
In Western Europe about 10-15% of the male population rely on the vasectomy procedure as a contraceptive method. Since divorce rates are increasing, the demand for vasectomy reversal is high: in 2-6.5% of the vasectomised men a vasovasostomy is being performed [5].

In this review we will discuss the different aspects of microsurgical treatment of obstructive male infertility and focus on recent modifications, refinements of the techniques of the vasoepididymostomy (VES) and vasovasostomy (VVS) procedure and on the value of the different prognostic factors involved in refertilisation surgery for obstructive azoospermia.

**DIAGNOSTIC PROCEDURES**

Before scrotal exploration is performed in men with suspected primary obstruction a testicular insufficiency should be ruled out. In men with small testis and high levels of FSH an impairment of spermatogenesis is always present and exploration is not indicated. Ultrasound investigations of the scrotum can be helpful in the diagnosis of obstruction (epididymal congestion) and in detecting other causes of male infertility, like varicocele. Transrectal ultrasound is indicated in men with a history of genital infections and in case of a consistent low seminal volume (<1.0 ml). In 33% of the men with obstructive azoospermia abnormalities can be found on transrectal ultrasound investigation [6].

The diagnosis CBAVD can be made by careful examination of the patient. The results of the semen analysis always show a low seminal volume and low pH. Fructose is absent in the ejaculate. Genetic testing for cystic fibrosis gene mutations in both the patient and his partner should be performed before a microsurgical epididymal sperm aspiration (MESA) and intracytoplasmic sperm injection (ICSI) is performed.

Vasography is performed during scrotal exploration and is indicated in men with a history of inguinal hernia repair and in primary infertility. In men with a history of gonorrhoea obstructions in both the vas deferens and the ejaculatory ducts may be present. For vasectomy reversal cases the patency of the vas deferens can simply be tested by a saline flushing through a small 24-gauge angiocatheter or by flushing with a methylene blue solution, which colours the urine green.

If sperm cryopreservation is considered the patient should be screened for sexual transmittable diseases, like HIV and Hepatitis, for several of these viruses can be transmitted into the oocyte during the ICSI procedure.
Figure 1. End-to-side vasotubulostomy
VASOEPIDIDYMOSTOMY

Vasoepididymostomy (VES) is a technically demanding procedure that requires advanced microsurgical skills. The end-to-side vasotubulostomy was popularised by Silber et al. [7]. A high scrotal delivery of the testis is performed by a longitudinal incision of the scrotum, which can be extended in the inguinal direction in case of an unexpected inguinal obstruction. The tunica vaginalis is opened anteriorly and the epididymis is inspected under the operation microscope. Starting in the tail of the epididymis, tubular fluid is aspirated for microscopic examination and cryopreservation. In case of patency a vasography with saline or methylene blue is performed in the distal part of the vas deferens. The vas deferens is then pulled through the tunica vaginalis and the serosa of the vas is fixated to the tunica vaginalis of the epididymis. A dilated part of the epididymal tubule is opened anteriorly under the microscope and a 10-zero nylon suture is used for the anastomosis between the opened tubule and the lumen of the vas deferens (Fig. 1). Finally, the serosa of the vas deferens is fixated to the tunica vaginalis of the epididymis.

The reported patency rates after the procedure range from 64-78%, spontaneous pregnancies occur in 31-56% [7,8,9]. Passage through a substantial portion of the epididymis is essential for the fertilising capacity of the spermatozoa. An anastomosis between the vas deferens and the head of the epididymis usually does not result in spontaneous pregnancy. IVF/ICSI procedures are indicated for these couples. Recently, Berardinucci et al. reported that anatomical abnormalities are often found in men with suspected epididymal obstruction [2]. In a series of 147 men evaluation of the reproductive tract resulted in 52.7% epididymal obstructions and 47.3% other anatomical abnormalities, like vasal obstruction and aplasia, epididymal atresia and intra-testicular obstructions. The outcome of microsurgical repair in men with other anatomical obstructions was poor and in 35% post-testicular obstructions were encountered that precluded successful VES. In men with an infectious etiology spermatozoa were found in the ejaculate postoperatively in 76%, in idiopathic cases this was only 32%. Late failure after initial patency was reported as high as 25%, and therefore the procedure should be combined with epididymal sperm aspiration and cryopreservation. Recently, Berger et al. introduced an invagination technique modification to facilitate the anastomosis between the epididymal tubule and the lumen of the vas deferens, with high initial patency results [10]. The dilated epididymal tubule is exposed through a small incision in the tunica vaginalis and slight pressure of the epididymis by the thumb and the index finger is applied.
Figure 2. Vasoepididymostomy by a tubular invagination technique, using 2 double-armed 10-zero nylon sutures. (From: Marmer et al., J Urol 2000, 163:483-486)
Three double-armed 10-zero nylon sutures are placed through the anterior wall of the tubule before opening the lumen and fixated insight-out to the mucosa of the vasal lumen in a triangular fashion. A tubulotomy is performed in the middle of the three sutures and the seminal fluid is examined for spermatozoa. The anastomosis is created by this invagination method at six sites for mucosal adaptation, using only 3 double-armed sutures. The procedure has recently been modified by Marmer et.al., using only 2 double-armed 10-zero sutures (Fig.2) [11]. Others have also shown the advantages of invagination techniques in an animal model [12]: it is easier to learn than the end-to-side procedure and shows equally effective. In small series the patency was as high as 90% and already established 3 months after the operation.

VASOVASOSTOMY

Microsurgical repair after vasectomy can be a very successful procedure, since both patency and pregnancy results are high under good surgical conditions. The advantage of microsurgery is that it enables the surgeon to perform a delicate and exact alignment between the proximal and distal part of the dissected vas deferens.

A high scrotal delivery of the testis is performed, allowing the surgeon to perform an anastomosis even in the lower inguinal region in case the site of the vasectomy is located here. A vertical incision is preferred because it can be extended into the inguinal region, if necessary. The site of the previous vasectomy is identified and both ends of the vas deferens are mobilised for a tension free anastomosis. Stripping of the vas should be avoided since this will cause circulatory problems and subsequent stenosis of the vas deferens. The patency of the distal (inguinal) part of the vas deferens is tested by flushing a small amount of saline through a 24-gauge angiocatheter into the lumen. In case of resistance a methylene blue solution can be used. Under the microscope all fibrotic tissue, which can be recognised by white scarring bands in the seromuscular layer of the vas deferens, should be excised with a microknife.

The proximal part of the vas deferens is cut in a similar way and any fluid from the testicular part of the vas deferens is examined for the presence of spermatozoa. Motile sperm can usually be found in clear or white seminal fluid. Creamy thick "tooth-paste" like fluid usually does not contain spermatozoa and is often seen in cases of epididymal blockage [13]. The absence of fluid or spermatozoa is an indication for epididymal inspection: if the epididymis is dilated entirely, the dissection of the vas deferens should be continued until good semen quality is found in a more proximal part. A VVS procedure in men with absent spermatozoa in the vasal fluid will lead to a patency of 50-60%, provided that there is no epididymal blockage or fibrosis. In case of
an epididymal obstruction a VES procedure is indicated. Patency can be expected in 71-86% and the pregnancy rate after 2 year was found to be 44-65% [13,14].

In case of a large defect between both ends of the vas deferens length can be gained by mobilising the tortuous proximal part of the vas deferens. The dissection should be between the tail of the epididymis and the testicular end of the vas deferens, leaving adequate tissue surrounding the vasal end for adequate nutritional supply. Unrolling of the tortuous part of the proximal vas deferens should be avoided, since this will cause vascular impairment and subsequent stenosis. More length can be gained by mobilising the tail of the epididymis from the testis. Small vessels between the testis and the epididymis should be cauterised using bipolar diathermia only.

The anastomosis can be performed by a two-layer technique [14] (fig.3) or a modified one-layer technique [15] (Fig.4). Although many authors prefer the two-layer technique, both procedures show similar results in terms of patency and pregnancy. The advantage of the modified one-layer procedure is that it requires less surgical skills and can be performed bilaterally within 2 hours. Identification of the lumen can be facilitated by using methylene blue or a marker pen for colouring the vasal mucosa on both sides. For the anastomosis double-armed monofilament non-absorbable 9-zero or 10-zero sutures are used, allowing the needles to be placed inside out on both sides of the vas deferens. Six stitches are used for the mucosal anastomosis (inner layer) using a 10-zero double-armed suture with a small round needle and another six stitches for the seromuscular layer, for which a 9-zero suture with a cutting needle can be applied.

The operative success of the vasectomy reversal procedure depends on several factors:
- Gentile tissue handling, taking good care of the nutritional vessels of the vas deferens.
- Accurate mucosa to mucosa approximation
- Tension free leak-proof anastomosis.
- Excision of fibrotic vasal tissue.
- The presence of spermatozoa in the proximal (testicular) vas deferens
- A modified on-layer or a two-layer microsurgical anastomosis of the vas deferens.
- Bipolar diathermia and non-absorbable 9-zero and 10-zero double armed sutures.

Late stenosis of the anastomosis occur in about 12-18% of the patients within one year. Failure after a first VVS is usually caused by stricture of the anastomosis and epididymal blockage. Only in a few men an epididymal dysfunction or a testicular insufficiency is the cause of the failure.
Figur 3. Two layer vasovasostomy
Figure 4. Modified one-layer vasovasostomy
PROGNOSTIC FACTORS

Silber et.al. was the first to describe an inverse relationship between the duration of the obstructive interval and the patency and pregnancy rates. The pregnancy rate after vasectomy progressively decreases with the duration of the obstructive interval [16]. Although, even in men with an interval >15 years patency rate can be as high as 60-70%, pregnancy rate is only 20-30%, mainly due to loss of epididymal function. In a personal series of 223 vasovasostomy procedures, 118 men had an interval of more than 10 years. Table 1 shows the results of the patency and pregnancy rates at different interval periods between vasectomy and VVS. In selective cases when no spermatozoa can be found in the fluid of the proximal vas deferens, a VES should be performed at a site where motile spermatozoa are identified in the epididymal fluid.

The age of the female partner is a strong predictive factor in the treatment of infertile couples [17]. The decline of ovarian function in aging woman results in a decrease in pregnancy rate, both spontaneous and with artificial reproductive techniques. From the results of ART in older woman it is estimated that the fertility potential of a woman aged 35 is only 50% of the fertility of a woman aged 25 years; by the age of 38 years this is further reduced to only 25%, and over the age of 40 years it is less than 5%. Silber et al. reported a dramatic decrease in delivery rates per ICSI cycle in women older than 36 years: the rate dropped from 34% in woman aged 30-36 to 13% for women aged 37-39 and only to 4% in woman 40 years and older [18].

It has been suggested that men with older female partners who seek treatment for post-vasectomy infertility should undergo sperm aspiration and ICSI rather than vasectomy reversal. Recently, Deck et. al. compared the results of vasectomy reversal in men with ovulating female partners older than 37 years to the results of sperm retrieval and ICSI for woman in the same age category [19]. The ongoing pregnancy rate was 22% after vasectomy reversal and 8% per cycle in the ICSI group. They concluded that vasectomy reversal is the treatment of choice, although pregnancy rates were low for both groups. Sperm retrieval and ICSI did not improve the final outcome of these couples.

Table 2 shows the results of semen analysis after 224 VVS procedures: in men with a long obstructive interval a decrease in both the sperm count and the progressive motility is seen. The low number of motile spermatozoa can be the consequence of epididymal insufficiency, reactive oxygen species (ROS) and antisperm antibodies [20,21,22]. The role of the epididymis is storage and maturation of spermatozoa, and passage through the organ is essential for normal fertilisation. Epididymal insufficiency is seen after a long obstructive interval with dilatation of the epididymal tubule, subsequent leakage of seminal fluid in the interstitium of the organ and
fibrosis. ROS are generated by leukocytes and spermatozoa are highly susceptible to oxidation, since enzymes for neutralisation of ROS are absent in germ cells. Leukocytospermia can be the consequence of the epididymal obstruction and a low-grade infection of the genital tract [20]. Antisperm antibodies are found in 20-50% of the ejaculates after the operation, but most of these men prove to be fertile [21,22]. Antisperm antibodies may cause sperm agglutination and low motility. IgA antibodies on the cell surface of the spermatozoa are bound to anti-IgA in the cervical mucus, preventing further progression of the spermatozoa into the uterine cavity. Sperm washings and intra-uterine insemination is indicated in couples with major IgG and IgA binding and low motility. Severe oligozoospermia with low sperm count and motility is usually the consequence of a stricture at the anastomosis due to imprecise microsurgical alignment of the vasal lumen, malnutrition of the vas deferens or leakage of seminal fluid causing granuloma and fibrosis [14]. These men should be treated by a repeat reversal procedure rather than ART, since both patency and pregnancy rates are acceptably high after a second procedure. The repeat reversal procedure is indicated for men with initial patency and late stenosis of the anastomosis resulting in severe oligozoospermia or azoospermia. Partial obstruction with oligozoospermia and azoospermia after an initial good semen analysis results occurs in 12-18%. A second reversal procedure will result in patency rate of 50-80% and spontaneous pregnancies were reported in 20-50% [23,24]. Therefore, a repeat reversal with intraoperative sperm aspiration and cryopreservation appears to be the first treatment option in these couples.

ART OR SURGERY?
Sperm retrieval and ICSI has been advocated as the treatment of choice for male infertility, regardless the etiology. However, the results of microsurgical treatment of obstructive male infertility as compared to IVF/ICSI results are much in favour for surgical treatment [25,26]. Pregnancy rates of 44-65% after vasectomy reversal are better than the ongoing pregnancy rate after MESA/ICSI of 29%. Also, the less successful VES procedure still results in a spontaneous pregnancy rate of 31-56% [25]. Furthermore, the cost per delivery after microsurgery and after MESA/ICSI is much in favour of surgical treatment [26]. With surgical treatment several complications and disadvantages of in vitro reproductive techniques can be avoided, like hormonal treatment of the partner, transvaginal ovum pick-up and embryo transfer and the higher risk for offspring with a (sex-) chromosomal abnormality after ICSI procedures. Therefore, microsurgical epididymal sperm aspiration and intracytoplasmic sperm injection should be reserved for couples not amenable to microsurgical reconstruction of obstructive azoospermia.
SUMMARY

Microsurgical repair of obstructive male infertility is a challenge for the physician, but training and experience are mandatory for good results. Fertility can often be restored or improved, thus avoiding the need for artificial reproductive techniques. The procedures can be combined with sperm aspiration and cryopreservation, to be used for ICSI in cases of failed microsurgery. Comparing microsurgery to MESA/ICSI, both the results and the costs are much in favour of surgery. Urologist with an interest in Andrology should be encouraged to learn microsurgery as a part of their surgical training. The operations are best performed in centres for reproductive medicine, allowing different options to be performed for selective cases.

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**TABLE 2**: results of the sperm count, sperm progressive motility and antisperm antibody (MAR) test according to the length of the obstructive interval between vasectomy and reversal.

<table>
<thead>
<tr>
<th>SPERM COUNT</th>
<th>&lt; 1.0 MILL/ML (%)</th>
<th>1.0-20 MILL/ML (%)</th>
<th>&gt; 20 MILL/ML (%)</th>
<th>&lt; 10% MOTILITY (%)</th>
<th>MAR-TEST POS. (%)</th>
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<tr>
<td>OBSTRUCTIVE INTERVAL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 10 YEARS</td>
<td>8/62 (12.9)</td>
<td>34/62 (54.8)</td>
<td>20/62 (32.3)</td>
<td>9/30 (30.0)</td>
<td>29/41 (70.7)</td>
</tr>
<tr>
<td>OBSTRUCTIVE INTERVAL</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>&gt; 10 YEARS</td>
<td>14/77 (18.2)</td>
<td>42/77 (54.5)</td>
<td>21/77 (27.3)</td>
<td>25/46 (54.3)</td>
<td>18/31 (58.1)</td>
</tr>
<tr>
<td>Student’s T-test</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>P = 0.025</td>
<td>N.S. (P=0.49)</td>
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</tbody>
</table>
Table 1. Patency and pregnancy rates after vasovasostomy at different intervals (Years) between vasectomy and reversal.

PERCENTAGE.
SURGICAL SPERM RETRIEVAL AND INTRACYTOPLASMIC SPERM INJECTION AS TREATMENT OF OBSTRUCTIVE AZOOSPERMIA.

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Abstract

Male genital tract obstructions may result from infections, previous inguinal and scrotal surgery (vasectomy) and congenital bilateral absence of the vas deferens (CBAVD). Microsurgery can sometimes be successful in treating the obstruction. In other cases and in cases of failed surgical intervention, the patient can be treated by microsurgical or percutaneous epididymal sperm aspiration (MESA, PESA) or testicular sperm extraction (TESE) and intracytoplasmic sperm injection (ICSI). We present the results of 39 ICSI procedures for obstructive azoospermia in 24 couples. The aetiology of the obstruction was failed microsurgery in 11 patients, CBAVD in nine and genital infections in four. Sperm retrieval was accomplished via MESA in four cases, PESA in 18 cases and via TESE in 11 cases. TESE was only applied when PESA failed to produce enough sperm for simultaneous ICSI. In six patients the ICSI procedure was performed with cryopreserved sperm after an initial PESA procedure. Fertilization occurred in 47% of the metaphase II oocytes; embryo transfer was performed in 92% and resulted in a clinical pregnancy in 13/39 procedures. Ongoing pregnancy was found in 10/39 procedures. One pregnancy was terminated early after prenatal investigation showed a cytogenetic abnormality (47,XX+18, Edwards syndrome). The other nine pregnancies resulted in a live birth of 10 children, without any congenital abnormalities. Epididymal and testicular retrieved spermatozoa were successfully used for ICSI to treat obstructive azoospermia, and resulted in an ongoing pregnancy in 10 of 24 couples (41.6%) after 39 ICSI procedures, a success rate of 25.6% per treatment cycle and of 27.7% per embryo transfer.

KEY WORDS: ICSI - MESA - PESA - TESE - OBSTRUCTIVE AZOOSPERMIA
Introduction
Azoospermia is found in 10% of male infertility cases and is caused by a testicular insufficiency in the majority of patients. In 20% a bilateral obstruction of the male genital tract is responsible for the azoospermia (Hendry et al., 1994). In the presence of a history of previous scrotal or inguinal surgery or recurrent genital infection an obstruction can be suspected. These patients are characterised by normal testis volume and normal levels of gonadotrophins [Luteinizing hormone (LH)/follicular stimulating hormone (FSH)]. In 1-2% of the infertile male population congenital bilateral absence of the vas deferens (CBAVD) is found (Anguiano and Oates, 1992). The definite diagnosis of obstructive azoospermia is made by performing a testicular biopsy, showing normal spermatogenesis.

Microsurgical repair of vas deferens obstructions or epididymal blockage can result in spontaneous pregnancies in 27% - 56% of cases (Belker et al., 1991; Jarow et al., 1997). The results are determined by several factors, including the site and the duration of the obstruction, epididymal function, recurrent genital tract infections and sperm antibodies. In cases of poor sperm quality after the operation assisted reproductive techniques (ART) have been applied successfully. Recently, intracytoplasmic sperm injection (ICSI) has become available for treatment of severe oligospermia (Palermo et al., 1992). This technique has also been applied to treat azoospermia if viable sperms could be retrieved from the epididymis or the testis, giving results similar to those of ICSI for oligospermia (Silber et al., 1994; Devroey et al., 1994).

We present the results of 39 ICSI procedures for obstructive azoospermia in combination with microsurgical epididymal sperm aspiration (MESA), percutaneous epididymal sperm aspiration (PESA) or testicular sperm extraction (TESE).

Materials and Methods
Patients. The combination of ICSI and sperm retrieval was offered to couples with male factor infertility due to bilateral genital obstruction. The mean age of the treated males was 38.1 years (SD 6.34), the mean age of the treated females was 34.1 years (SD 4.2). All males had normal testicular volume, normal serum FSH concentrations and, on testicular biopsy, a normal sperm count according to the Johnsen scoring system (Johnsen, 1970). Azoospermia was found on at least two occasions. In men with CBAVD genetic analysis of common cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations was performed of the patient and his spouse. These couples were counselled for genetic risks concerning cystic fibrosis inheritance before the procedure. In cases of pregnancy all patients were offered
prenatal screening, amniocentesis and ultrasound examination. Informed consent was obtained in all couples after explanation of the experimental nature of the treatment with known and unknown medical and genetic risks.

**Ovulation induction and oocyte retrieval.** Pituitary down-regulation was achieved using a gonadotrophin releasing hormone analogue (GnRHa) Triptoreline (Decapeptyl\textsuperscript{R}, Ferring, Hoofddorp, The Netherlands) subcutaneously once a day. Multiple follicle development was induced with Human Menopausal Gonadotrophins (Humegon\textsuperscript{R}, Organon, Oss, The Netherlands). Depending on the size of the follicles and the serum oestradiol concentrations a single injection of 10,000 IU of Human Chorionic Gonadotrophin (HCG)(Pregnyl\textsuperscript{R}, Organon) was administered and a transvaginal ultrasound-guided follicular aspiration was performed 34-36 h later.

**Oocyte processing.** Oocyte preparation was performed according to the protocol as described by Palermo (Palermo et al., 1992) with a modification to a maximum of 3 min. hyaluronidase treatment. Only intact oocytes showing a polar body were microinjected.

**Sperm retrieval.** The MESA procedure was performed under general anaesthesia as an outpatient procedure. After exposure a dilated tubule of the epididymis was microsurgically opened and its fluid examined for the presence of motile spermatozoa. After four MESA procedures had been performed, the procedure was abandoned in favour of PESA in all subsequent cases. Percutaneous aspiration of the epididymal head (Tsirigotis et al., 1995) was performed under local anaesthesia of the spermatic cord (figure 1). In the case of an unsuccessful PESA procedure a testicular excisional biopsy (TESE) was performed for extraction of viable spermatozoa (Silber et al., 1995).

**Sperm processing.** Following MESA/PESA, the epididymal aspirates were diluted in 10 ml in ivf cultivation (IVF) medium and centrifugated for 10 min at 1500g. The supernatant was removed and the pellet was resuspended again in 10 ml IVF medium and the same centrifugation procedure was performed. After removal of the supernatant the cell-pellet was incubated until the start of the ICSI procedure. Approximately 1 1 of this suspension was added to 50 1 of polyvinylpyrrolidone (PVP) solution or diluted in 50 1 IVF medium in case of very poor motility.

During the TESE procedure, the testicular biopsies were performed under local or general anaesthesia. After obtaining a testicular sample, its adequacy of the sample was immediately assessed: depending on the outcome of the first sample (i.e. the presence or absence of spermatozoa) more testicular tissue might be necessary. In the laboratory the tissue sample was shredded by means of two sterile glass slides and a suspension of the cells was made in
IVF medium. During an incubation period of at least 2 h, spermatozoa were allowed to gain motility. After the incubation period the suspension was centrifuged for 5 min at 300g and the supernatant was removed and recentrifuged for 10 min at 1500g. Shortly before the injection procedure ~ 1% of the cell pellet suspension was added to 50% of IVF medium. During the MESA/PESA and TESE laboratory procedures none of the suspensions or washings media was discarded. If spermatozoa were absent in the pellet, they might still be found in one of the other suspensions.

**ICSI procedure.** Commercially available injection pipettes (Gynotec®, Malden, The Netherlands) were used with a outer and inner diameter of 7 μm and 5 μm respectively. The oocyte holding pipette had an outer and inner diameter of 90 μm and 10 μm respectively. One drop of sperm-PVP solution was placed in the centre of a Petri dish, surrounded by 5 drops of IVF medium containing metaphase II oocytes. All drops were covered by paraffin oil. The ICSI procedure was performed under a 400 x magnification, using hydraulic micromanipulators and microinjectors. A single spermatozoon was isolated, immobilized and aspirated tail-first into the injection pipette. The oocyte was fixed by the holding pipette and the injection pipette was pushed through the zona at the opposite side. Breakage of the ooplasmic membrane was initiated by gentle suction with the injection pipette, and one spermatozoon was injected immediately after membrane breakage was observed.

**Fertilization.** 16-18 h after microinjection, the oocytes were microscopically examined. Normal fertilization was reached by the presence of two pronuclei and a second polar body. Depending on the day of embryo transfer, embryo cleavage was judged 3, 4 and 5 days after the ICSI procedure. A maximum of 2 embryos was transferred into the uterine cavity and supernumerary embryos of good morphological quality were cryopreserved. Pregnancy was confirmed 18 days after oocyte retrieval by a positive pregnancy test. A clinical pregnancy was defined as the presence of at least one gestational sac with a fetal heart beat. An ongoing pregnancy was defined as a clinical pregnancy beyond 12 weeks of gestation.

**Results**

The combined sperm retrieval-ICSI procedure included 39 treatment cycles in 24 couples. Azoospermia was present in all men in at least two semen samples. CBAVD was present in nine patients, a history of recurrent infections in four and a previous surgical intervention of the scrotum or the inguinal region, including failed microsurgery, in 11.
Figure 1. Percutaneous epididymal sperm aspiration (PESA). A 19 gauge butterfly needle is inserted into the epididymal head. A mild vacuum is applied and the butterfly needle is moved gently within the epididymal head. Clear epididymal fluid is aspirated into the collecting tube for microscopical examination.
MESA was performed in the first 4 patients only, and was successful in all. Subsequently, 
PESA was introduced as the first approach for epididymal sperm retrieval for future cases. 
PESA showed successful in 18/29 (62%) procedures. In 11 cases of failed epididymal sperm 
retrieval an excisional testicular biopsy was performed under local or general anaesthesia in 
the same procedure, showing viable sperm in 9/11 (82%) biopsies. In one patient a repeated 
biopsy had to be performed on the same day, which yielded viable spermatozoa, which were 
successfully used for ICSI. Only in one case of TESE no viable sperm could be found after 
incubation. Sperm cryopreservation was performed only after epididymal retrieval and was 
successfully used in 6 patients as a sperm source where the first combined cycle had failed. 

Table 1 summarizes the results of the 39 treatment cycles. Oocyte retrieval resulted in a total 
number of 454 oocytes, of which 67.7% had extruded the first polar body (metaphase II oocy­
tes). Fertilization was found in 47% of the metaphase II oocytes after the ICSI procedure. The 
fertilization rate and embryo transfer after MESA/PESA and TESE was similar. Transfer of 2 
embryos into the uterine cavity was performed in 36/39 (92%) cases, resulting in a clinical 
pregnancy in 13/39 (33,3%). In 8 cycles, 26 supernumerous embryos of good morphological 
quality were cryopreserved. Thawing of the embryos was performed in 5 patients and 
transferred in 2 couples. This, however, did not result in clinical pregnancies. MESA/PESA 
produced 27% ongoing pregnancies, TESE resulted in 36% ongoing pregnancies. Finally, 10 
ongoing pregnancies occurred in 24 couples (41,1%), a pregnancy rate of 25,6% per treatment 
cycle and 27,7% per embryo transfer. Frozen-thawed epididymal spermatozoa were succes­
sfully used for a second ICSI procedure in 6 couples. Unfortunately, no pregnancy occurred 
in these patients. Amniocentesis was performed in eight out of 10 pregnancies, and gave 
normal karyograms in seven pregnancies. One pregnancy was terminated early for trisomy 18 
(47XX+18, Edwards syndrome). Nine deliveries occurred at term, resulting in 10 babies with 
no obvious congenital malformations. Birth weight was low in two children (<p50; 
Kloosterman, 1970), one singleton and one twin baby (Table 2).

Discussion

Ductal obstruction occurs in 20% of azoospermic patients. Acquired ductal obstructions 
account for most of the cases of obstructive azoospermia and can sometimes successfully be 
treated by microsurgery. Infections of the ductal system and surgery of the scrotum and the 
groin are the main causes of this type of obstructions. 
Surgical sperm retrieval has been introduced as an alternative for microsurgery and for 
treatment of failed vasectomy reversal. Initial attempts of IVF with epididymal sperm showed
limited results, due to poor sperm quality after retrieval (Temple-Smith et al., 1985; Pryor et al., 1984). In 1988 Silber introduced a microsurgical technique of sperm aspiration, the MESA procedure, which showed successful in 10 of 32 cases of CBAVD (Silber et al., 1988). The combined MESA/IVF procedure showed a pregnancy rate of 10 - 14% (The sperm microaspiration retrieval techniques study group., 1994). Recently, the ICSI procedure has been introduced for the treatment of severe male factor infertility (Palermo et al., 1992). Combining the ICSI procedure with the MESA procedure has resulted in high fertilization rate and pregnancies in 31 - 34% per treatment cycle (Devroey et al., 1994; Tournaye et al., 1994).

We have performed the ICSI procedure with surgically or percutaneously retrieved sperm from the epididymis (MESA/PESA) or the testis (TESE) in 39 treatment cycles. PESA was successful in 62% of cases and was introduced as a minimal invasive technique of sperm retrieval in an outpatient clinic setting under local anaesthesia (figure 1). PESA has been proved successful in several reports in the majority of cases of obstructive azoospermia (Tsirigotis et al., 1995; Collins et al., 1996; Craft et al., 1995). The advantages of PESA are: minimal discomfort for the patient, low complication rate compared to open surgery, repeatable procedure showing clear aspirated fluid with usually minimal blood contamination and less debris. PESA does not require microsurgical skills, is easy to learn and can be performed as an outpatient clinic procedure. In a recent report PESA was shown to be as effective as MESA with comparable pregnancy rates (Meniru et al., 1997).

In a series of 47 men PESA was unsuccessful in 11 cases and a subsequent MESA procedure was performed, showing viable sperm in only 2 cases (Tsirigotis et al., 1995). In cases of non-obstructive azoospermia MESA and PESA were usually unsuccessful and a testicular biopsy was performed.

Testicular biopsy could be an alternative for PESA as a source of sperm. Successful harvesting of spermatozoa has been reported with TESE using both the open biopsy technique and more recently the testicular fine needle aspiration (TEFNA), although needle biopsies showed less effective as compared to open biopsies (Friedler et al., 1997). Late complications have been described after testicular sperm retrieval techniques, including inflammation, haematoma and even devascularization of the testis (Schlegel et al., 1997). Therefore we believe that PESA should be performed first in cases of obstructive azoospermia.

We have chosen to treat only obstructive cases of azoospermia because of unknown medical and genetic risks of treating azoospermia due to testicular insufficiency. It has been shown that non-obstructive azoospermia can be associated with cytogenetic abnormalities, such as a
high number of sex chromosome aneuploidy in these men (Yoshida et al., 1995; Persson et al., 1996) and (micro)deletions of the Y chromosome (Reijo et al., 1995).

In this study nine cases of CBAVD were selected for this treatment. CBAVD is found in 2% of the infertile men and has recently been described as a primary genital form of cystic fibrosis. CBAVD is highly associated with mutations of the cystic fibrosis transmembrane conductance regulator gene (CFTR, Oates and Amos, 1994). Since CBAVD is accompanied by the absence of the seminal vesicles these men consistently produce a low volume and a low pH semen. Spermatogenesis in CBAVD appears normal, but sperms derived from the caput epididymis show to have a low fertilizing capacity, because of their short passage through the epididymis (Silber et al., 1988). In couples with CBAVD-related infertility genetic screening of the patient and his partner is mandatory before MESA/ICSI procedures are performed. The risk of inheritance of CF or CBAVD is mainly determined by the presence of a CFTR-gene mutation in the partner (1:25): if she also carries a mutation there is a 25-50% chance of a form of CF (severe, mild, CBAVD) in the offspring (Anguiano and Oates, 1992).

In all treated cases no common mutations of the CFTR gene were found in the female partners.

In conclusion, ICSI has been shown to give high number of fertilization rates with ejaculated, epididymal and testicular spermatozoa. We have combined ICSI with surgical and percutaneous sperm retrieval in men with bilateral obstruction of the genital tract, resulting in an ongoing pregnancy rate of 25.6% per treatment cycle. Non-obstructed cases of azoospermia were excluded from this treatment for potential genetic risks.

References


Table 1: Results of 39 ICSI cycle with epididymal (MESA/PESA), testicular (TESE) retrieved spermatozoa and cryopreserved (CRYO) spermatozoa after initial PESA procedure.

<table>
<thead>
<tr>
<th>Number of Cycles</th>
<th>Oocytes</th>
<th>MII oocytes</th>
<th>Fertilization (%)</th>
<th>Embryo transfer (%)</th>
<th>Pregnancy (%)</th>
<th>Ongoing pregnancy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MESA/PESA</td>
<td>22</td>
<td>275</td>
<td>186</td>
<td>101/186 (54)</td>
<td>21/22 (95)</td>
<td>9/22 (41)</td>
</tr>
<tr>
<td>TESE</td>
<td>11</td>
<td>127</td>
<td>90</td>
<td>32/90 (36)</td>
<td>9/11 (82)</td>
<td>4/11 (36)</td>
</tr>
<tr>
<td>CRYO (after PESA)</td>
<td>6</td>
<td>52</td>
<td>27</td>
<td>9/27 (33)</td>
<td>6/6 (100)</td>
<td>0/6 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>454</td>
<td>303</td>
<td>142/303 (47)</td>
<td>36/39 (92)</td>
<td>13/39 (33)</td>
</tr>
</tbody>
</table>

Table 2: Pregnancy and delivery results of 39 ICSI-procedures combined with surgical or percutaneous spermatozoa retrieval.

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Prenatal cytogenetics</th>
<th>Delivery terms</th>
<th>M/F</th>
<th>Birth weight (g)</th>
<th>Percentile (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>46,XX</td>
<td>38 weeks</td>
<td>F</td>
<td>3555</td>
<td>P90</td>
</tr>
<tr>
<td>2</td>
<td>46,XY</td>
<td>41 weeks</td>
<td>M</td>
<td>4110</td>
<td>P95</td>
</tr>
<tr>
<td>3</td>
<td>46,XY</td>
<td>37 weeks</td>
<td>M</td>
<td>2435</td>
<td>P15</td>
</tr>
<tr>
<td>4</td>
<td>46,XY</td>
<td>36 weeks</td>
<td>M</td>
<td>2660</td>
<td>P50</td>
</tr>
<tr>
<td>5</td>
<td>46,XX</td>
<td>35 weeks</td>
<td>F</td>
<td>2270</td>
<td>P40</td>
</tr>
<tr>
<td>(twin)</td>
<td>46,XX</td>
<td>35 weeks</td>
<td>F</td>
<td>2370</td>
<td>P50</td>
</tr>
<tr>
<td>6</td>
<td>47,XX+18 (trisomy 18)</td>
<td>Early termination</td>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>46,XX</td>
<td>42 weeks</td>
<td>F</td>
<td>4160</td>
<td>P95</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>41 weeks</td>
<td>F</td>
<td>3990</td>
<td>P90</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>41 weeks</td>
<td>F</td>
<td>4190</td>
<td>P90</td>
</tr>
<tr>
<td>10</td>
<td>46,XY</td>
<td>41 weeks</td>
<td>M</td>
<td>3760</td>
<td>P75</td>
</tr>
</tbody>
</table>

* Kloosterman et al., 1970.
GENERAL DISCUSSION
“Counseling in reproductive medicine start by obtaining an accurate diagnosis. The medical history, physical examination and various laboratory tests of both partners are generally required” (Rowe et al., 1993).

Aim of the thesis
The aim of this thesis was to optimize the diagnoses of obstructive male infertility and to evaluate treatment options and risk factors in these couples. Therefore, several studies were performed in men with either severe oligozoospermia or azoospermia with suspected obstruction of the male reproductive tract. Diagnostic studies focused on the true incidence of obstructions of the seminal path, the genetic background of severe male factor infertility and the associated abnormalities found in these men. Furthermore, the current treatment options of obstructions of the male reproductive tract were critically analyzed.

The introduction
A review is presented on the different factors associated with male infertility and the possible causes of unexplained male infertility are discussed. The etiology of male infertility is partially known and more research on the pathogenesis and treatment of unexplained male infertility is needed.

Male infertility is most often caused by testicular and epididymal failure and for this non-obstructive male infertility a new concept of testicular dysgenesis was introduced recently: a disruption of embryonal programming and gonadal development during fetal life can result in both reduced fertility, an increased risk of cryptorchidism, hypospadias and testicular malignancy in adulthood. Evidence for this concept comes from epidemiological observations (Skakkebaek et al., 2001). An increasing incidence of testicular dysgenesis, including testicular cancer, may reflect adverse effects of environmental factors, in particular those capable of disrupting hormonal balance in the developing testis. Future epidemiological studies in male reproductive health should focus on the different aspects of environmental and genetic influence on gonadal function and development (Rajpert-De Meyts et al., 2001). Despite marked progress that has been made in elucidating the genetic basis of male infertility, our current knowledge of the genes involved in gametogenesis and meiosis is still incomplete. Many genes are involved in the complex process of spermatogenesis, both autosomal and sex-chromosomal genes, and we are beginning to understand their functions from animal models (Cooke et al., 1998).
Gene defects, which might normally be lost or eliminated by natural means can be transmitted with ICSI to the offspring, since this technique bypasses the normal physiological mechanisms related to fertilization (Chandley et al., 1998). Also, in men with severe male factor infertility the number of aneuploid spermatozoa was found increased, particularly for the sex chromosomes (Martín et al., 1996). Infertile men, even with a normal karyotype, showed to have decreased chromosomal recombination and pairing during meiosis, resulting in meiotic arrest and non-disjunction of the sex chromosomes (Speed et al., 1990). Genetic studies, including karyotyping and counseling are essential in the work-up of these couples: if pregnancy is achieved, especially by ICSI, prenatal cytogenetic and DNA testing can be offered.

Azoospermia was reviewed, including epidemiology, classification, etiology, genetic factors and treatment. Obstructive and non-obstructive azoospermia as well as the congenital and acquired causes of obstructions of the seminal path were detailed. An algorithm of the diagnostic work-up of azoospermia and severe oligozoospermia is provided, used as the basis for the studies presented in this thesis.

Obstructions of the reproductive tract
Obstructions in azoospermic men are defined as men presenting with normally sized testes, normal hormonal evaluation and a testicular biopsy revealing normal spermatogenesis.
Subtotal obstruction of the reproductive tract is less well defined and, therefore, we prospectively investigated all men presenting with severe oligozoospermia for possible partial obstruction of the seminal path. Subtotal obstruction was found highly prevalent in men with severe oligozoospermia, normal size testes and normal FSH. Microsurgical repair next to epididymal sperm aspiration and ICSI may be considered in these couples. This concept needs further evaluation, confirming these first results in 78 men and comparing the results of microsurgery to ICSI, with and without surgical sperm retrieval, in men with severe oligozoospermia.

The cystic fibrosis gene and obstructive male infertility
Congenital bilateral absence of the vas deferens became defined as a genital form of cystic fibrosis, since 85% of these men are found to have at least 1 CFTR gene mutation, but most of them are without obvious non-genital symptoms of CF (Anguiano et al., 1992). We analyzed 21 men with CBAVD for symptoms of CF by performing an extensive medical history taking, physical examination, pulmonary function tests, sweat tests and rectal suction biopsies for
measuring interstitial chloride excretion. At least half of these men also have non-genital symptoms of CF. The group was quite heterogeneous in the different abnormalities found and no straightforward relationship between phenotype and genotype for CFTR was found. CFTR gene mutations are related to obstructive azoospermia with low seminal volume and pH (Mak et al., 2000). Some patients in our study had only partial absence of the vas deferens in the abdominal region. A wide spectrum of phenotypical variability of anomalies in the reproductive tract associated with both CF and CBAVD was recently described (Jarvi et al., 1998). Although virtually all CF males have CBAVD and are azoospermic, a scrotal vas deferens is present in 20% of men with CF. Furthermore, CFTR gene mutations were found in 47% of men with idiopathic epididymal obstruction. Thus, it seems that a broad spectrum of Wolffian duct abnormalities may be associated with CFTR gene mutations, even in men with ejaculatory duct obstruction (Meschede et al., 1998). Therefore, it seems prudent to screen all men with unexplained obstructive azoospermia, with and without CBAVD for CFTR gene mutations.

Impaired spermatogenesis in men with CBAVD was recently described (Meng et al., 2001). Apparently, defective CFTR function might influence spermatogenesis. Currently, we are investigating men with severe oligozoospermia for CFTR gene mutations by an extensive screening method of the CF-gene.

**Genetic risk factors associated with severe male infertility**

We were surprised to find 24% genetic risk factors in 150 infertile men screened for chromosomal abnormalities, Y chromosome deletions and CFTR gene mutations. Two men showed to have more than one risk factor. Our aim was to define subgroups of patients who need specific genetic tests by comparing the genetic screening results to the andrological evaluation. However, risk factors were found in men with and without male infertility associated factors.

A nationwide cytogenetic study in the Netherlands of 1792 males with severe oligozoospermia and azoospermia showed 4.0% chromosomal abnormalities (Tuerlings et al., 1998). Numerical sex chromosomal aberrations and Robertsonian translocations predominated. Y chromosomal deletions were found in 5-15% of the infertile men, depending on the patients selection and the number of sequence tagged sites studied (Simoni et al., 1999). These microdeletions have been linked to variable defects in spermatogenesis, ranging from Sertoli cell-only syndrome to spermatogenic arrest. Transmission of these mutations to male offspring was recently reported (unpublished results).
We showed that combining different genetic tests for screening purposes results in a high number of men carrying one or more genetic defects. The high ratio of anomalies found warrants genetic screening in all men applying for ICSI and not exclusively in case of unexplained male infertility.

**Ejaculatory duct obstructions**
The results of endoscopic treatment of ejaculatory duct obstructions were evaluated: simple incision of the prostatic cyst in men with ejaculatory duct obstruction resulted in only temporarily improvement of the semen in some men, but no spontaneous pregnancies occurred. Others achieved better results by combining deroofing of the midline prostatic cyst with transurethral opening of the ejaculatory ducts (Jarrow *et al*., 1996). More recently, reflux of urine into the seminal vesicles was reported after this more extensive procedure, with risk of infection of the reproductive glands. We therefore advise caution with this procedure as to prevent permanent damage to the reproductive glands. Until now, sperm aspiration with ICSI seems a better solution for these couples.

**Microsurgery**
A review on the different microsurgical approaches of obstructions of the male reproductive tract and their prognostic factors is presented. Our experience with 214 procedures showed that with adequate training patency can be expected in 80-90% of the men. Spontaneous pregnancies occur in about half of the patent couples, and in 25% of the men with older female partners, slightly better than the results of sperm retrieval and ICSI in these couples. In men with a long obstructive interval concomitant obstructions in the epididymis may be found and a vasoejididymostomy is indicated. Sperm motility was significantly reduced in men with a long obstructive interval, resulting in less spontaneous pregnancies and more indications for artificial reproductive techniques. Efforts are needed to improve microsurgical techniques and urologists with an interest in andrology should be encouraged to learn the different microsurgical techniques. The operations are ideally performed in a center for reproductive medicine, thus allowing different options to be performed in selective cases.

**Sperm retrieval and ICSI**
We analyzed the first results in our center of surgical and percutaneous sperm retrieval with ICSI in men with CBAVD and failed microsurgery. Percutaneous sperm retrieval of epididymal sperm showed successful in 60-70% of the men and became the method of choice.
for epididymal sperm harvesting. Microsurgical epididymal sperm aspiration (MESA) was abandoned in favor of percutaneous epididymal sperm aspiration (PESA). In 1996 it was decided to stop this treatment in the Netherlands, because of uncertainties about the possible transmission of genetic abnormalities from sperm derived from the epididymis and the testis. This treatment moratorium lasted for 5 years and was recently partially lifted in favor of epididymal sperm aspiration and ICSI in case of obstructive azoospermia. Research on epididymal spermatozoa showed no increase in DNA damage in epididymal spermatozoa compared to ejaculated spermatozoa from oligozoospermic men.

The role of the andrologist
Andrology is the subspecialty that deals with male reproductive health. The main topics of andrology are male infertility, male sexual dysfunction, male contraception and hormonal replacement therapy in ageing males. Andrology can contribute to reproductive medicine by studying the pathophysiology of disturbed spermatogenesis, epididymal insufficiency and obstructions of the male reproductive tract. This should lead to causative treatment for male infertility, risk assessment for transmission of genetic abnormalities and preventive measurements for optimal gonadal development. For the future, treatment of couples with infertility should be concentrated in joint clinics for reproductive medicine, combining experts in the field of reproductive health, clinical genetics and sexology for optimal patient care.

References


Obstructive male infertility
Male infertility due to obstructions of the reproductive tract is present in 10-20% of the men with azoospermia. In this thesis diagnosis and treatment of obstructive male infertility are discussed.

Obstructions of the male reproductive can be classified into congenital and acquired forms of ductal obstruction. Epididymal obstruction can be the consequence of blocks in the capital part of the organ, also known as Young's syndrome, infections of the epididymis and surgical damage to the organ. Vas deferens obstructions are usually the result of previous surgery, like vasectomy, hernia repair and orchidopexia. In 1-2% of the men with azoospermia congenital bilateral absence of the vas deferens is found (CBAVD), a genital form of cystic fibrosis. Ejaculatory duct obstruction can be caused by cystic lesions in the prostate, like Mullerian cysts, and prostatitis.

Clinical aspects
The clinical picture of men with bilateral obstruction of the reproductive tract shows azoospermia, normal testicular volume and normal gonadotrophins. Sometimes, severe oligozoospermia is found together with normal physical examination and normal follicle stimulating hormone (FSH). We routinely performed testicular biopsies in men with less than 1 million spermatozoa/ml in their semen analysis and found normal spermatogenesis to be present in 50%. Subtotal obstruction of the male reproductive tract was found to be a frequent cause of severe oligozoospermia, especially in men with normal testicular volume and normal FSH. Next to artificial reproductive techniques (ART), like intracytoplasmic sperm injection (ICSI), surgical treatment with vasography and vaso-epididymostomy should be considered in these cases. Microsurgical repair can be performed in 80% of the men with normal spermatogenesis and azoospermia or severe oligospermia and is effective in 50-60% of cases. Natural conception and less invasive forms of ART are feasible in these couples with lower costs and less complications during pregnancy. Also, the potential risks of transmitting an inheritable disease is not increased as in ICSI, due to the absence of natural sperm selection. Therefore, surgical sperm retrieval and ICSI in case of obstructive male infertility should be reserved for patients that appear surgically irreparable.

Congenital bilateral absence of the vas deferens
A form of irreparable obstruction is congenital bilateral absence of the vas deferens. Since CBAVD and cystic fibrosis (CF) share the same genetic background we investigated men
with CBAVD for symptoms of CF and found that defective cellular chloride transport to be present in 11/21 patients. Furthermore, CF-related symptoms, like obstructive lung disease and pancreatic insufficiency was found in 6 men, thus showing that a majority of men with CBAVD actually have a subclinical form of cystic fibrosis. Cystic fibrosis gene mutations were found in 66% of CBAVD patients. Genetic testing and counselling is clearly indicated for these couples seeking pregnancy through epididymal or testicular sperm aspiration and ICSI. If the partner of the CBAVD patient also carries a CF-gene mutation the situation becomes complex, as no precise prediction can be made on the different phenotypes of CF (CBAVD, mild CF, severe CF) for most CF-gene mutations.

Other genetic risk factors involved in infertile men with severe oligozoospermia and azoospermia are constitutive chromosomal abnormalities and microdeletions of the Y chromosome (Azoospermia factor region, AZF). In a cohort of 150 men screened for CF-gene mutations, cytogenetic abnormalities and AZF deletions of the Y chromosome, a genetic risk factor was found to be present in 17% of men with severe oligozoospermia and 34% of men with azoospermia. Application of ICSI in the couples can result in offspring with an enhanced risk for unbalanced chromosome complement, male infertility due to the transmission of a Y chromosome microdeletion, and a form of cystic fibrosis (CF, CBAVD). Therefore, genetic testing and counselling is indicated for all couples seeking reproduction through ICSI.

**Ejaculatory duct obstruction**

Obstructions of the ejaculatory ducts are characterized by severe oligozoospermia or azoospermia and a low seminal volume. On transrectal ultrasound investigation cystic lesions of the prostatic gland and dilatation of the seminal vesicles can be found. In a follow-up study of 11 cases treatment of obstructions of the ejaculatory ducts in men with midline prostatic cysts showed unsuccessful. Although improvement of seminal volume was observed in some men, transurethral deroofing of the midline prostatic cyst did not show any effect on sperm concentration and did not result in any durable effect or spontaneous pregnancy. The poor efficacy of this operation might be due concomitant epididymal obstructions or secondary stenosis/fibrosis of the ejaculatory ducts after marsupialization of the cyst. Also, the prostatic cyst might not be the cause of the obstruction. Resection of the wall of the cyst might improve the results of transurethral resection of the ejaculatory ducts in some men.

**Microsurgery**
Vasectomy is a popular procedure in Western Europe: about 10-15% of the men rely on this form of definitive contraception. Due to the high divorce rate in most western countries the demand for vasectomy reversal is increasing. A vasovasostomy (VVS) is performed in 6% of the vasectomised men. We have presented our results of the microsurgical VVS procedure in a review of the literature on microsurgical repair of the male reproductive tract. In a series of 223 VVS procedures patency was accomplished in 84% and spontaneous pregnancy occurred in 50% of the couples, if the interval between vasectomy and reversal was less than 10 years and the age of the female partner was <37 years. The procedure was less successful in couples with a long interval and with older female partners; patency was still 66%, but spontaneous pregnancy decreased to 22%. These results are similar to the results of surgical sperm retrieval and ICSI, but the cost are much in favour of microsurgery. Also, simultaneous treatment of the partner and multiple pregnancies are avoided as in IVF/ICSI. Microsurgical repair of obstructive male infertility is a challenge for the physician, but training and experience are required for good results. Fertility can often be restored, thus avoiding the need for ART. The procedure can be combined with sperm aspiration and cryopreservation, to be used for ICSI in cases of failed microsurgery. Urologists with an interest in Andrology should be encouraged to learn microsurgery as part of their surgical training. The operations are best performed in centres for reproductive medicine, allowing different options to be performed in selective cases.

**Sperm retrieval and ICSI**

Finally, we analysed the results of surgical sperm retrieval and ICSI in 39 cycles performed in 24 couples. The sperm aspiration procedure was performed in men after failed microsurgery, in men with CBAVD and in men with a history of genital infections, which resulted in multiple obstructions of the reproductive tract. Initially, we performed microsurgical epididymal sperm aspiration (MESA) as a primary procedure for sperm harvesting and testicular sperm extraction (TESE) in case no spermatozoa could be aspirated from the epididymis. The MESA procedure was abandoned in favour of percutaneous epididymal sperm aspiration (PESA), because this procedure showed equal results in sperm harvesting with less discomfort for the patient. Fertilization occurred in 47% of the metaphase II oocytes; embryo transfer was performed in 92% of the procedures and resulted in ongoing pregnancies in 10/39 (25%) procedures. One pregnancy was terminated early for a fetal cytogenetic abnormality. The other nine pregnancies resulted in the live birth of 10 children, without any
congenital abnormalities. Non-obstructed cases of azoospermia were excluded from this treatment due to their potential genetic risks for the offspring.
SAMENVATTING
Obstructies van de mannelijke tractus genitalis

Ongewenste kinderloosheid wordt gevonden bij circa 15% van de paren. In de helft van de gevallen is er sprake van een mannelijke stoomis en meestal gaat het om een afwijking van het sperma. Bij 10-20% van de mannen met vruchtbaarheidsstoornissen zijn er geen zaadcellen aanwezig in het sperma als gevolg van een verstopping van de zaadwegen. Dit proefschrift behandelt de diagnostiek en behandeling van obstructies van zaadwegen, de tractus genitalis.

Obstructies van de tractus genitalis van de man kunnen worden onderverdeeld in aangeboren afwijkingen en verkregen vormen van zaadwegverstoppling. Verstoppingen van de bijbal (epididymis), waar de zaadcellen uitrijpen om uiteindelijk de eicellen te kunnen bevruchten, kunnen het gevolg zijn van een aangeboren aanlegstoornis van de afvoergangen in de kop van het orgaan, het zgn. Young's syndroom. Verkregen vormen van bijbalobstructie zijn vaak het gevolg van infecties of eerder uitgevoerde operaties in het scrotum. Obstructies van de zaadleiders worden meestal veroorzaakt door chirurgische behandelingen, zoals sterilisatie (vasectomie), liesbreukcorrectie en operaties voor niet-ingedaalde testikels. Van alle mannen met vruchtbaarheidsstoornissen wordt 1-2% geboren zonder zaadleiders (vas deferens), een genitale vorm van taaislijmziekte (cystische fibrose, CF). Verstoppingen van het laatste deel van de zaadwegen, de ductus ejaculatorius worden veroorzaakt door cystes van Müllér in de prostaat, die de afvoergang van het sperma geheel of gedeeltelijk kan dichtdrukken, en door ontstekingen van de prostaatklier.

Klinisch beeld

Kenmerkend voor totale obstructie van de zaadwegen is het volledig ontbreken van zaadcellen in het sperma, terwijl het volume van de testikels normaal is en hormoononderzoek geen afwijking oplevert. Wij hebben onderzoek gedaan naar mannen met een zeer gering aantal zaadcellen in het sperma om te onderzoeken of er wellicht sprake is van een bijna totale obstructie. Bij alle mannen met minder dan 1 miljoen zaadcellen/ml in het sperma werd weefselonderzoek (biopsie) verricht van de testikels. Bij 50% van de mannen was er sprake van een normale spermaproductie in de testis. Obstructies van de zaadwegen zijn dus een vaak voorkomende oorzaak van ernstige sperma-afwijkingen, vooral bij mannen met normale testikels en normaal hormoononderzoek. Naast geassisteerde voortplantingstechnieken, zoals intracytoplasmatige sperma injectie (ICSI), bestaat bij deze mannen ook de mogelijkheid om operatief de obstructie te behandelen en zo de spermakwaliteit te verbeteren, zodat een spontane zwangerschap tot de mogelijkheden behoord en ook minder ingrijpende
voortplantingstechnieken, zoals inseminatie, kunnen worden toegepast. Microchirurgische behandeling van obstructies is mogelijk bij ongeveer 80% van de mannen met een normale spermaproductie en leidt bij 50-60% van deze mannen tot een verbetering van de spermakwaliteit. Naast een reële kans op een spontane zwangerschap en lagere totale kosten, is ook het risico voor overdracht van erfelijke aandoeningen kleiner dan bij de ICSI behandeling. ICSI dient daarom alleen te worden toegepast bij obstructies die niet toegankelijk zijn voor chirurgische behandeling of na een mislukte operatieve ingreep.

**Congenitale afwezigheid van het vas deferens**

Een vorm van aangeboren obstructie die niet chirurgisch gecorrigeerd kan worden is de congenitale bilaterale afwezigheid van het vas deferens (CBAVD), een genitale vorm van cystische fibrose. Omdat CBAVD en CF genetisch verwant zijn, is door ons onderzoek gedaan naar verschijnselen van taaislijmziekte bij mannen zonder zaadleiders. Het bleek dat 11/21 onderzochte mannen op subklinisch niveau verschijnselen hadden van CF en dat bij 6 mannen ook afwijkingen er ook afwijkingen van de luchtwegen of de alvleesklier aanwezig waren. CBAVD is dus bij een belangrijk deel van deze mannen een milde vorm van cystische fibrose. Bij 66% van de onderzochte mannen werd een of twee afwijkingen van het CF-gen gevonden, die overdraagbaar zijn aan het nageslacht. Het is daarom van groot belang om genetisch onderzoek van de patiënt en zijn partner te verrichten, voorafgaand aan zaadcelaspiratie en ICSI. Als ook de partner draagster blijkt te zijn van een CF-gen mutatie, zoals voorkomt bij 3-4% van de westersse bevolking, wordt de situatie complex: er bestaat dan een kans van 25-50% op een kind met een vorm van taaislijmziekte. Helaas is niet nauwkeurig te voorspellen welke vorm van CF (ernstig, mild, CBAVD) past bij welke mutaties.

Andere genetische risicofactoren bij mannen met azoospermie en ernstige oligozoospermie zijn cytogenegetische afwijkingen en deleties van het Y-chromosoom, de "Az oospermia factor" (AZF) regio. In een cohort van 150 mannen werd genetisch onderzoek verricht naar mutaties van het CF-gen, cytogenegetische afwijkingen en deleties van het Y-chromosoom: er werden 34% afwijkingen gevonden bij mannen met azoospermie en 17% afwijkingen bij mannen met ernstige oligozoospermie. In deze situatie bestaat een verhoogde kans op nageslacht met een vorm van taaislijmziekte, onvruchtbaarheid bij mannelijke nakomelingen die de AZF deletie overerven en multipale congenitale afwijkingen bij sommige cytogenegetische afwijkingen. Daarom dient in geval van ICSI altijd genetisch onderzoek te worden verricht, voorafgaande aan de behandeling.
Obstructies van de ductus ejaculatorius

Obstructies van de ductus ejaculatorius worden gekenmerkt door azoospermie of ernstige oligozoospermie en een klein spermavolume. Transrectaal onderzoek van de prostaat en de zaadblaasjes laat bij deze mannen regelmatig een cyste zien in de prostaat en stuwing van de zaadblaasjes. Een vervolgonderzoek van 11 mannen die werden behandeld voor een midline cyste in de prostaat liet weinig of geen verbetering zien van de spermakwaliteit: alleen bij enkele mannen werd een tijdelijke verbetering gezien van het spermavolume. Enige blijvende verbetering of zwangerschap werd niet waargenomen. Het teleurstellende resultaat van deze behandeling kan worden verklaard doordat bij een aantal mannen er mogelijk ook een obstructie van de epididymis bestaat of omdat de behandeling heeft geleid tot een nieuwe vernauwing door littekenvorming. Ook kan het zo zijn dat de prostaatcyste niet de oorzaak van de obstructie was. Mogelijk is het resultaat te verbeteren door niet alleen de cyste te openen, maar ook de wand te openen van de cyste waar de ductus ejaculatorius loopt.

Microchirurgie

Vasectomie (sterilisatie van de man) is een populaire vorm van definitieve anticonceptie in de meeste West-Europese landen: 10-15% van de mannen laat zich steriliseren. Als gevolg van de toename van het aantal echtscheidingen neemt ook de vraag naar hersteloperaties na vasectomie, de zgn. vasovasostomie, toe: bij 6% van de gesteriliseerde mannen wordt deze hersteloperatie uitgevoerd. In een literatuurstudie naar de resultaten van microchirurgische behandeling van mannelijke onvruchtbaarheid hebben wij de resultaten gepresenteerd van 223 hersteloperaties: doorgankelijkheid werd bereikt bij 84% van de mannen en een spontane zwangerschap trad op bij 50% van de partners, indien de sterilisatie korter dan 10 jaar geleden was uitgevoerd en de partner jonger was dan 37 jaar. Bij een langer interval en een oudere partner daalde de resultaten naar 66% doorgankelijkheid en 22% spontane zwangerschappen, vergelijkbaar met de zwangerschapsresultaten bij in vitro fertilisatie/ICSI. De voordelen van microchirurgie zijn echter: lagere kosten per zwangerschap, geen belastende hormonale behandeling van de partner en waarschijnlijk minder genetische risico’s voor het nageslacht. Microchirurgische behandeling van mannelijke infertiliteit is een uitdaging voor de uroloog en goede resultaten worden pas verkregen na voldoende ervaring en zorgvuldige techniek. Urologen met belangstelling voor Andrologie dienen gestimuleerd te worden om microchirurgie te leren als deel van de opleiding. Deze operaties dienen bij voorkeur te
worden verricht in een centrum voor voortplantingsgeneeskunde, zodat verschillende opties aanwezig zijn voor specifieke probleemgevallen.

**Sperma aspiratie en ICSI**

Tot slot, hebben wij de resultaten geanalyseerd van 39 gecombineerde zaadcelaspiratie en ICSI behandeling bij 24 paren. Deze behandeling werd toegepast bij mannen met een mislukte microchirurgische behandeling, bij congenitale afwezigheid van de zaadleiders en bij mannen die onvruchtbaar zijn geworden als gevolg van een genitale infectie met meerdere obstructies van de zaadwegen. In eerste instantie werd gekozen voor de microchirurgisch epididymale sperma aspiratie (MESA) om zaadcellen te oogsten of voor de testiculaire sperma extractie (TESE) indien er geen zaadcellen werden gevonden in de bijbal. Echter, de MESA werd al na 4 behandelingen vervangen door de even effectieve percutane epididymale sperma aspiratie (PESA), die als belangrijk voordeel heeft de minimale belasting voor de patiënt. Bevruchting trad op bij 47% van de metafase II eicellen en bij 92% van de paren werd 1 of meerdere embryo’s terug geplaatst. Een doorgaande zwangerschap trad op bij 10/39 paren (25%) en resulteerde in 9 bevallingen van 10 gezonde kinderen. Een zwangerschap werd voortijdig beëindigd vanwege een cytogenetische afwijking van de foetus, ontdekt door middel van een vruchtwateronderzoek. Deze behandeling werd alleen toegepast bij mannen met een aangetoonde obstructie van de zaadwegen.
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List of publications
International


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