Issue 3

# Infertility and its management

*Approaches and perspectives*

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† Schindler AE. Progestational effects of dydrogesterone *in vitro, in vivo* and on the human endometrium. *Maturitas*. 2009;65(1):S3-S11. * Data on file. ‡ Internal calculations based on Quintiles IMS database, IMS Health Analytics Link MAT03 2017.

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**Contraindications:** Known hypersensitivity to the active substance or to any of the excipients. Known or suspected progestogen dependent neoplasms. Undiagnosed vaginal bleeding. Contraindications for the use of estrogens when used in combination with dydrogesterone. **Warnings and Precautions:** Before initiating dydrogesterone treatment for abnormal bleeding, the etiology for the bleeding should be clarified. Breakthrough bleeding and spotting may occur during the first months of treatment. If breakthrough bleeding or spotting appears after some time on therapy, or continues after treatment has been discontinued, the reason should be investigated, which may include endometrial biopsy to exclude endometrial malignancy. **Pregnancy and Lactation:** Pregnancy: It is estimated that more than 10 million pregnancies have been exposed to dydrogesterone. So far there were no indications of a harmful effect of dydrogesterone use during pregnancy. Some progestogens have been reported in the literature to be associated with an increased risk of hypospadias. However due to confounding factors during pregnancy, no definitive conclusion can be drawn regarding the contribution of progestogens to hypospadias. Clinical studies, where a limited number of women were treated with dydrogesterone early in pregnancy, have not shown any increase in risk. No other epidemiological data are hitherto available. Effects in non-clinical embryo-fetal and post-natal development studies were in line with the pharmacological profile. Untoward effects occurred only at exposures which exceeded the maximum human exposure considerably, indicating little relevance to clinical use. Dydrogesterone can be used during pregnancy if clearly indicated. Breastfeeding: No data exist on excretion of dydrogesterone in mother's milk. Experience with other progestogens indicates that progestogens and the metabolites pass to mother's milk in small quantities. Whether there is a risk to the child is not known. Therefore, dydrogesterone should not be used during the lactation period. Fertility: There is no evidence that dydrogesterone decreases fertility at therapeutic dose. **Adverse Reactions:** The most commonly reported adverse drug reactions of patients treated with dydrogesterone in clinical trials of indications without estrogen treatment are migraines/headache, nausea, menstrual disorders and breast pain/tenderness. Undesirable effects that are associated with an estrogen-progestogen treatment : Breast cancer, endometrial hyperplasia, endometrial carcinoma, ovarian cancer • Venous thromboembolism • Myocardial infarction, coronary artery disease, ischemic stroke. Issued on: 3/4/14. Source: Prepared based on full prescribing information (version 03) dated 13/03/2015.

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# Preface

Infertility is a medical condition which is estimated to affect 8-12% of the couples in the reproductive age group. Apart from emotional challenges and stress it adds to interpersonal relationships, there is also social stigma that is attached to childless couples, particularly in the developing nations such as India. However, over the last couple of years the science of reproductive medicine has experienced a true revolution, thereby making it possible for several childless couples to realize their dream of parenthood.

"Infertility and its Management-Issue-3" is an educational initiative which has been developed to impart up-to-date information on various aspects related to the diagnosis and management of infertility in men and women. Fibroids are common benign gynecological tumors which can cause infertility. The first chapter offers guidelines on when to treat and how to treat fibroids. The technology of assisted reproduction is rapidly evolving and its latest advances are enhancing the possibility of pregnancy in situations which were previously considered impractical for pregnancy. The comprehensive overview of the available ARTs and their suitability, advantages, and disadvantages are discussed in the next chapter. Anovulation, which is majorly attributed to polycystic ovarian syndrome, is the primary cause of infertility in 25% of the women seeking medical assistance. In the third chapter, the experts discuss a novel method called "hormonal wedge resection" to treat functional infertility associated with PCOS. The last chapter discusses preoperative preparation and postoperative care for patients undergoing sperm retrieval procedures, as well as the principles of selection among different sperm retrieval techniques for patients with obstructive and non-obstructive azoospermia.

We sincerely hope that the valuable insights provided in this input will help stimulate new ideas and perspectives and assist fertility specialists in providing the best care possible to childless couples.

Happy reading!

# Contents

# Uterine Fibroids in the Setting of Infertility: When to Treat, How to Treat?

**Erin I. Lewis[1,2], Antonio R. Gargiulo[1,2]**

We aim to provide insight on the treatment of fibroids in the infertile patient. Specifically, we discuss which fibroids, based on size and location within the uterine wall, have the most impact on fertility outcomes. In addition, we demonstrate which methods are best for treatment of fibroids in the infertile patient, focusing on minimally invasive techniques. Current research demonstrates that, in addition to submucosal fibroids, also intramural fibroids can have a negative impact on fertility via molecular and mechanical disruption of the endometrium and of normal uterine peristalsis. Certain intramural fibroids should be considered for removal or treatment in the infertile patient, depending on size and patient history. We also provide a large body of evidence demonstrating the safety and clinical advantages of minimally invasive techniques, such as hysteroscopy, laparoscopy, and robot-assisted laparoscopy in the treatment of uterine fibroids. All submucosal and many intramural fibroids interfere with uterine

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function. In the evaluation of the infertile patient, accurate fibroid mapping within the uterus is essential to identify those submucosal and intramural fibroids that are likely to have the most impact of fertility outcomes. The mainstay of treatment is surgery for those fibroids with the most detrimental impact. Nonsurgical alternatives such as magnetic resonance-guided focused ultrasound (MRgFUS) and radiofrequency volumetric thermal ablation (RFVTA) need further validation before their widespread adoption in infertile patients.

**Keywords**
Fibroids, Leiomyoma, Infertility, Myomectomy, Intramural fibroids, Submucosal fibroids

## Introduction

The contributory role of uterine fibroids in the etiopathogenesis of infertility has long been debated. Their presence among reproductive age women is particularly common, with an estimated 20–40 % women harboring one or more of these benign solid tumors [1]. By age 40, approximately 50 % of women have fibroids, and by menopause, almost 70 % of white women and 80 % of African American incur this pathology [2•, 3]. Literature from the 1980s estimated that fibroids existed in 5–10 % of infertile women by physical examination, but only 2–3 % of subfertility could be attributed to their presence [4]. In the last 30 years, we have advanced our diagnostic ability through ultrasonography and magnetic resonance imaging (MRI), with more recent literature estimating 12.6 % of women undergoing IVF treatment and over 25 % of older women receiving donated oocytes have fibroids [5]. The impact of fibroids on fertility is becoming increasingly relevant given that since 2007, the largest increase in birth rates (15 %) is seen in women aged 40–44 years and is at its highest rate since 1966 [6]. This is a fundamental concept in modern gynecological care: we now diagnose more fibroids in more women who have not yet completed childbearing. The conundrum of when, and to what extent, to intervene

to remove this pathology has become one of the most common in our practice. Patient counseling is complex, and it must be based on updated clinical and scientific knowledge. Indeed, even if conservative surgery for uterine fibroids is generally considered to be one of the most complex in gynecology, it is correct patient counseling that is the hardest to provide.

With our advances in the diagnosis of fibroids through imaging, we have expanded our understanding of how fibroids may affect fertility through molecular and genetic mechanisms and physiologic changes. Most importantly, we have elucidated which fibroids have the most impact on reproductive outcomes by evaluating their specific location within the uterine wall and size. With the advent of the International Federation of Gynecology and Obstetrics (FIGO) classification system, we are able to better study and delineate different subtypes of fibroids (Fig. 1) [7•]. Along with updates on the above topics, we describe in this review the most effective treatment of fibroids for reproductive outcomes, emphasizing minimally invasive approaches. In this advancing field, we have still not answered all the questions regarding fibroids and infertility with finality but have improved upon our ability to know when to treat and how to treat.

## Mechanisms of Action: Molecular and Physiologic Effects of Fibroids

The explanation for the detrimental effect of uterine fibroids on fertility and pregnancy outcome has long remained elusive, until molecular research began describing specific changes in the endometrium of women with fibroids. The homeobox-containing transcription factor essential for embryonic uterine development and endometrial receptivity, *Hoxa10*, has been found to be critical for implantation and is expressed throughout the menstrual cycle in humans. In *Hoxa10* deficient mice, embryos are produced and can implant in wild-type surrogates, but these same embryos are unable to obtain successful implantation in *Hoxa10* deficient mouse uteri [8]. In 2010, Rackow et al. described substantially decreased *Hoxa10* mRNA in endometrial biopsies from women with submucosal fibroids compared to women without fibroids in the proliferative stage of the menstrual cycle. Endometrial tissue from women with intramural fibroids showed a trend towards less *Hoxa10* mRNA, which was not

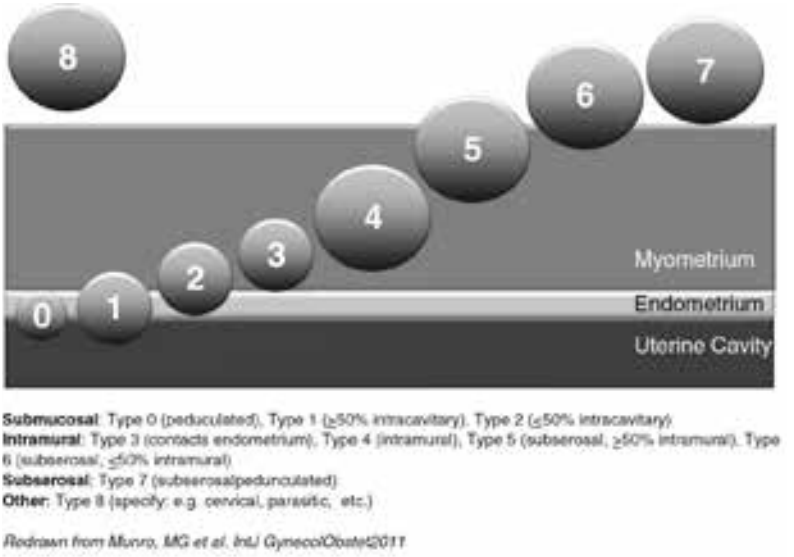


**Fig. 1.** FIGO classification of uterine fibroids.

statistically significant [9]. A more recent study in 2016 by Makker et al. analyzed biopsies from mid-secretory endometrium in women with a single intramural fibroid (mean size 5.57 ± 0.37 cm) alongside fertile controls, looking at *Hoxa10* and *Hoxa11*, which have both been described as endometrial receptivity markers. Those participants with an intramural fibroid had a significant decrease in *Hoxa10* mRNA [10]. Three months after myomectomy for intramural fibroids, Hoxa10 and Hoxa11 mRNA expression in endometrial tissue has also been shown to increase 12.8- and 9.0-fold, respectively [11].

Other studies have investigated bone morphogenetic protein 2 (BMP-2), a growth factor regulating cell proliferation and differentiation found to be critical to endometrial decidualization. Endometrial stromal cells (ESC) isolated from 12 women with either submucosal or intramural fibroids were found to secrete threefold less BMP-2 than ESC from healthy controls [12]. These recent studies demonstrate that both submucosal and intramural fibroids alter the molecular make-up of the endometrium, largely decreasing the production of endometrial factors important for implantation. One important factor to consider when looking at these studies is that FIGO type 3 myomata, which touch the endometrium but

not enter the endometrial cavity, are usually considered as part of the intramural myoma population but are probably a different condition altogether. They are likely more similar to FIGO types 0, 1, and 2 under the standpoint of the molecular impact on the overlying endometrium. Future studies will have to focus on FIGO type 3 myomata as a unique and separate entity.

Another theory to explain the deleterious effect of fibroids on reproductive outcomes focuses on the altered contractility and mechanics of the uterus. Specifically, alterations in blood flow, lower resistance index (RI), and pulsatile index (PI) have been found in women with fibroids distorting the cavity on day of embryo transfer. This alteration was also correlated with decreased clinical pregnancy rate compared to women with non-cavity-distorting fibroids [13]. Along with vascular changes, abnormal peristalsis of the uterine wall has been detected in uteri with fibroids, not just cavity-distorting fibroids. In the normal nonpregnant uterus, peristaltic waves run from the fundus to the cervix to clean out the cavity in the early follicular phase. In the late follicular phase and periovulatory phase, peristalsis occurs in the opposite direction, from the cervix to the uterus, likely for transport of sperm to the fallopian tube. Finally, in the mid-luteal phase, the uterus appears to slow down peristaltic movements to facilitate embryo implantation [14]. Two studies have utilized ultra-fast MRI to compare fibroid uteri with normal uteri and have found abnormal peristaltic patterns. Specifically, uterine peristalsis was significantly decreased in women with fibroids, supporting the theory that fibroids prevent "subtle wave conduction" in myometrial muscle [15]. In addition, uterine peristalsis was noted during the mid-luteal phase or "implantation window" in women with fibroids whereas was absent in controls [16]. Yoshino et al. found that women with fibroids who exhibited high levels of uterine peristalsis during the implantation window (luteal phase days 5–9) and who subsequently underwent myomectomy ceased to have abnormal peristalsis. These previously infertile women ($n$ = 15) who had a myomectomy and resumed normal uterine peristalsis attempted conception after surgery with a 40 % success rate [17]. Current research suggests that fibroids, including intramural fibroids, alter the physical environment of the uterus, making it much more difficult for implantation to occur due to abnormal vascular flow and peristaltic movement.

## Fibroids and Infertility: Location Matters

The location of the fibroid within the uterine wall has been a critical part of deciding whether or not to treat in the setting of infertility. Several studies have tried to elucidate which fibroids need treatment in the setting of infertility, but their findings are often disparate, largely due to methodologic flaws such as small sample size and inconsistent diagnostic imaging and evaluation of the uterine cavity [18•]. Another factor in the often contradictory literature regarding which fibroids should be treated stems from the fact that infertility is often multifactorial, and fibroids might be just one of many variables impeding conception in a given couple. Utilizing the FIGO classification for myomas (Fig. 1), we are making steps to advance our understanding which fibroids have the most effect on implantation and are getting closer to outlining a more definitive treatment algorithm for fibroids in the setting of infertility.

### Submucosal Fibroids (FIGO 0–2)

The most definitive research involves the effect of submucosal (FIGO 0–2) fibroids on infertility. Three systemic reviews in the last decade have all demonstrated that submucosal fibroids are associated with infertility [5, 18•, 19]. Klatsky et al. in 2008 showed that submucosal fibroids had the strongest association with lower ongoing pregnancy rates, compared to other types of fibroids, with an odds ratio (OR) of 0.5 and 95 % confidence interval (CI) of 0.3–0.8. Specifically, implantation rates decreased from 11.5 to 3.0 % and ongoing pregnancy rates decreased from 30 to 14 % in women with submucosal fibroids. Miscarriage rates for this group were also increased from 22 to 47 % [5]. In the largest systematic review to date, Pritts et al. in 2009 confirmed that clinical pregnancy rate, implantation rate, and ongoing pregnancy/live birth rate were all significantly decreased by submucous fibroids, while spontaneous abortion rate was increased [18•]. Lastly, Somigliana et al. in 2011 showed that submucosal lesions appear to strongly interfere with the chance of pregnancy with the OR (95 % CI) for conception and delivery at 0.3 (0.1–0.7) and 0.3 (0.1–0.8), respectively. What is lacking in these studies is the separate analysis of FIGO type 3 fibroids (abutting but not entering the endometrial cavity) [19]. Given the new molecular data implicating both submucosal

and intramural fibroids having deleterious effects on the endometrium, no doubt these FIGO type 3 fibroids would also negatively impact implantation.

Unfortunately, the one randomized matched control trial evaluating removal of submucous myomas on pregnancy rates was published in 2010 but retracted in 2011 [20]. We have yet to see other prospective randomized trials published in this area, but given the strong and consistent data from retrospective and observational studies in the last decade, we recommend removal of submucosal fibroids in the setting of infertility or recurrent pregnancy loss.

## Intramural (FIGO 3–5) and Subserosal Fibroids (FIGO 6–7)

Early literature from the 1990s repeatedly found that intramural fibroids did not affect clinical pregnancy rates. In fact, a meta-analysis in 2001 by Pritts et al. failed to find an adverse effect on pregnancy rates in women with fibroids not encroaching on the endometrial cavity [21]. When Pritts et al. reanalyzed this subgroup in a 2009 systematic review of 23 studies, intramural fibroids did in fact negatively affect implantation rate, ongoing pregnancy/live birth rate, and increased spontaneous abortion rate [18•]. Subserosal fibroids have never been linked to infertility, with a systematic review of 11 trials in 2007 evaluating the effect of subserosal tumors, demonstrated no effect on clinical pregnancy rates or delivery rates [22]. Sunkara et al. in a 2010 systematic review focused on 19 observational studies focusing on intramural fibroids and found a significant decrease in the live birth (RR = 0.79, 95 % CI 0.70–0.88) and clinical pregnancy rate (RR = 0.85, 95 % CI 0.77–0.94) in women with these fibroids [23]. In conclusion, more recent literature has affirmed that intramural fibroids do have an adverse effect on fertility.

The debate still remains regarding the tumor size at which intramural fibroids have the most negative effect on fertility. In 2004, Oliveira et al. compared 245 women with intramural fibroids to healthy controls undergoing IVF-ICSI and found that when fibroids were >4 cm, there were significantly lower pregnancy rates [24]. Guven et al. in 2013 in a similar study found that intramural fibroids negatively affected implantation, with the mean fibroid diameter in his study population at 4.96 ± 1.3 cm [25]. Most recently, Yan et al. in 2014 found that when the largest diameter

of the fibroid was >2.85 cm, clinical pregnancy rate was not affected but delivery rate was [26]. Somigliana et al. in 2011 published that small intramural fibroids (mean diameter in his study was 2.2 ± 1.0 cm) did not impact clinical pregnancy rate or delivery rate [19]. Previous literature had found that fibroids >5 cm also affect birth outcomes, causing increased risk of prematurity, fetal malpresentation, and labor dystocia [27]. Given the convincing data regarding intramural fibroids, we recommend removal of these fibroids when they are >4 cm in the setting of infertility or failed ART cycles. Although data is less convincing, it might not be unreasonable to remove intramural fibroids >2.85 cm in the setting of recurrent pregnancy loss or multiple failed cycles with transfer of high-quality or euploid embryos.

## Surgical Treatment of Fibroids

Myomectomy is the mainstay of fibroid treatment in symptomatic women wishing to preserve their fertility. It is also the chosen treatment if fibroids are thought to be affecting a woman's fertility. Although evidence is mounting that both submucosal and intramural fibroids affect fertility, we lack definitive studies to indicate myomectomy improves a woman's ability to conceive. This is largely due to the heterogeneity of studies and nonrandomized nature of the literature at the present time. In 2012, a Cochrane review examined the surgical treatment of fibroids for subfertility found there was, "currently insufficient evidence from randomized controlled trials to evaluate the role of myomectomy to improve fertility" [28]. More recently, a Cochrane review in 2015 examining the effect of hysteroscopy in treating subfertility for uterine cavity anomalies concluded that a large clinical benefit cannot be excluded given that if 21 % of women with fibroids achieve a clinical pregnancy having timed intercourse only, with evidence suggesting that 39 % of women will achieve pregnancy after hysteroscopic fibroid removal [29]. The only well-controlled prospective (though nonrandomized) trial by Buletti et al. in 1999 suggests a beneficial role of laparoscopic surgery in fibroid removal. Women with 1–5 intramural fibroids (with at least one >5 cm) who underwent myomectomy had clinical pregnancy rates of 33 %, compared to 15 % in women who did not have surgical intervention [30].

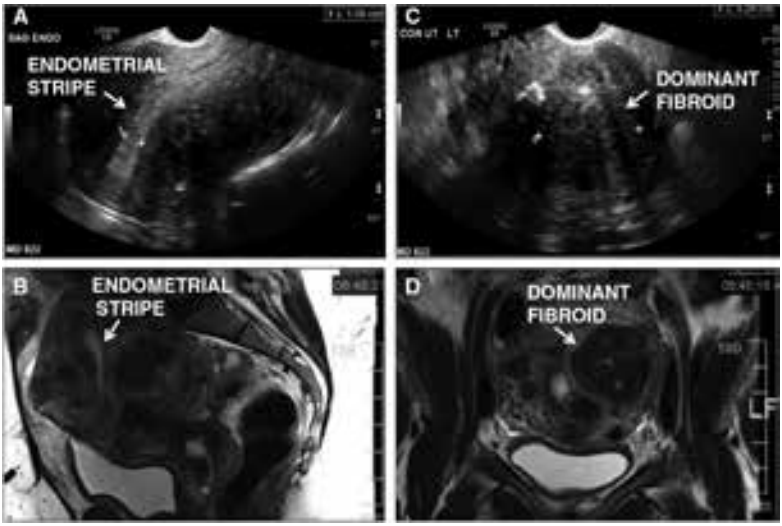


**Fig. 2.** Comparison of ultrasound and MRI in the diagnosis of fibroids in the same patient. **A** Ultrasound with depiction of endometrial strip. **B** MRI with depiction of the endometrial stripe and surrounding fibroids. **C** Ultrasound image of dominant fibroid. **D** Coronal view of MRI showing clear delineation of dominant fibroid as well as multiple other fibroids.

Although multiple reviews on fibroids and infertility agree that submucosal fibroids should be removed, they caution against the removal of intramural fibroids purely for infertility purposes, citing lack of evidence [31, 32]. We recommend that each case be evaluated individually. A recent study from Japan found that the beneficial effect on fertility with conservative treatment of submucosal and intramural fibroids plateaued at 1 year [33]. Given that reproductive age is still the most important determinant of successful live birth, we recommend myomectomy be performed sooner rather than later in women with intratumoral fibroids >4 cm with decreased ovarian reserve or advanced maternal age. At the same time, decision making regarding when to operate should only be done after thorough preoperative evaluation, and it is often dictated by the clinical presentation. All data and controversies aside, we must be intellectually honest and agree that basically every gynecologist in the world will look at the same exact 5-cm intramural myoma as an "innocent bystander" in a 28-year-old woman with a year of unsuccessful attempts at conception but as a "likely contributor to implantation failure" in a 38-year-old who failed two euploid embryo transfers: same tumor, same species, different clinical scenarios. This should bring all

of us to pause and drop our personal biases when faced with these clinical scenarios. In the age of patient-centered, personalized, medicine, the decision is the patient's. And the patient needs solid, simplified, data to make the decision. Communication of data in simple fashion is not easy, nor fast, and requires adequate imaging, for a start. We have long been strong advocates of high-quality pelvic imaging to assist in our surgical strategy: MRI is the imaging of choice for uterine fibroids, followed by high-quality 3D ultrasonography and sonohysterography, limited to smaller pathology [34•, 35] (Fig. 2). We make extensive use of MRI imaging in our counseling sessions, given their operator independent nature, which allows every person with basic knowledge of human anatomy to actually understand the relative size and location and tissue distortion caused by their tumors.

The decision whether to perform a hysteroscopic myomectomy versus a laparoscopic or robot-assisted laparoscopic myomectomy depends on thorough imaging. Transvaginal ultrasound (TVUS) is our first-line imaging modality, best done cycle days 5–9, for a detailed evaluation of the uterus [36]. TVUS has its limitations in that it cannot capture multiple large fibroids in the same plane (Fig. 2). We recommend utilizing ultrasound for imaging of relatively small uteri (contained within the bony pelvis) with four or fewer fibroids. Saline sonohysterograms and office hysteroscopy are particularly helpful in delineating intrauterine pathology [37]. With regard to when to operate with intramural fibroids, MRI has proven to be the most sensitive in detecting submucosal fibroids [38]. In addition, MRI is more reproducible compared to TVUS, which is provider dependent [39]. MRI allows for adequate fibroid mapping, enabling surgeons to plan the route of fibroid removal, and excludes non-fibroid pathology such as adenomyosis. The decision to proceed with surgery for infertility purposes should also be done with generous counseling reviewing the risks and limitations of undergoing surgery, with the knowledge that it might not improve fertility (or ART outcome) in some cases.

## Hysteroscopic Myomectomy

Fibroids with the FIGO classification 0, 1, or 2 have the optimal pathology for hysteroscopic resection. Limits of fibroid size depend on the comfort and experience of the operator, with most sources recommending the limit for hysteroscopic resection at 5 cm [40•]. Given hysteroscopic fluid limits,

there might need to be an interval resection of the fibroid (i.e., a second operation) if it cannot be safely removed in one sitting [40•]. When comparing the instrumentation of hysteroscopic fibroid removal, one recent meta-analysis compared hysteroscopic intrauterine morcellator (first described in 2005) versus the more traditional hysteroscopic resectoscope which has been used since the 1970s [41]. Shazly et al. found that hysteroscopic morcellation was associated with less incomplete removal of the fibroid and shorter operating times, although studies were small and meta-analysis was limited by heterogeneity [42]. At this time, we recommend that gynecologic surgeons employ the hysteroscopic device they have the most comfort with, until more concrete data demonstrates superior efficacy for one method versus the other.

Hysteroscopic myomectomy (HM) has the potential to cause further uterine cavity distortion with the formation of intrauterine adhesions (IUA). The patients with the highest risk of forming IUA are those that have hysteroscopic resection of two submucosal myomas that are opposing one another. One small study found that out of nine patients with two or more apposing submucous myomas undergoing diagnostic office hysteroscopy after surgery, seven (78 %) had IUA [43]. Another study found that IUA were found in 31.3 % of patients after removal of a solitary fibroid and in 45.5 % of patients after removal of a multiple intracavitary fibroids [44]. Meanwhile cold resection, without thermal energy, for small fibroids has been reported as having a 4 % rate of postsurgical adhesions [45]. If the size of the fibroid and/or bleeding during the surgery necessitates use of thermal energy, we recommend to use the least amount possible, with the goal to maintain as much normal endometrium as possible. In addition, an early postoperative look with office hysteroscopy, 2–4 weeks after myomectomy surgery, has been shown to be a preventative as well a therapeutic strategy to prevent long-lasting intrauterine adhesions [46]. We recommend this be a prerequisite before commencing infertility treatments.

## Minimally Invasive Myomectomy: Laparoscopic Versus Robot-Assisted Laparoscopic Myomectomy (RALM)

For those fibroids inoperable by hysteroscopy, namely FIGO types 3–5, and FIGO type 2 with minimal free myometrial margin, minimally

invasive surgery is recommended over open abdominal surgery. A large number of studies have detailed the improved surgical outcomes seen in laparoscopic myomectomy compared to abdominal myomectomies [47]. In addition, Palomba et al. in 2007 described improved reproductive outcomes with a minimally invasive approach. Those women undergoing laparoscopic versus minilaparotomic myomectomy were found to have higher cumulative pregnancy rates in the laparoscopic group (52.9 %) versus the mini-laparotomy group (38.2 %) [48•]. Similarly, a 2006 retrospective study looked at obstetric and delivery outcomes after laparoscopic myomectomies reported 158 pregnancies and no uterine ruptures during deliveries [49]. The risk of uterine rupture with laparoscopic myomectomies has been reported as quite low, with only one reported rupture in 2000 cases over 6 years [50]. Furthermore, analysis of cases of uterine rupture has been attributed to overuse of electrocautery and inadequate closure of the myometrial defect [40•, 51]. It appears that laparoscopic myomectomies may improve fertility over abdominal myomectomies, and there is no evidence that they have a negative impact on obstetric outcomes.

Laparoscopic surgery has made advances in terms of surgical and reproductive outcomes but is ergonomically challenging and technically difficult—and has yet to become widely adopted [52]. The introduction of RALM has enabled more surgeons to provide minimally invasive myomectomies to patients given its relatively fast learning curve [53]. Reproductive outcomes have consistently demonstrated the importance of incorporating RALM in the armamentarium of fibroid removal techniques in the setting of infertility. In women who underwent RALM for deep, symptomatic fibroids and unexplained infertility, the pregnancy rate after recovery was reported to be as high as 68 % [54]. In a 3-year follow-up after RALM surgery for the purpose of infertility, the pregnancy rate was 80 % in symptom-free patients [55]. RALM may also allow for a finer dissection of the fibroid tumor, with preservation of its pseudocapsule. A true intracapsular myomectomy has been shown to be critical for myometrial healing [56•]. Regardless of whether conventional or robot-assisted laparoscopy are utilized, we recommend attempting fibroid removal with a minimally invasive approach whenever safely feasible, to maximize reproductive outcomes and patient recovery.

### Medical Therapy: Presurgical Treatment

Preoperative treatment with gonadotropin releasing hormone agonists (GnRH-a) and selective progesterone receptor modulators (SPRMs) has been proposed to shrink the size of the fibroid prior to HM. Studies utilizing pretreatment with GnRH-a 2 or 3 months prior to surgery report operating times were reduced, and there was decreased hysteroscopic fluid resorption [57, 58]. SPRMs have also been used prior to HM and have demonstrated reduction in size of fibroids and decreased vaginal bleeding preoperatively [59]. When SPRMs (ulipristal acetate) and GnRH-a were compared head to head, there was no difference in operative time, amount of resection completed, or fluid deficit in hysteroscopic procedures [60].

In laparoscopic myomectomies, GnRH-a and SPRMs have also demonstrated utility in presurgical treatment. Most recently, a 3-month pretreatment with ulipristal acetate decreased intraoperative blood loss, hemoglobin drop, need for postoperative blood transfusion, and length of surgery compared the no pretreatment arm [61]. Similarly, Chang et al. found that pretreatment with GnRH-a analogue reduced intraoperative blood loss, operating time, formation of pelvic hematomas, and need for blood transfusion [62]. More studies are needed to confirm these findings and pharmacologic doses, but these medications have a potential to make surgeries less challenging and safer for patients.

## Alternatives to Surgical Treatment

### Uterine artery Embolization

Since the 1990s, uterine artery embolization (UAE), whereby the fibroids shrink due to blockage of arterial blood flow and its resulting necrosis, has been touted as a surgical alternative to myomectomy. The procedure utilizes fluoroscopic guidance to pass a catheter from the femoral vessels to the uterine arteries where embolizing agents are then released. This therapy leads to shrinkage of the dominant fibroid by about–40 %, and about 80 % of women have relief of symptoms such as menorrhagia, dysmenorrhea, and bulk symptoms by 11 months post-procedure [63]. When comparing surgery versus UAE, a Cochrane meta-analysis from 2014 found that there were no differences in major complications between the

two procedures; however, UAE had a higher likelihood of having minor complications and requiring surgery in 2–5 years from the procedure [64]. For women of reproductive age, perhaps, most concerning is the documentation of impairment of fertility after UAE. Although there are documented pregnancies after UAE [65], other studies have found very low pregnancy rates, with only 1 documented pregnancy out of 31 women with severe symptomatic fibroids, which ultimately failed to result in a live birth [66]. A randomized controlled trial comparing surgery versus UAE found that 2 years out from either procedure patients who had myomectomies had higher pregnancy rates (78 vs 50 %) and lower miscarriage rates (23 vs 64 %) [67]. Given the data indicating decreased fertility after UAE, the first-line treatment for fibroids in women desiring to conceive is myomectomy. Patients of reproductive age who desire UAE need to be counseled extensively about the risks of decreased fecundity and poor obstetric outcomes [68].

## Magnetic Resonance-Guided Focused Ultrasound

Other noninvasive fibroid-removing techniques include the magnetic resonance-guided focused ultrasound (MRgFUS) fibroid treatment. In 2004, the ExAblate® 2000 device (InSightec, Haifa, Israel) received U.S. FDA approval for fibroid treatment. Focused ultrasound therapy causes thermal injury of the fibroid tissue by absorption of sound wave energy, vibratory effects, and cavitation through generation of microbubbles [2•]. A T1-unenhanced and gadolinium-enhanced MR imaging study is performed to calculate the degree of ablation or "nonperfused volume." There is a lower likelihood of needing further treatments if the "nonperfused volume" is >50–60 %. With just >30 % "nonperfused volume" achieved, patients report improved symptom control and greater reduction in fibroid volume [69]. Although study patients report improved symptom control, the maximal reduction in fibroid size at 12 months is only approximately 25 % [70]. The effects on pregnancy and fertility are still being studied but there appears to be successful reproductive outcomes after MRgFUS. A prospective registry of all known pregnancies after MRgFUS treatment for conservative treatment of clinically significant fibroids maintained by the manufacturer revealed 54 pregnancies in 51 women with live births in 41 % of pregnancies and a 28 % miscarriage rate. Fifty-seven percent of the

pregnancies had no neonatal or maternal complications [71]. Although further research still needs to be done to verify the safety of MRgFUS in reproductive age women, the results appear promising for nonsurgical treatment of fibroids.

### Laparoscopic Radiofrequency Volumetric Thermal Ablation

The newest noninvasive treatment of fibroids is laparoscopic radiofrequency volumetric thermal ablation (RFVTA) which was approved in 2012 for patients who desired conservative treatment and quick recovery (Acessa procedure; Halt Medical, Inc., Brentwood, CA, USA) [72]. The procedure occurs under laparoscopic guidance where an ultrasound probe delineates fibroids and real-time imaging monitors insertion of the electrodes into the fibroids, resulting in fibroid ablation [72]. Outcomes of initial studies report sustained relief from fibroid symptoms and improvement in quality of life during the 36 months after ablation. In addition, only 11 % of patients at 36 months needed repeat intervention [73]. In a randomized controlled trial, outcomes of laparoscopic myomectomy to RFVTA improvements in the severity of symptoms were shown to be significantly improved in both groups [74]. Despite the requirement that women enrolled in early premarket RFVTA studies were to have completed childbearing, nine women were between ages 31 and 40 years of age. Six subjects became pregnant within 15 months of treatment, with five having successful live births and one miscarrying [75]. Larger studies are needed to confirm the efficacy and safety of this fibroid treatment, but initial studies are promising for the treatment of fibroids in the setting of infertility.

## Conclusion

Our understanding of how and why fibroids contribute to infertility and adverse pregnancy outcome has vastly grown. We have clearly identified submucosal fibroids as having the most direct and negative effect on implantation. While our data on the impact of intramural fibroids on fertility is more recent, there is now no doubt that they too can affect the endometrium at a molecular level and physically disrupt uterine peristalsis. Moreover, through the compilation of studies, we have determined

that certain intramural fibroids above 4 cm are very likely to be detrimental to fertility. Treatment modalities for fibroids have vastly improved as well. Hysteroscopic myomectomy, a routine surgery in general gynecology, has proven to enhance pregnancy rates when cavity-distorting fibroids are removed. Advances in minimally invasive surgery, employing RALM, have demonstrated successful reproductive outcomes and are poised to be adopted more widely than laparoscopy myomectomy, due to their ergonomic advantages. Employment of GnRH-a analogues and SPRMs has improved surgical outcomes by allowing reliable shrinkage of fibroid tumors, making both hysteroscopic and laparoscopic operations more often feasible. New technologies such as MRgFUS and RFVTA have emerged as possibilities in the nonsurgical treatment of fibroids, although their safety in the reproductive age patient has yet to be confirmed and is needed before their widespread adoption. The most recent evidence allows us to give clearer guidelines on when to treat and how to treat fibroids. For now, surgery remains the gold standard for fibroid removal for infertility treatment.

**Compliance with Ethical Standards**

**Conflict of Interest**
Erin I. Lewis declares no conflicts of interest.
Antonio R. Gargiulo is a consultant for Medicaroid, which builds robotic equipment for medical use, and a consultant for OmniGuide Holdings, which builds laser and ferromagnetic energy tools for medical use.

**Human and Animal Rights and Informed Consent**
This article does not contain any studies with human or animal subjects performed by any of the authors.

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# Assisted Reproductive Technologies in Infertility Treatment: Opportunities and Challenges

**Pawan K. Dubey, Anima Tripathi, Akhtar Ali**

A tremendous rise in the fertility clinics providing ART services is seen worldwide with the birth of first IVF baby (Louise Joy Brown) in 1978. ART comprises various types of medical treatments designed to assist in achieving pregnancy. IVF and other ART-associated technologies of fertilization (ICSI, IUI, PZD, SUZI, MESA, and PESA) offer an opportunity to become parent even in severe cases of infertility. These technologies have allowed millions of individuals to fulfill their parenting wish. A positive attitude combined with an appropriate treatment can help most of the infertile couples experience the joy of parenthood. This chapter provides a thorough overview of the assisted reproductive technologies with opportunities for patients and challenges for clinical professionals or researchers.

**Keywords**
Assisted reproductive technologies (ARTs), In vitro fertilization (IVF) Intracytoplasmic sperm injection (ICSI), Surrogacy, Infertility treatment

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## Key Points

- In 1978, for the first time, manipulation of the gametes was done under in vitro conditions by conventional IVF methods that resulted in the successful birth of Louise Joy Brown.
- In a number of cases, failure of other treatments leaves the patients with the option of ART as the last hope.
- ARTs in the form of IUI, IVF, and ICSI are used very commonly because of a variety of reasons.
- Other variants of ARTs, such as SUZI, ZIFT, GIFT, and PZD are good alternative techniques to routine ARTs.
- ARTs have revolutionized the field of infertility treatment as theoretically even men with one or few sperm can have a hope to father a child.

## Introduction

Infertility can be considered as inability of a female individual to conceive pregnancy for the full term. Infertility occurs mainly because of two factors, the male factor and the female factor. One third of both the male and the female factors are responsible for infertility, and the remaining one third is because of unexplained infertility. Despite the progresses in the field of reproductive biology, the etiology of infertility is still unknown, and about 50% of the cases are termed as "idiopathic." The diagnosis and treatment of infertility may involve targeted or empirical therapies depending upon the nature of infertility, depth of investigations, and success in identifying the underlying cause. Unfortunately, a large number of individuals who are suffering from the infertility do not get benefit from the traditional medications or treatments; therefore, they need to move for the next line of therapy, i.e., assisted reproduction.

For a number of infertile couples, leaving the few exceptions, assisted reproductive technologies (ARTs) are the only effective treatments that allow conception even in severe infertility cases, including azoospermia. These technologies include in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI), intrauterine insemination (IUI), percutaneous epididymal sperm aspiration (PESA), microsurgical epididymal sperm aspiration (MESA), testicular sperm extraction (TESE), partial zona

dissection (PZD), and subzonal sperm injection (SUZI). Earlier, infertile men were dependent on sperm donor insemination or adoption, but at present, even in more severe infertility cases, IVF and other ART fertilization technologies (ICSI, IUI, PZD, SUZI) provide them an opportunity to become parent. This chapter provides an overview of the available ARTs for infertile individuals with focus on their suitability, advantages, and disadvantages.

## ARTs are a Boon

Assisted reproductive technologies acted as a boon for millions of people worldwide by providing them the opportunity to become the parent of their biological children that would rather not been possible ever. According to the European Society of Human Reproduction and Embryology (ESHRE), three million babies have born with the help of ARTs in the last 30 years which suggest that ARTs can be used to treat any form of infertility either related to women and men, lesbians and gays, or transgender couples. The arrival of these fascinating technologies in the form of ARTs has changed the opinion about the reproductive world and has generated new hopeful possibilities for infertile couples to have their own baby. The following ARTs may be considered for the treatment of infertility-related problems (Table 1).

Before undertaking an ART procedure, a number of investigations must be completed in order to know the symptoms, cause, and type of infertility that would help them choose the best possible therapy (Table 2). Traditionally, the gynecologist or reproductive endocrinologist starts

**Table 1. ARTs Used in Treatment of Infertility.**

| Cause of infertility | ART | Outcome |
|---|---|---|
| Ejaculatory disorders (oligo-, azoo-, and zoospermia) | In vitro fertilization (IVF) | Pregnancy |
| Repeated fertilization failure by natural method or IVF | Intracytoplasmic sperm injection (ICSI) | Pregnancy |
| Repeated embryo transfer failure | | |
| Asthenozoospermia (progressive motility), teratozoospermia, oligozoospermia | Partial zona dissection (PZD) and subzonal sperm injection (SUZI) | Pregnancy |

**Table 2. Different Parameters of Male Fertility Considered for the Diagnosis of Infertility.**

| Parameters | Clinical examination of male infertility |
|---|---|
| History | Time and duration of infertility, any previous pregnancy, medical details of female partner, intercourse frequency and timing, any existing and past disease, alcohol, smoking, and drug consumption |
| Examination | Details of treatment for testicular maldescent, size of testis, vas deferens diameter and blockage, epididymis diameter and blockage, hydrocele/varicocele, semen analysis by CASA system |
| Investigation | Endocrine profile (T3, T4, TSH, FSH, LH, testosterone) |

observing and evaluating the infertility-related problems mainly with the female partner in comparison with a little analysis of the male partner. Analysis of male factors which are also equally responsible for infertility must be done by a urologist with specialization in male infertility. In fact, it is very necessary to diagnose and identify the real problem in order to suggest the best-suited ARTs to cure infertility-related problems. Hence, infertile couple should opt for a complete clinical checkup by a physician specialized either in male or female infertility, respectively.

## Sperm Recovery Techniques

The preference of sperm retrieval technique and its success rate is based on the type of male infertility either obstructive or non-obstructive. Some of the important preoperative tools for diagnosis are clinical history, physical examination, and endocrine assessment like measurement of follicle stimulating hormone (FSH) and testosterone levels in patient serum. In conditions like vasectomy, failed vasectomy reversal, primary testicular failure, or congenital obstruction of the sperm ducts where there is no spermatozoa in the patient's semen, different types of technologies are available to retrieve sperm for ART purposes. This includes electroejaculation/vibratory stimulation, percutaneous epididymal sperm aspiration (PESA), microsurgical epididymal sperm aspiration (MESA), and testicular sperm extraction (TESE). For azoospermic patient where there is no obstruction, PESA and MESA are extremely useful to retrieve sperm for ART purposes. In contrast, MESA is a more advanced technology in which a number of sperms can be retrieved with less epididymal damage (Kim and Lipshultz 1997). In TESE, an open surgical procedure is adopted

**Table 3. Common and Useful Methods for Sperm Retrieval.**

| Technique | Common name | Indications |
|---|---|---|
| Percutaneous epididymal sperm aspiration | PESA | Obstructive azoospermia |
| Microsurgical epididymal sperm aspiration | MESA | Obstructive azoospermia |
| Testicular sperm aspiration | TESA | Failed PESA, epididymal agenesis, non-obstructive azoospermia |
| Microsurgical testicular sperm extraction | Micro-TESE | Non-obstructive azoospermia |

**Table 4. The Approximate Cost of Different ARTs.**

| ART | Approximate cost/cycle (USD) | Success rate |
|---|---|---|
| **IUI** | $120–$400 | 5–30%, depending on the age of woman |
| **IVF** | $10,000–$14,000 | 34% successful pregnancies per cycle |
| **ICSI** | $12,000–$16,000 | 31% success rate |
| ZIFT/GIFT | $12,000–$20,000 | 39–45% success rate |
| PGD | $2500–$5000 | Success rates are 90% for testing for medical conditions and close to 100% for sex selection |

to retrieve the sperm. The procedure is performed in those cases where the process of epididymal sperm retrieval fails. However, it provides similar pregnancy rates with micro-assisted fertilization technologies (Ghazzawi et al. 1998). Considering the number of sperm retrieval methods which plays an important role to achieve pregnancy, each infertile male partner must be carefully examined to determine the best sperm retrieval method because it would affect the overall outcome of ART. The most common and useful methods for sperm retrieval are summarized in Table 3 with their approximate costs summarized in Table 4.

## Intrauterine and Donor Insemination

Intrauterine insemination (IUI) is the simplest form of assisted reproductive technology where, at the time of egg ovulation, washed ejaculated sperm is placed in the uterus, beyond the cervix. This technology is used

to treat infertility problem in males related to low sperm count or low motility, antisperm antibodies, and erectile dysfunction. In this technology, if the husband's sperm is used, it is considered as AI (artificial insemination), and if the donor sperm is used, it is considered as DI (donor insemination). Donor insemination is an alternative form of AI that offers an effective advantage to those couples who fail to conceive despite repeated clinical therapies. In case of the absence of persisting female factor infertility, IUI technology is extremely successful (70%). However, donor insemination is unacceptable at social level, and it may be considered an illegal practice by some societies. Moreover, where adoption is not desired and the male wants to have his own genetic offspring, assisted reproductive technologies may prove to be a good option.

In recent years, IUI along with superovulation technology has become a famous method for the treatment of male-related infertility. Insemination using a high number of motile and morphologically normal sperms after superovulation by gonadotropins has a theoretical advantage and maximum chance of successful pregnancy. The success rate of IUI varies widely and is closely related to female age and reproductive potential as, for example, the IUI success rate is higher for younger women. The IUI is very beneficial in case of male infertility; however, if the ovaries are stimulated with drugs to increase the number of eggs obtained every month, we can further enhance the overall pregnancy rate, for example, if the IUI is performed with superovulation in comparison with the IUI alone, a fourfold increase in the pregnancy rate will be observed (Kemmann et al. 1987). Furthermore, a study conducted by Serhal et al. (1988) showed that pregnancy rate/cycle is significantly greater for the combination of IUI and gonadotropin superovulation (26.4%) as compared to IUI (2.7%) or superovulation alone (6.1%). Further, a review by Dodson and Haney (1991) showed that the fecundity rate with IUI and superovulation in male factor infertility is 8.7% as compared to 17% for unexplained infertility. It appears that combination of IUI and superovulation may offer some limited benefits to infertile men but may improve the success rate of IUI in infertility due to female factors.

## IUI Procedure

The IUI can be conducted mainly using three procedures, viz., natural, clomiphene, or gonadotropin stimulation. Natural cycle is recommended

when treatment is with donor sperm or infertility is secondary to difficulties with intercourse. Cycles of fertility drugs such as clomiphene (Clomid) or gonadotrophins (Gonal-F, Puregon, and Menopur) are generally prescribed if there is a case of unexplained or mild male factor infertility. In case of IUI, lower doses of drugs are used than in IVF with an aim to increase the incidences of successful fertilization by stimulating the production of more than one follicle, for example, production of two or three follicles.

After induction of ovulation by injection of HCG or detection of natural ovulation by urine, freshly prepared sperm from male partner or donor is prepared as per the established procedure and drawn into a syringe with small amount of culture medium. In insemination procedure, a fine plastic catheter is used to transfer the processed sperm through female partner's cervix into the uterus (Fig. 1). Following IUI, there is no need to take time off or limit work, but the patient is advised to visit an embryologist and IVF clinic for post-insemination checkup to assure the pregnancy. The IUI procedure is more advantageous because of its less invasive nature and is better tolerated as compared to IVF. However, the major disadvantage of IUI is that its success rate is low and there is a high chance of occurrence of multiple pregnancies as compared to the IVF.

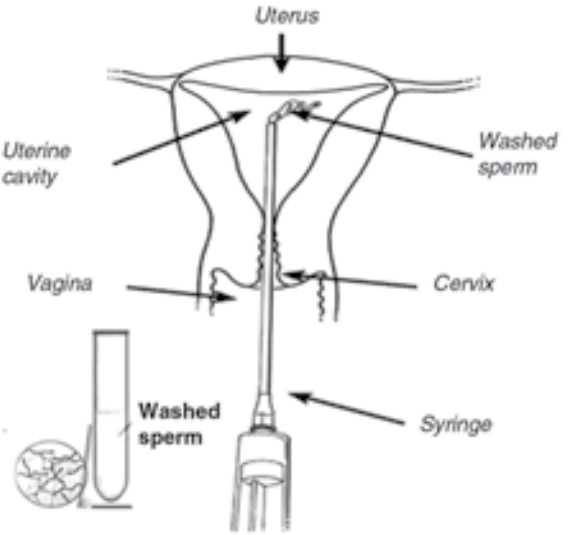


**Fig. 1.** Diagrammatic representation of IUI procedure.

## In Vitro Fertilization (IVF)

Among the various ART treatments, IVF is the most popular and invasive technology. It has been seen that in general, women who are trying for the pregnancy or live birth adopt other methods first and finally move on to the IVF when those methods become unsuccessful. In contrast to artificial insemination, fertilization in IVF gets done outside of the woman's body in which eggs (retrieved from the woman trying to get pregnant or from an egg donor) are fertilized with the sperm (from a male partner or donor-derived sperm) in a petri dish. In 1978, for the first time, manipulation of the gametes has been done under in vitro conditions by conventional IVF methods that resulted in the successful birth of Louise Joy Brown. Since the delivery of the first IVF baby some 38 years ago, the technology has spread worldwide and is still in high practice because of its consistent results. Here, we summarize the basic procedures of IVF.

### IVF Procedure

#### Step 1: Controlled Ovarian Hyperstimulation (COH)

For ovarian hyperstimulation, GnRH agonist (Lupron) protocol is used to suppress the secretion of gonadotropin hormone to avoid premature ovulation. The next stage is the multiple follicular recruitment by the use of gonadotropin injections daily once the suppression of gonadotropic hormone is achieved to optimum level. The follicular development is monitored by the use of the technologies like ultrasound imaging and hormone assessments. Physician using ultrasound examinations and blood testing can determine whether the follicles are ready for egg retrieval or not. Generally, 8–14 days of stimulation are required. The hCG administration is given for final maturation of the egg when the follicles are ready and reach an appropriate size. Egg retrieval is scheduled 34–36 h after hCG injection.

#### Step 2: Egg Retrieval

Egg retrieval is usually performed by transvaginal ultrasound aspiration, a minor surgical procedure, which is performed for egg retrieval process. To

retrieve eggs from the patients, clinicians generally administer some pain medications. During egg retrieval process to identify the follicles, an ultrasound probe is inserted into the vagina, and a needle is guided through the vagina into the follicles. Thereafter, to locate all the available eggs, the follicular fluid is scanned by the embryologist. The cumulus-oocyte complexes (COCs) consisting of the first polar body (PB) (Fig. 2) are placed in a special media and cultured in a $CO_2$ incubator until insemination. Laparoscopy technique is also used to retrieve the eggs using a small telescope placed in the umbilicus. For more information on laparoscopy, consult with an ART center or a specialized doctor.

### Step 3: Fertilization and Embryo Culture

After assessment of maturity and quality, the retrieved eggs are placed in an IVF culture medium. For fertilization, simultaneously sperms are processed from a male's partner or donor-derived semen. Alternatively, if the male's partner is azoospermic or having any kind of obstruction, sperm can be obtained from the testicle, epididymis, or vas deferens using sperm retrieval technology as described above. For fertilization purposes, approximately 50,000–100,000 motile sperms are mixed with the meiotically competent eggs under in vitro condition. After 16–18 h of co-incubation of the egg and sperm, fertilization is assessed by visualization of two pronucleus formation. Once the fertilization is confirmed, the presumptive zygotes (fertilized eggs) are cultured into a specially formulated culture medium that supports the growth and development of embryos. After 24 h of postfertilization, the fertilized eggs

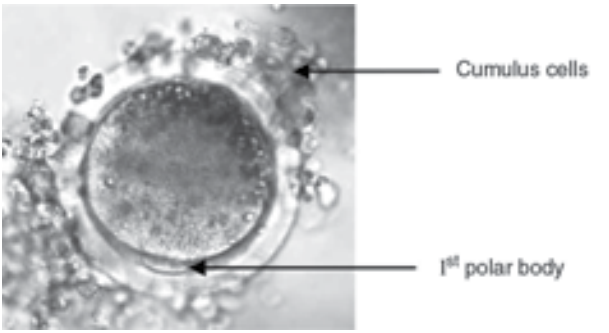


**Fig. 2.** Cumulus-enclosed mature oocyte showing first polar body extrusion.

are divided into two to four cell embryos (Fig. 3). For transfer purpose, the embryos are grown till the blastocyst stage which have higher potential for implantation.

### Step 4: Embryo Transfer

In general, 4–8 cell stage embryos (day 3 of postfertilization) are used to transfer for implantation purpose. Although, the later stages like 8–16, morula or blastocyst stage (Fig. 4) of embryo can be transferred into female partner or surrogate mother to get pregnancy. However, before transfer, clinicians must perform the preimplantation diagnosis to examine the embryo for any fragmentation or diseases. In practice, transferable embryos should be free from any kind of diseases and must be classified into grades 1–4 on the basis of several parameters where grade 1 represents the best-quality embryos to maximize the chance of successful pregnancy.

### Assisted Hatching (AH)

In general, after transfer of embryo in the uterus, embryo must expand and rupture the zona pellucida (ZP) allowing to implant. In some cases, embryo does not hatch out from ZP and as a result implantation does not occur. In such cases, assisted hatching (AH) is used to overcome this problem. AH is a technology which is used to create a hole in the ZP

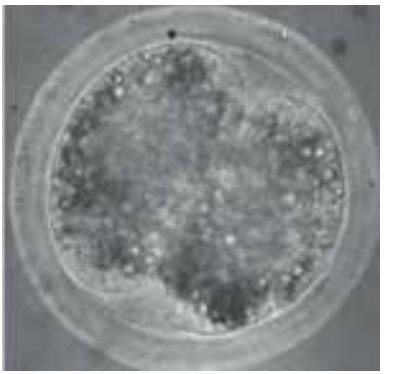

**Fig. 3.** Cell stage embryo.

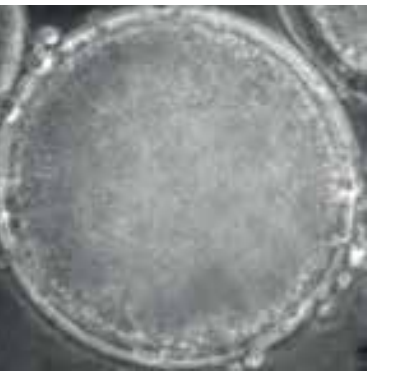

**Fig. 4.** Blastocyst stage of embryo.

with the help of an instrument prior to embryo transfer which facilitates hatching in utero. However, it has been seen that AH does not improve the rates of live birth though it is useful for aged women or couples to get maximum chance of pregnancy.

## Preimplantation Genetic Diagnosis (PGD)

PGD is a technology which is used to diagnose inherited diseases by screening of preimplantation embryo. In this procedure, one or two cells called blastomere are retrieved from the presumptive zygote and diagnosed the probability for different genetic disease using different molecular approaches. After diagnosis, embryos that are free from any type of disease are selected for transfer in the uterus. However, for conducting these procedures, a specialized clinician and equipment are needed. PGD is helpful for couples who are carriers of some genetic diseases, and these couples must perform embryo screening to reduce the risk of having an affected child. There are some other methods like chorionic villus sampling (CVS) and amniocentesis which can be used for diagnosis of genetic diseases during gestational period.

## Cryopreservation

Cryopreservation is a technology in which any type of cells, tissues, or body organs can be preserved at very low temperature (−196 °C) in a natural state for future use. In ART, cryopreservation technology can be used to store or preserve extra embryos or oocytes for future use. The most significance of this technology is that ART clinicians may use the preserved oocyte or embryo for the fertilization or embryo transfer purposes rather than initiating a new IVF cycle in infertile patient. Moreover, live births that have been reported using frozen embryos showed the importance of this technology. However, there are some risks like chromosomal aberrations or biochemical level associated with frozen embryo. Therefore, it is advisable for infertile couples and clinicians both that before using cryopreserved embryos, they must ensure that embryos are healthy and free from any type of aberrations at cellular and molecular level. In general, in the field of ART, cryopreservation plays an important role because it is less expensive, time saving, and an invasive procedure (Fig. 5).

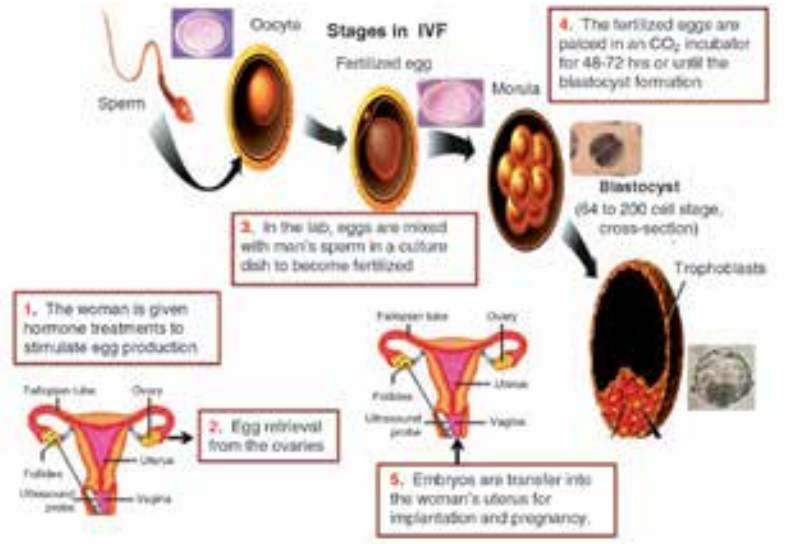


**Fig. 5.** Diagrammatic representation of stages used in IVF procedure.

## Advantages of IVF

- This technology may help infertile couple to get a baby of their own.
- IVF can be performed with less number of motile sperms (50,000–100,000/oocyte) compared to natural (2–6 million/oocyte) fertilization.
- It has higher success rate as compared to IUI and other ARTs.
- Preimplantation genetic diagnosis can be done for identification and prevention of genetic abnormalities.
- The procedure is relatively safe and has been utilized for a long time to produce a baby via egg or sperm donors.
- Sperm as well as embryos which are unused can be cryopreserved and may be utilized for stem cell research that would help cure various kinds of degenerative diseases in the future.

## Disadvantages of IVF

- The main drawback of IVF is multiple births, i.e., delivery of more than one baby. To get higher success rate, clinics and doctors generally transfer more than one embryo that can result undesired multiple births.

- IVF may cause ovarian hyperstimulation syndrome due to heavy use of hormones and drugs during the procedure. IVF can lead to ectopic pregnancy in which implantation of embryo occurs outside the uterus.
- The success rate of IVF depends on the age of the female, the quality of eggs, the quality of sperm, the quality of the uterus, etc. IVF can cause some abdominal pain due to the use of some minor surgical procedure besides the use of drugs and hormones.
- IVF technique is little costly and it may not be affordable for some.

Failure of IVF could be devastating for any infertile couple. If it happens, what would be offered to the patient? In recent years, many advanced technologies have been developed in the field of ART, some of which promise to provide positive results even in severe infertility cases, such as severe oligospermia, asthenospermia, and teratospermia. In case of IVF failure, infertile couple can choose other options such as gamete intrafallopian transfer (GIFT), zygote intrafallopian transfer (ZIFT), and micro-assisted fertilization.

## Gamete Intrafallopian Transfer (GIFT) and Zygote Intrafallopian Transfer (ZIFT)

Gamete intrafallopian tube transfer (GIFT) and zygote intrafallopian transfer (ZIFT) are variants of IVF which are used in the case of female infertility or some other infertility treatments that have been unsuccessful. From the last few years, both the technologies have increased attraction of the clinicians because these technologies reasonably increased the clinical pregnancy rates in comparison with IVF. It is predicted that higher clinical pregnancy rate is due to the in vivo environment of the fallopian tube. As like IVF, ZIFT and GIFT technologies begin with ovarian stimulation and egg retrieval. In ZIFT, retrieved eggs are fertilized outside of the body, and the resulting zygote(s) is directly transferred into the woman's fallopian tube by the help of laparoscopic surgery. In contrast to ZIFT, in GIFT, the processed eggs and sperms are both transferred directly into the woman's fallopian tube. Principally, GIFT is more useful for the type of unexplained infertility. However, the disadvantage of GIFT is we can't confirm whether transferred eggs become fertilized or not if the pregnancy is not achieved. Therefore, ZIFT is better than GIFT, and mostly ART

clinicians preferred ZIFT over GIFT for the treatment of unexplained infertility. Moreover, some studies have been randomized comparing the ZIFT and IVF and found no advantage of ZIFT over IVF for the treatment of male-related infertility (Tournaye et al. 1992a, b). Furthermore, Tournaye and associates conducted a comparative study between IVF, GIFT, and ZIFT and stated that take-home baby rates are 13.5%, 7%, and 20%, respectively (Tournay et al. 1991). Remarkably, IVF is still the first choice of infertile couple when compared to other ARTs due to the ease of access, availability, cost, and success rate. However, as per the report, the technology like ZIFT also may be useful for treating infertile couple with little more success rate.

## Micro-Assisted Fertilization

Though IVF, IUI, GIFT, and ZIFT are very useful for infertility treatment in the field of ART with variable success rate, however, still in many severe or unexplained infertility cases, these technologies are not able to treat the patient. Therefore, there is a need to treat such cases with more advanced technologies. When there is severe deficiency of sperm number and/or limited ability of sperm to fertilize during IVF, GIFT, and ZIFT, adjunctive micromanipulation technologies such as intracytoplasmic sperm injection (ICSI) may be useful in providing a reasonable chance of pregnancy.

### Micromanipulation Technique

Micromanipulation is an advanced technology which is used to manipulate the gametes (egg and sperm) under in vitro condition for different purposes. Micromanipulation technology have revolutionized the field of ART where maximum pregnancy rate can be achieved. In this technology, single oocyte and sperm can manipulated as per the need with the help of a holding and injection pipette equipped with an inverted microscope. First, processed oocyte is immobilized by the holding pipette and then injected a single sperm or even any other chemicals into cytoplasm of oocyte with the help of injection pipette. This technology can be divided into zonal, subzonal, and intracytoplasmic procedure (Fig. 6). In zonal procedure, a tiny hole is created in zona pellucida, an acellular layer surrounding the egg by the help of laser-guided beam.

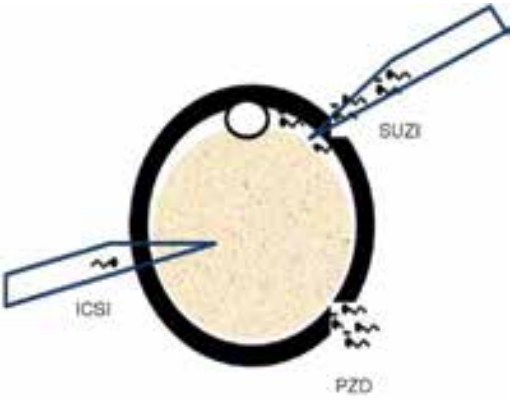


**Fig. 6.** Diagrammatic representation of different micromanipulation technologies which are used to fertilize meiotically-competent egg.

Basically, this procedure has been broadly termed as "zona drilling" which is successfully adopted for treating male patient-related infertility. This method is also called partial zona dissection (PZD). Subzonal procedure of micromanipulation technology directly facilitate sperm-egg interaction are known as subzonal insertion of sperm (SUZI). In SUZI, sperm is directly placed into the perivitelline space of egg for the fertilization purposes. The third and most invasive form of microsurgical fertilization is the microinjection of a single sperm into the cytoplasm of oocyte, referred to as intracytoplasmic sperm injection (ICSI).

In the field of male factor infertility where sperm production is nil or zero sperm count, another micromanipulation technology known as round spermatid nucleus injection (ROSNI) can be used. In ROSNI, round spermatid is directly extracted from male testicles and after removing the nucleus injected into the female partner's eggs. However, this process has yet to give live birth and has to be clinically validated, though clinicians believe that it will eventually become a successful technology that will allow men, who previously had no hope, to be a father of a biological child.

## Intracytoplasmic Sperm Injection (ICSI)

One of the major leading technologies for the treatment of male factor infertility is ICSI where a single sperm is injected into the cytoplasm of

an egg. ICSI is performed in case of low sperm count or when there is no sperm present in the ejaculate, in case of abnormally-shaped sperm, low sperm motility, as well as when the IVF has been previously unsuccessful. ICSI has become the ART of choice for male infertility and is much more effective therapy than other assisted fertilization technologies. ICSI is carried out using automated instrument called as micromanipulator, which is equipped by a holding and injection pipette. In ICSI, first a single healthy and motile sperm and then meiotically-competent egg are immobilized by the help of injection and holding pipette, respectively. After insuring that everything is right, then single sperm is injected into the cytoplasm of the egg by the help of injection pipette (Fig. 7). However, this technology has the possibility of transmitting genetic defects of spermatogenesis or other genetic defects to a future offspring.

In a study, fertilization rate of 55% for ICSI versus 17% for SUZI has been reported (Van Steirteghem et al. 1993). ICSI has become the most quickly adopted technology for those couples who are unable to conceive from conventional IVF. Further, a study conducted by Palermo and associates showed 69% fertilization rate and a 38% ongoing pregnancy rate using ICSI (Palermo et al. 1995). The use of ICSI may prevent such complete failures; however, fertilization failure may still occur even when ICSI is used. Therefore, taking into consideration the added expenses and the potential risks of the procedure, it is still debatable whether ICSI should be exclusively used for all the patients in place of the conventional IVF method.

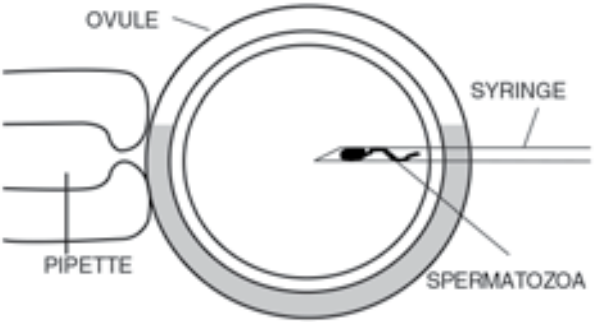


**Fig. 7.** Intracytoplasmic sperm injection.

In addition, concerns regarding disruption of chromosomal or cytoskeletal elements or fertilization consequences with genetically abnormal sperm remain to be a matter of further discussion and research. Approximately, half of the 7% of infertile men that harbor major sex chromosome abnormality accounts for a mosaic Klinefelter condition. The incidence of sex chromosome abnormality rises from 2% in men with normal sperm concentration to 20% in those with azoospermia (Baker et al. 1993). Despite these data and an apparently high risk for chromosome abnormalities in ICSI fetuses, Bonduelle and colleagues found the risk of chromosomal abnormalities to be approximately 1%, similar to the general newborn population (Bonduelle et al. 1996). There is a growing concern of germ line mutation that may result in heritable defects because ICSI allows fertilization by sperm, which under natural conditions is incapable of ZP penetration and oocyte-sperm fusion. The inheritance of susceptibility to infertility is another concern that remains unrecognized until late in the next generation. Once sexing of the spermatozoa becomes routinely available, prevention of sex-linked diseases may be prevented by selecting the healthier gender. Thus, during the ICSI procedure careful evaluation, genetic consultation with the couples, as well as follow-up of the pregnancies, is necessary.

Five important steps in the ICSI procedure involve:

1. The sperm sample is either surgically removed from the testes or epididymis or taken from male partner's semen.
2. Eggs are collected by surgical method from hormonally-induced ovarian follicles. Single motile sperm is injected carefully from male partner into meiotically-matured egg of the female partner by using a tiny hollow needle.
3. The fertilized egg is observed for growth and development after injection.
4. Once the normal growth is seen, the presumptive embryo is delivered into the female uterus where it has a chance to implant and grow.

## Advantages and Disadvantages of ICSI

Although ICSI has become an established ART procedure, however, many concerns have been raised on the resultant embryos and children over

its potential detrimental effects. First and foremost, ICSI is a procedure, where the sperm cells are directly introduced into an egg which effectively eliminates male infertility. One of the major concerns is that ICSI bypasses the natural selection of sperm for fertilization and so the sperm having defects that would have been prevented from fertilizing oocytes may do so with the aid of ICSI that ultimately passes the defects on to the next generation. Apart from facilitating the transmission of genetic defects, the sperm injection process may inevitably cause physical damage to the oocyte that finally interferes with subsequent embryo development. The ICSI-generated embryos have been found to less likely attain the blastocyst stage in vitro and have a greater chance of developing fragments in comparison with embryos from the conventional IVF method. Despite the observed and potential detrimental effects on the embryos at the genetic and cellular levels, ICSI has not been associated with increased incidence of birth defects (Van Steirteghem et al. 2002; Hansen et al. 2002). Public concerns over the safety of ART were raised again by a report showing a higher incidence of birth defects in IVF babies (Hansen et al. 2002). The limited information available suggests that ICSI children may have a small delay in mental development although it is unknown if this mental impairment is caused by the ICSI procedure or by factors inherent to the patients who require ICSI in the first place (Bowen et al. 1998). Obviously, further studies on the long-term effects of ICSI on the offspring involving multiple centers with well-controlled study designs are needed to minimize confounding variables, such as operator/technical variations and population variations. Until conclusive data becomes available, patients should be counseled carefully before ICSI is offered as an ART treatment.

## Surrogacy

In severe cases of infertility, the infertile couple may choose surrogacy. In surrogacy, the infertile couple does the legal contract with fertile women in which fertile woman becomes pregnant and gives birth to a child. If the surrogates used their own egg for the fertilization purposes, then the condition is referred to as "genetic surrogate." On the other hand, if embryos are generated using another woman's eggs and then implanted into the surrogate, condition is referred to as "gestational surrogate" and has no genetic tie with the child. From the last decade, it has been seen that hiring

a surrogate becomes a business to earn money worldwide. In the United States alone, for surrogacy, ART clinic can charge $40,000–$100,000, including the surrogate fee, insemination or IVF costs, and costs related to medical care, transportation, and legal services. Due to the high cost, recently it has been seen that some of the couples started to hire women in the developing countries. In Indian subcontinent, hiring a surrogate costs from $5000 to $12,000, and the surrogate gets paid $3000–$6000.

## Regulation of ART

In the United States alone, it is estimated that ART is a $3–5 billion industry. Considering the modern lifestyle and increasing infertility rate, ART clinics are rapidly expanding including egg brokers, sperm banks, and surrogacy services worldwide. As many ethical, social, and critical issues are associated with the ARTs, it is necessary that ART clinics must be regulated by the government agencies; otherwise, it would be at risk. There also must be some international law which can regulate individuals, couples, and ART clinics to find the quality services whether in their own country or another.

## Risks in ART

Beyond the fact that ARTs offer the possibility for infertile couples to attain pregnancy, however, it poses potential risk in health issue for the mother as well as the infant. As in majority of ART procedures, multiple embryos are transferred, and there is a risk of multiple gestational pregnancy and multiple births. The risk of multiple births at maternal interface includes high rates of cesarean deliveries, maternal hemorrhage, pregnancy-related high blood pressure, and gestational diabetes. At fetal interface, it includes prematurity, low birth weight, infant death, elevated risk for birth defects, and developmental disability. Further, even singleton infants conceived with ART have a higher risk for low birth weight compared with singleton infants conceived with normal procedures. If during ART a maximum of two embryos are transferred rather than multiple embryos, the risk of high-order multiple births can be restricted. For patients who are seeking ART, twin pregnancy can be treated as necessary but manageable complication of infertility treatment. Double embryo transfer for the patient

undergoing ART may be an option provided their health is good and they are in proper condition to conceive a twin pregnancy for 34 weeks and wish to have more than one child. ART programs should not be penalized for providing patients the option of double embryo transfer, by not counting twin births when reporting IVF "success". Nevertheless, IVF and ICSI technologies have revolutionized the treatment of male infertility with new hope of having their own genetic offspring as well as disease-free newborns.

## Conclusion

ART has become one of the widely accepted and most desirable technologies since last one decade. ART is a rising hope to millions of couples facing the problem of infertility. In the coming years, advancing technology is likely to exacerbate ethical, legal, and social concerns associated with ART. Further, due to the rapidly evolving nature of the ART, legislation is often unable to keep pace and address all of the ethical and legal issues that are constantly emerging in the field. It is therefore incumbent upon physicians to continuously monitor these issues and ensure that ART technologies are offered and delivered in a manner that balances patient care with social and moral responsibility. Furthermore, medical professionals should be keenly aware of their professional as well as social and ethical responsibilities in the pursuit of technical advancement. Of course, the latest advances in ART have not only enhanced the possibility of pregnancy but have also made today's women conceive in situations which would not have been possible decades ago.

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# "Hormonal Wedge Resection": An Effective Treatment Method of Anovulatory Infertility Associated with Polycystic Ovary Syndrome

**György Siklósi**

## The Role of Folliculo-Luteal Insufficiency in the Failed Treatment of Anovulatory Conditions

The primary cause of infertility in 25 % of women seeking medical advice is the lack of ovulation, 80 % of which is accounted for by polycystic ovary syndrome (PCOS) (WHO; Berger and Bates 2014). Clomiphene citrate (CC) therapy is the first-line treatment of PCOS since its introduction into clinical practice (1967) (ESHRE/ASRM 2007; ASRM-ESHRE 2012). The greatest contradiction in the CC treatment of polycystic ovary syndrome is that despite achieving 70–80 % actual ovulation, only 30–40 % of patients will conceive (Sirmans and Pate 2013; Conway et al. 2014).

The latest study comparing the efficacy of letrozole and CC treatment involved 750 patients in a multicentred, double-blind, placebo-controlled study design (Legro et al. 2014a, b). At least one fallopian tube was permeable in the treated patients, and each male partner had normospermia. Letrozole (aromatase-inhibitor) treatment was applied with an initial dose of 2.5 mg and lasted 5 days, and the dose was increased up to 5 × 7.5 mg until ovulation took place. CC treatment was initialised with a dosage of 5 × 50 mg, which was increased up to 5 × 150 mg if needed. Ovulation was

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achieved in 61.7 % and 48.3 % of patients in the letrozole and CC group, respectively, out of which 27.5 % and 29.1 %, respectively, gave birth. Pregnancies ended with miscarriage in 31.8 % and 29.1 %, respectively, in the two groups. At the same time, some authors achieved better results with letrozole compared to CC treatment: ovulation in 65.7 % and 61.8 % of cases, respectively, and pregnancy in 17.4 % and 12.4 %, respectively, per cycle (Badawy et al. 2009). There is currently no consensus on the value of letrozole treatment, and most scientists view it as an equivalent of CC therapy (ESHRE/ASRM 2008).

In the case of unsuccessful CC treatment, FSH/HCG treatment is usually applied, but due to the high risk of hyperstimulation (5–12 %), this requires great care. The high prevalence (20 %) of multiple pregnancies in FSH treatment also has to be taken in account (Dickey et al. 1998). FSH treatment resulted in higher cumulative pregnancy rates compared to CC therapy: 52.1 and 36.9 % (Sirmans and Pate 2013). By CC therapy, or in the case of failure of this, FSH treatment resulted in successful birth in 60 % of patients within a year and in 78 % within 24 months (Veltman-Verhulst et al. 2010). After unsuccessful CC treatment, 2 years of FSH/HCG treatment led to successful birth in 70 % of patients (Berger and Bates 2014).

The efficacy of metformin treatment is also often investigated in PCOS-related infertility as, according to the currently accepted view, insulin resistance plays a key role in the development of PCOS. Comparing the results of CC + metformin and CC + placebo treatment, the supplemental metformin failed to improve therapeutic results; the two-studied groups had ovulatory rates of 64 % and 72 %, respectively, and pregnancy rates of 40 % and 46 %, respectively (Moll et al. 2006). The resolution of the ESHRE/ASRM (2008) stated that metformin therapy is ineffective in PCOS and the associated infertility. It is only indicated in glucose intolerance, and for this purpose, it is also suitable during pregnancy. IVF is only considered a third-line treatment of PCOS, in the event that the aforementioned therapies all fail (ESHRE/ASRM 2008); nevertheless, lately it has been used more and more frequently.

The currently accepted view is that surgical wedge resection or the laparoscopic multiple coagulation of the ovarium surface can only be considered in the management of PCOS if pharmacological treatment is ineffective (ESHRE/ASRM 2008), particularly due to the high (10–50 %) prevalence of postsurgical pelvic adhesions (Buttram and Vaquero 1975;

Adashi et al. 1981). In studies involving large populations of patients who underwent surgery, wedge resection resulted in ovulation in 70–80 % of patients and pregnancy in 26–60 % (Adashi et al. 1981; Coney 1984), but this beneficial effect is merely temporary in 30–40 % of cases (Buttram and Vaquero 1975).

Inducing ovulation fails in about 25 % of patients despite 5 × 150 mg CC treatment for up to 3 months (CC-resistant PCOS). However, many authors achieved positive results in CC-resistant cases by combining CC treatment with prednisolone or dexamethasone (DEX). In CC-resistant PCOS patients, 7 days of CC treatment (150 mg/day) combined with 5 mg prednisone every evening induced ovulation in 73 % of patients and resulted in pregnancy in 46 % (Isaacs et al. 1997). CC treatment (daily 5 × 100 mg administered between the 3rd and 7th day of the cycle) combined with 2 mg per day DEX between the 3rd and 12th day of the cycle produced significantly better results compared to 5 × 100 mg CC alone: ovulation occurred in 75 % and 15 % of cases, respectively, and pregnancy in 40 % and 5 %, respectively (Elnashar et al. 2006). Similar results were obtained in CC-resistant PCOS when complementing 5 × 200 mg CC with DEX (2 mg DEX per day for 5–14 days) compared to 5 × 200 mg CC only: ovulation rates were 88 % and 22 %, respectively, and the pregnancy rate was 40.5 % and 4.2 %, respectively (Parsanezhad et al. 2002).

The aforementioned treatment methods of PCOS have rather poor efficacy, and the monthly and cumulative pregnancy rates achieved using them are nowhere near the physiological rates. The current professional opinion on these methods reflects the general view that the occurrence of ovulation alone is sufficient to diagnose intact fertility (ASRM 2012a, b). However, about half of the patients with confirmed ovulation and without any other alterations affecting fertility fail to conceive, and though the other half becomes pregnant, approximately one-third of pregnancies are aborted and only the remaining fraction ends in birth. Ovulatory cycles can differ greatly from each other from the aspect of fertility.

It is also FLI (grade II) that underlies recurrent miscarriage, and the normalisation of FLF prior to conception results in successful birth in 95 % of patients. The primary cause of preterm birth, IUGR and PE is also the mild form of FLI (grade I), the prevalence of preterm birth and IUGR both decrease to 0.7 % with physiological FLF, while PE did not occur at all. In light of this, we concluded that various degrees of FLF insufficiency

might underlie the phenomena observed during the aforementioned treatment methods of PCOS. The average *P* value is typically under 11 ng/ml in infertility, between 11 and 17 ng/ml in miscarriage and over 17 ng/ml in birth (the authors do not provide data about the pregnancy outcome in the aforementioned studies).

We regularly examined FLF in the event of ovulation during CC treatment of PCOS. In cases where the average luteal *P* fell behind the physiological ($P > 23$ ng/ml) value, we gradually increased CC dose using *P* control. We complemented CC treatment with continuous low-dosage corticoid therapy if necessary (0.5 mg DEX every evening or if DEX in not available 4 mg of methylprednisolone). Using this protocol, we achieved physiological FLF in almost every case, and if no other pathologic alteration was present (i.e., normospermia, at least one intact tuboovarian unit), conception took place at physiological monthly and yearly cumulative pregnancy rates. These therapeutic results seem to confirm our primary hypothesis that the main cause of failure in the currently applied therapies is the various degree of FLI in ovulatory cycles.

One of the aims of this chapter is to draw attention to the importance of regular FLF evaluation when treating anovulatory disorders with ovulatory cycle induction therapies. This does not apply only to PCOS treatment, but monitoring FLF seems essential in other, rare anovulatory conditions as well. The WHO also found the setting of physiological FLF very important and effective in the treatment of Class 2 normogonadotropic oligo- and amenorrhea that do not originate from PCOS. Although in hyperprolactinaemia-induced amenorrhea the administration of prolactin-decreasing drugs is the primary therapy, in some cases ovulatory cycles are still not physiological, despite adequate prolactin suppression. We also achieved excellent therapeutic results in such patients using supplemental, controlled CC treatment.

During PCOS treatment, 20–25 % of patients fail to ovulate even with a $5 \times 150$ mg CC dosage (Legro et al. 2014a, b). The other purpose of this chapter is to present the method we used for the treatment of CC-resistant PCOS and associated infertility, which we later used also in the general therapy of PCOS owing to its success.

PCOS is the most common endocrinopathy in women. It affects 5–15 % (Nestler 2008a, b; Bozdag and Yildiz 2013), although this data is significantly influenced by the diagnostic criteria that is used to declare

PCOS (Berger and Bates 2014). We used the criteria accepted on the consensus conference of the ESHRE and ASRM (2003) in the diagnostics of PCOS: ovarian hyperandrogenism, anovulation and typical ultrasound image of the ovaries. Based on this, out of 1,000 unselected infertile married couples in our patient population, the primary cause for infertility was anovulation in 18 % and out of this, PCOS in 10 %.

PCOS exhibits a very diverse clinical manifestation and it is the most common cause of anovulatory infertility (Homburg et al. 1996; WHO). Its main characteristic is ovarian hyperandrogenism associated with chronic anovulation, the main clinical symptoms of which are oligomenorrhea, hirsutism and obesity in varying incidence. Major features of PCOS in test results: typical ultrasound image of the ovaries (at least 10 follicles with the diameter of 2–10 mm in the cortical region and/or enlarged [>10 ml] stromal volume), hyperandrogenaemia, elevated LH level and LH/FSH ratio in most patients and hyperinsulinaemia plus insulin resistance are detected in 50–70 % of patients (Martikainen et al. 1996). Patients suffering from PCOS develop diabetes mellitus in 10–15 % and hypertension in 40 % some time in their life. The functional androgen hypersecretion of the ovaries is universally considered as a fundamental sign of PCOS. Generalised adrenal hyperfunction can be demonstrated in approximately 50 % of patients with PCOS (Martikainen et al. 1996).

## Studies to Better Understand The Pathogenesis of PCOS and Associated Anovulation

The pathogenetic mechanism of PCOS is unclarified in many aspects (Barthelmess and Naz 2014). PCOS is characterised by the complex interactions of gonadotropic hormones, androgens and insulin (Nestler 2008a, b). The most approved theory today regards insulin resistance (IR) as a core element in the emergence of these alterations. PCOS is a disorder that arises from genetic and environmental factors. The primary factor in its development is hyperandrogenism, which also worsens IR, and vice versa, insulin resistance worsens hyperandrogenism (Barthelmess and Naz 2014). Insulin resistance is present in most patients with PCOS (50–70 %, Sirmans and Pate 2013) regardless of their weight, and its cause is not known. The concurrent presence of obesity enhances IR, while weight loss ameliorates it. IR and compensatory hyperinsulinaemia act

as key factors in altering ovarian function, increasing androgen production and thus the emergence of anovulation, and they inhibit the SHBG production of the liver. Increased insulin secretion might have a role in the altered ratio of LH and FSH hormones produced by the hypophysis. IR elevates the levels of free fatty acids (Nestler 2008a, b). The IR-related increased insulin secretion may contribute to the development of type 2 diabetes, dyslipidaemia and hypertension and the emergence of cardiovascular complications in the long run.

A factor that further supports the role of IR is that as a result of metformin treatment, the level of serum androgens shows a long-lasting drop, cycle irregularities improve and the risk of type 2 diabetes decreases (26 and 31 %). Nonetheless, metformin therapy is incapable of influencing the occurrence of pregnancy (Moll et al. 2006; ESHRE/ASRM 2008, 2013; Nestler 2008a, b).

A more direct explanation of the development of PCOS and associated infertility is given in the pathomechanism described by Yen et al., which had been before the role of IR was discovered (Yen et al. 1976; Yen 1980) (Fig. 1). According to this, the primary factor in the development of PCOS is the elevated androgen secretion of the adrenal cortex, which is caused by increased stimulation and/or obesity.

A large proportion of androgens are peripherally converted into oestrogens (particularly androstenedione into oestrone). Androgens suppress both LH and FSH secretion, while oestrogens increase LH and decrease FSH production. Elevated LH levels cause increased function of the theca, stromal and hilus cells of the ovarium that are capable of producing androgens, and low FSH levels impair folliculogenesis. Folliculogenesis is further disturbed by the elevated intraovarian androgen effect and the increasing effect of extraovarian oestrogens. The androgens produced by the ovaries and the adrenal androgens add up, and this gradually leads to a self-sustaining vicious cycle. The elevated oestrogen effect acts back on the adrenal cortex, where it partially impairs the function of the 3-beta-HSD enzyme, thus altering the DS/testosterone and DS/androstenedione ratios: the result is an increase in DS together with a decrease in testosterone and androstenedione production. This pathomechanism is confirmed by the serum hormonal levels that we obtained in PCOS (see later).

If the primary cause of PCOS is the enhanced activation of the adrenal cortex, we expect that by reducing the activation PCOS will improve.

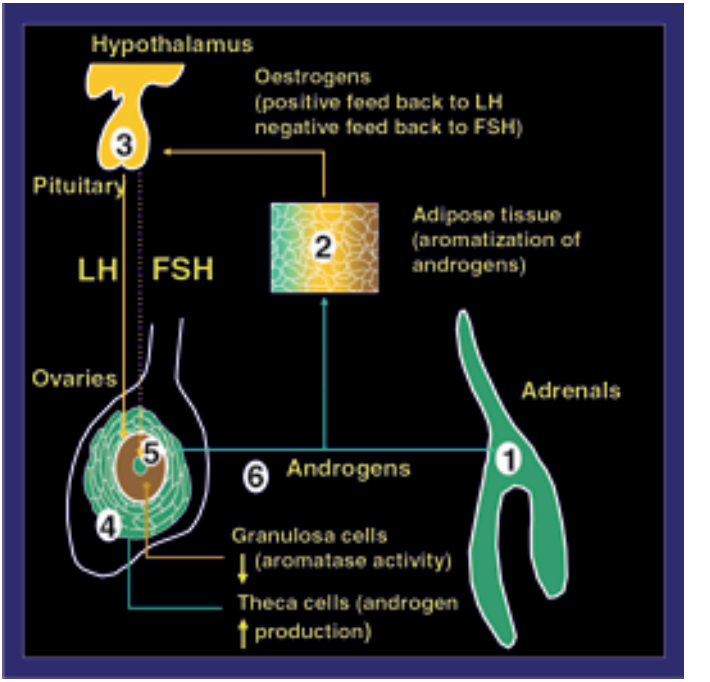


**Fig. 1.** The simplified pathogenetic mechanism of PCOS by Yen et al. (1976).

Therefore, based on the hypothesised pathomechanism proposed by Yen et al., we investigated the effect of long-term low-dosage (0.5 mg per evening) DEX suppression of the adrenal cortex – after a 7-day DEX trial – in CC-resistant PCOS. The levels of serum cortisol, DS, androstenedione (ANDR) and total testosterone (TT) decreased significantly ($p < 0.001$), while testosterone-estradiol-binding globulin (TEBG), free testosterone (FRT) and albumin-bound-testosterone (ALB-T) levels reduced only moderately. However, we failed to induce ovulation even after 50 days of treatment (Fig. 2). We determined hormone values (including androgens) each time from a mixture containing equal amounts of serum samples obtained on three different days to reduce the diagnostic error resulting from the episodic hormonal secretion and day-to-day variation. We followed this protocol during examinations performed later in our clinical practice. The determination of *P*, E2, LH and FSH in ovulatory cycles were exempt from this routine. We used the average of three measured *P* and E2 values in the luteal phase, after we confirmed the time of sampling.

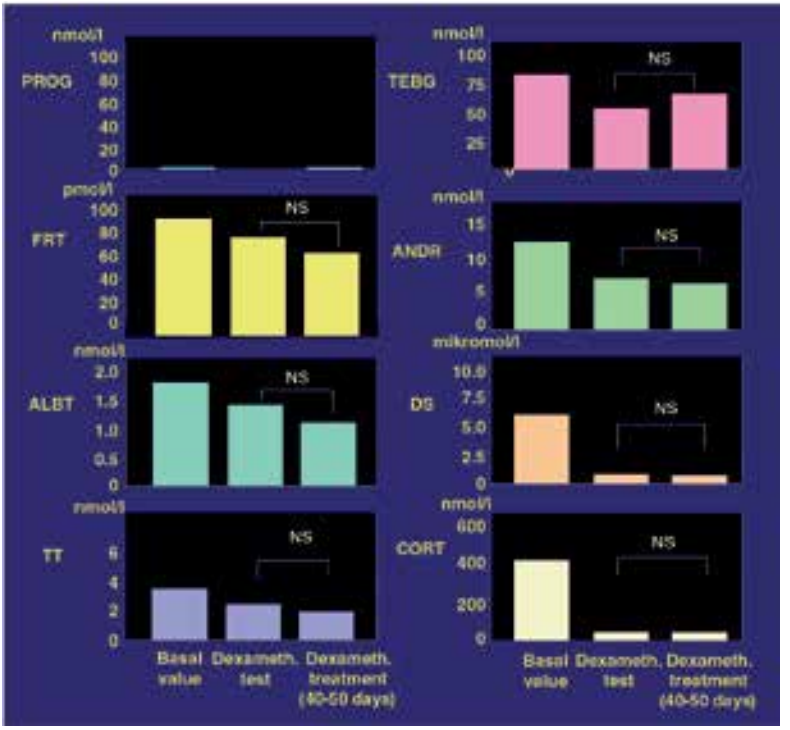


**Fig. 2.** Dexamethasone treatment of PCOS *(N* = 20).

After this we investigated the hormonal effects of the combined administration of oestrogen-gestagen in CC-resistant PCOS. Together with the moderate suppression of DS and DHEA ($p < 0.01$), androstenedione and TT underwent a significant and considerable decrease ($p < 0.001$) and FRT ($p < 0.001$) – despite the increase in TEBG – decreased to the greatest extent. After the cessation of treatment, every hormonal level returned to its original value and ovulation failed to occur within 50 days (Fig. 3).

We subsequently investigated the hormonal changes in CC-resistant PCOS resulting from combined adrenal and ovarian suppression followed by continued adrenal suppression only, after the cessation of the oestrogen-gestagen effect. During the combined effect of oestrogen-gestagen and low-dosage ($p < 0.001$) corticoid, the free fractions of T and every androgen decreased to a remarkable extent (to their minimum), while the level of TEBG increased significantly ($p < 0.01$). After the cessation of ovarian suppression and with the continuation of dexamethasone

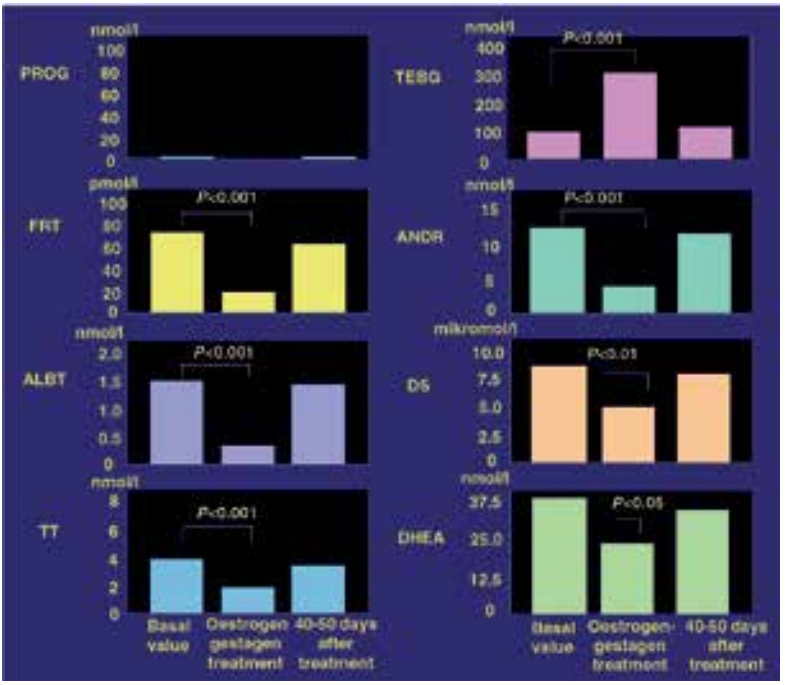


**Fig. 3.** Combined oestrogen-dexamethasone treatment of PCOS (*N* = 20).

treatment, the ovarian hypersecretion of androgens did not recur – unlike treatment without dexamethasone – but remained physiological and 80 % of CC-resistant patients ovulated between the 15th and 22nd days of the cycle (Fig. 4). In cases in which ovulation failed to occur, E2 levels also increased significantly (400–600 pmol/l). This observation formed the basis of our new treatment protocol.

Our studies seem to confirm that the primary cause of ovarian androgen overproduction and anovulation typical in PCOS is elevated androgen secretion of the adrenal cortex – including extraovarian oestrogen production–which is capable of sustaining itself after it has fully developed. *Whereas if we end the androgen hypersecretion of the ovaries, the hyperfunction of the adrenal cortex is required for its recurrence*. Without this, ovarian functions usually normalise and ovulatory cycles take place. The aforementioned studies and results provided the foundation for our treatment method for PCOS and the associated anovulation and infertility (Fig. 4). For the sake of simplicity and based on its similarity

to surgical wedge resection (while traditional wedge resection decreases ovarian androgen hypersecretion through surgery, our method works hormonally), we called our method "hormonal wedge resection".

## "Hormonal Wedge Resection": an Effective Treatment Method for Polycystic Ovary Syndrome

During oestrogen-gestagen-DEX treatment, the activity of the adrenal cortex and the ovaries are collectively suppressed, as are (concurrently) the secretion of LH and FSH. The cessation of suppressing the gonadotropic hormones and ovarian function (i.e., administering oestrogen and gestagen) is followed by approximately physiological FSH and LH secretion, which in turn results in the induction of ovulatory cycles in most patients (Fig. 5). We aimed to develop a new therapeutic method for the

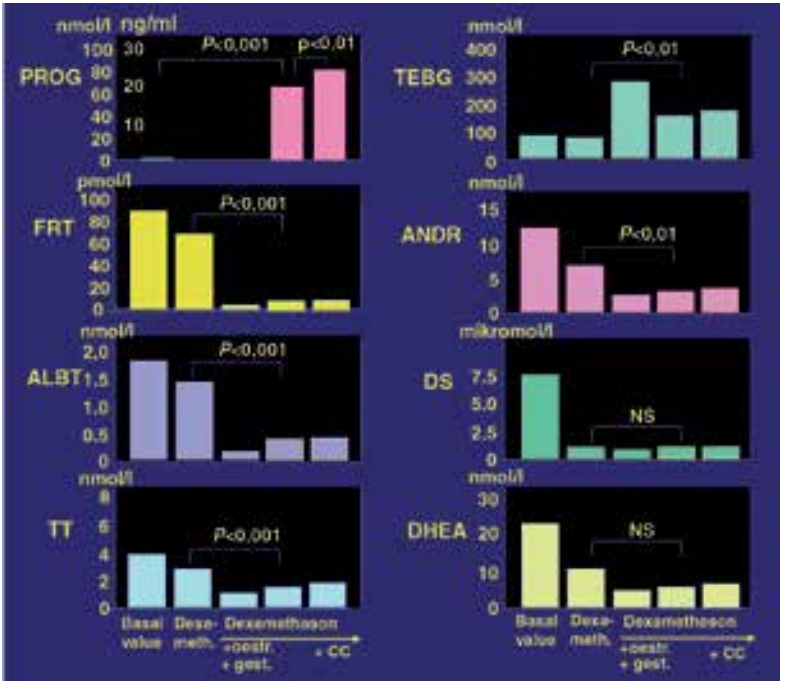


**Fig. 4.** Combined oestrogen-dexamethasone treatment followed by continuous dexamethasone treatment alone in PCOS ($N = 40$): the basis of "hormonal wedge resection".

treatment of PCOS and the associated infertility that surpasses the currently available methods in simplicity, efficacy and safety.

## Patients and Methods

To assess the clinical value of hormonal wedge resection, we evaluated our therapeutic results in patients suffering from PCOS where other factors causing infertility could be ruled out: at least one intact tuboovarian unit and andrologically confirmed normospermia presented. To diagnose PCOS, we used the criteria accepted on the consensus conference of the ESHRE and ASRM (2003): ovarian hyperandrogenism, anovulation and typical ultrasound image of the ovaries. Out of the patients, 93 % had primary infertility and 7 % had secondary infertility (length between 2 and 14 years, 4.41 ± 2.1 years, average ± SD). The typical clinical features

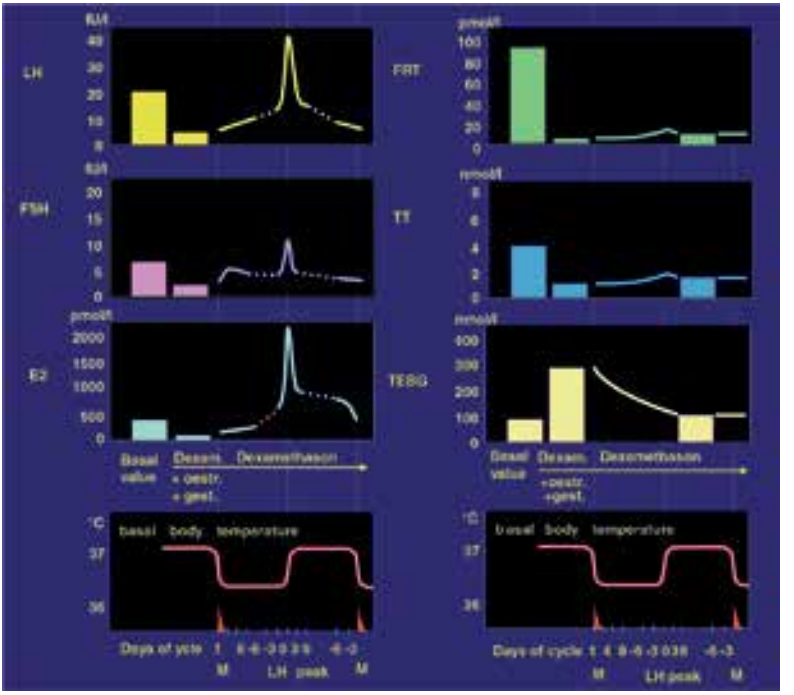


**Fig. 5.** The effective mechanism of "hormonal wedge resection".

**Table 1. Clinical Features of Patients with PCOS (*N* = 123).**

| | |
|---|---|
| Age (years) (average ± SD) | 30.5 ± 6.4 |
| Weight (kg) (average ± SD) | 65.7 ± 9.6 |
| Height (cm) | 164 ± 4.7 |
| BMI (kg/m$^2$) (average ± SD) | 23.6 ± 3.57 |
| Optimal weight (%) (average ± SD) | 122.2 ± 2.7 |
| Hirsutism grade* | 5.5 ± 2.6 |
| Infertility years (average ± SD) | 4.41 ± 2.1 |
| Range | 2–14 |
| Primary | 93 % |
| Secondary | 7 % |
| Overweight BMI > 25** | 39 % |
| Primary | 13 % |
| Secondary | 26 % |
| Menstrual cycle | |
| Eumenorrhea | 3 % |
| Oligomenorrhea | 65 % |
| Amenorrhea | 32 % |
| Anovulation | 100 % |

*According to Ferriman and Gallwey (1961)
**Primary: since the menarche, secondary: developed later in life

in patients with secondary infertility developed after pregnancies followed by considerable weight gain.

First we applied our treatment method on 40 patients with CC-resistant PCOS, but after seeing its effectiveness, we extended its use to other PCOS patients as well. The clinical features presented here summarise the data from every treated patient (Table 1).

The therapeutic scheme of "hormonal wedge resection" is shown in Fig. 6. During oestrogen-gestagen-DEX treatment, the activity of the adrenal cortex and the ovaries are collectively suppressed, as are (concurrently) the secretion of LH and FSH. As the continuous administration of DEX results in the permanent decrease of adrenal cortex function within 10 days, it is sufficient to initiate it 10 days before the end of combined oestrogen-gestagen therapy. The cessation of suppressing the gonadotropic hormones and ovarian function is followed by approximately physiological FSH and LH secretion, which in

turn results in the induction of ovulatory cycles in 80 % of CC-resistant patients. However, the ovulatory cycles that result do not always have physiological FLF (average luteal $P = 17.0 \pm 5.0$ ng/ml, average ± SD) (that equals $54.1 \pm 15.9$ nmol/l), and in 58 % (23/40) of cases, *P* shows values under 23 ng/ml, typical of FLI. To induce physiological ovulation and to avoid obstetrical complications caused by FLI (miscarriage, preterm birth, etc.), we combined the continuous DEX treatment with 100 mg CC administered daily for 5 days right from the fifth day of the first cycle, with the application of luteal *P* control.

The initial dose of $5 \times 100$ mg CC led to $25.6 \pm 5.7$ ng/ml ($81.4 \pm 18.0$ nmol/l) average luteal *P* values. If the serum *P* failed to result in the physiological value with this dosage, we increased the CC dose by $5 \times 50$ mg per cycle, controlled by regular luteal *P* measurements until physiological FLF was achieved. DEX treatment complemented with individually adjusted CC dosage resulted in physiological luteal function (except for three patients), similarly to cases where DEX treatment alone failed to induce ovulation. We recommended traditional contraceptive methods for our patients until the physiological FLF was achieved, but despite this, conception still took place in some cases before reaching this goal (see later). Since the presence of impaired glucose metabolism in PCOS is known, we combined our treatment with metformin therapy in the case of glucose intolerance. We considered it worth continuing metformin therapy throughout pregnancy to prevent or treat gestational diabetes (ASHRE/ASRM 2010, 2013).

In some patients we experienced that if conception failed to take place within 3–6 ovulatory cycles, the secretion of ovarian androgens partially recurred (hypertrophic theca, hilus and stromal cells might produce excess androgens even in the case of physiological LH level, especially during LH peak), and the patients remitted to anovulation. In these cases, intercalating a single month of contraceptive administration allowed the successful continuation of the treatment with the previously set CC dosage. Applying a contraceptive for 1 month already suppresses ovarian androgen hyperproduction, although in definite cases of androgen hypersecretion, it appears best to use oestrogen-gestagen therapy for several months before beginning "hormonal wedge resection". Presumably this is why permanent inhibition of the activity of androgen-producing ovarian cells leads to their partial atrophy, not solely the decrease of function.

When using combined contraceptives for the first time, breakthrough bleeding during the last week of application may occur in about 30 % of cases. This is the result of a relative decrease in oestrogen levels (constant exogenous oestrogen intake, declined endogenous oestrogen production). We recommend the use of sequential contraceptives to avoid this or alternatively complementing combined contraceptives with gestagen for the last 10 days of application (Fig. 6).

## Results

Looking at the clinical features of the patients (Table 1), it is striking that their average weight is 10.5 kg higher than the physiological control: 55.2 and 65.7 kg (criteria for the physiologic control group: eumenorrheal cycles, physiological FLF and BMI of 19–22). The BMI values of the patients exceeded 25 in 39 % of cases. In two-thirds of cases, being overweight and typical clinical features of PCOS did not emerge in connection with the menarche, but later in life. Only 3 % of patients had eumenorrheal cycles (cycle length between 25 and 35 days), most of them had oligomenorrheal

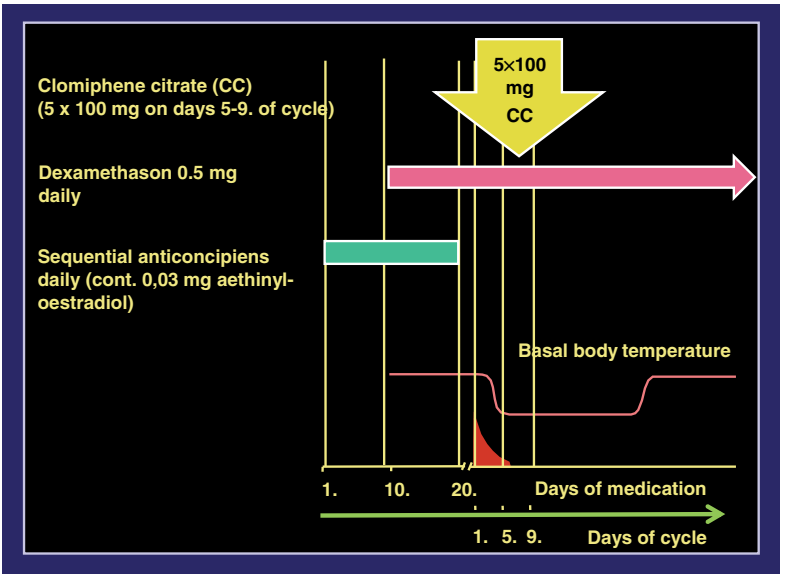


**Fig. 6.** The therapeutic scheme of "hormonal wedge resection".

(bleeding at intervals between 35 and 90 days) or amenorrheal bleeding (less often then 90 days), and ovulation could not be detected in any of them.

Hormonal characteristics show the well-known and expected increase of LH and decrease of FSH levels and the increased LH/FSH resulting from this (Table 2). In PCOS patients, elevated oestrone levels and an increased oestrone/oestradiol ratio is prominent, which implicates enhanced extraovarian oestrone production. Although the biological activity of oestrone is only a third of that of oestradiol, its free fraction amount is three times more, which means it can exert a considerable oestrogen effect. The decreased TEBG levels also result in the upsurge of biologically available free oestradiol fractions. In PCOS, prolactin levels are elevated as well.

The free fractions of every androgen and testosterone increased significantly ($p<0.001$) in PCOS, particularly the biologically active free (and albumin-bound) testosterone, which was three times the physiological value. The serum levels of DS and cortisol originating exclusively or almost exclusively from the adrenal cortex were also significantly ($p<0.001$) increased, which indicates exaggerated adrenal cortical activity (Table 3).

Hormonal wedge resection produced ovulation in all patients but three. Ovulation took place between the 15th and 22nd day of the cycles

**Table 2. Serum Levels of Gonadotropic Hormones and Oestrogens in PCOS.**

| Characteristics | Physiological control group $N=40$, average ± SE | PCOS patients $N=123$, average ± SE | Significance |
|---|---|---|---|
| LH (IU/l) | 10.9 ± 0.41 | 19.1 ± 1.19 | $p<0.001$ |
| FSH (IU/l) | 8.13 ± 0.39 | 5.89 ± 0.29 | $p<0.001$ |
| LH/FSH | 1.42 ± 0.07 | 3.60 ± 0.26 | $p<0.001$ |
| Prolactin (mIU/l) | 237.0 ± 10.4 | 303.0 ± 17.4 | $p<0.001$ |
| Oestrone (pmol/l) | 177.9 ± 9.9 | 393.1 ± 18.1 ($N=91$)* | $p<0.001$ |
| Oestradiol (pmol/l) | 247 ± 17.1 | 285.6 ± 16.9 | NS |
| Oestrone/oestradiol | 0.78 ± 0.04 | 1.58 ± 0.09 ($N=91$)* | $p<0.001$ |
| Free oestradiol (pmol/l) | 5.43 ± 0.36 | 8.11 ± 0.55 ($N=91$)* | $p<0.01$ |

*Number of cases where it is different from the usual

**Table 3. Levels of Serum Androgens in PCOS.**

| Characteristics | Physiological control group $N = 100$, average ± SE | PCOS patients $N = 123$, average ± SE | Significance |
|---|---|---|---|
| Free testost. (pmol/l) | 23.85 ± 0.47 | 77.72 ± 3.19 | $p < 0.001$ |
| Albumin testost. (pmol/l) | 488.2 ± 10.7 | 1,558.3 ± 59.7 ($N = 91$)* | $p < 0.001$ |
| Testosterone (pmol/l) | 1,819.9 ± 41.0 | 3,447.5 ± 111.8 | $p < 0.001$ |
| SHBG (nmol/l) | 131.2 ± 3.9 | 66.2 ± 4.2 | $p < 0.001$ |
| Androstenedione (nmol/l) | 5.38 ± 0.14 | 10.8 ± 0.51 ($N = 91$)* | $p < 0.001$ |
| DHEA (nmol/l) | 13.04 ± 0.37 | 24.93 ± 1.6 ($N = 91$)* | $p < 0.001$ |
| DHEA-S (μmol/l) | 3.97 ± 0.13 | 6.17 ± 0.28 | $p < 0.001$ |
| Cortisol (nmol/l) | 217.3 ± 5.2 | 410 ± 13.9 | $p < 0.001$ |

*Number of cases where it is different from the usual

with 28–36-day intervals. Out of the three patients, ovulation failed to occur despite the application of DEX + 5 × 200 mg CC in two cases and DEX + 5 × 250 mg CC in one case (in this case, the later histological test of the ovaries confirmed hyperthecosis syndrome).

Ovulation took place in 97.6% of patients as a result of hormonal wedge resection, which led to pregnancy in 98.3% (118/120) of patients. Two patients failed to conceive within 5 and 6 months, despite physiological FLF.

Out of the 118 patients with ovulation, 167 pregnancies were conceived as a result of hormonal wedge resection and hyperstimulation did not occur. In 13 of these cases, pregnancy took place before physiological FLF ($P < 17$ ng/ml) was achieved. This resulted in miscarriage in 12 cases and preterm birth in one case (Table 4). Ten patients who had miscarriages with insufficient FLF received repeated treatment, after which pregnancies were conceived with physiological FLF and ended in mature birth. Initially we considered the average luteal *P* value of 17 ng/ml as physiological. However, after recognising the strong correlation between FLF and pregnancy outcome, we approved the luteal *P* values regarding mature, singular and eutrophic newborns as physiological ($P > 23$ ng/ml). This partly explains the high conception rates with *P* values between 17 and 23 ng/ml (28%, 43/154). Pregnancy outcomes in PCOS also confirmed our expectations (Table 4).

The necessary dosage of CC combined with 0.5 mg DEX treatment per evening to achieve physiological during hormonal wedge resection is

**Table 4. Pregnancy Outcome in PCOS with "Hormonal Wedge Resection" in Terms of Progesterone Values.**

| Characteristics | Total pregnancies *N* = 167 | *P* < 17 ng/ml *N* = 13 | *P* > 17 and ≤23 ng/ml *N* = 43 | *P* > 23 ng/ml *N* = 111 | National average |
|---|---|---|---|---|---|
| Abortion/ pregnancy | 9.6 % | 92.3 % | 2.3 % | 2.7 % | 15.1 % |
| Patients | 16/167 | 12/13 | 1/43 | 3/111 | |
| First trimester abortion/ pregnancy | 7.2 % | 61.5 % | 2.3 % | 2.7 % | |
| Patients | 12/167 | 8/13 | 1/43 | 3/111 | |
| Second trimester abortion/ pregnancy | 2.4 % | 30.8 % | – | – | |
| Patients | 4/167 | 4/13 | | | |
| Extrauterine pregnancy/ pregnancy | 0.6 % | – | 2.3 % | – | 1.1 % |
| Patients | 1/167 | | 1/43 | | |
| Birth/pregnancy | 89.8 % | 7.7 % | 95.4 % | 97.3 % | 83.8 % |
| Patients | 150/167 | 1/13 | 41/43 | 108/111 | |
| Mature birth/ singular pregnancy | 98.6 % | – | 100 % | 99.0 % | 90.5 % |
| Patients | 137/139 | | 40/40 | 97/98 | |
| Preterm birth/ singular pregnancy | 1.4 % | 100 % | – | 1.0 % | 9.5 % |
| Patients | 2/139 | 1/1 | | 1/98 | |
| IUGR/singular pregnancy | 5.6 % | – | 17.5 % | 1.0 % | 10.1 % |
| Patients | 8/139 | | 7/40 | 1/98 | |
| Birth weight <2,500 g// singular pregnancy, | 2.8 % | 100 % | 7.5 % | – | 9.3 % |
| – patients | 4/139 | 1/1 | 3/40 | | |

*Continued*

*Continued*

| | | | | | |
|---|---|---|---|---|---|
| Preeclampsia/ pregnancy<br>Patients | – | – | – | – | – |
| Twin birth/ birth<br>Patients<br>Preterm birth, IUGR, Average birth weight <2,500 g | 7.3 %<br>11/150<br>4/11 3/11<br>7/11 | – | 2.4 %<br>1/41<br>1/1 0/1<br>1/1 | 9.3 %<br>10/108<br>3/10 3/10<br>6/10 | 1.6 % |
| Singular newborns | | | | | |
| Weight g (average ± SD) | 3,351 ± 461 | 1,400 | 2,993 ± 363 | 3,511 ± 380 | |
| Length cm (average ± SD) | 54.5 ± 2.3 | 45 | 53.2 ± 1.7 | 56.0 ± 2.3 | |
| Weight percentile % (average ± SD) | 53 ± 27 | 2 | 35 ± 22 | 62 ± 24 | |
| Multiple newborns | | | | | |
| Weight g (average ± SD) | 2,374 ± 542 | – | 1,550 | 2,454 ± 493 | |
| Length cm (average ± SD) | 50.0 ± 3.1 | | 44 | 50.5 ± 2.5 | |
| Weight percentile % (average ± SD) | 21 ± 15 | | 25 | 20 ± 16 | |

5 × 100 mg in 44 % of patients, 5 × 150 mg in 39 % of patients, 5 × 200 in 10 % and 5 × 250 in 6 % (we failed to achieve ovulation in three patients, see above). In 16 patients where pregnancy did not occur within 3 months despite physiological FLF, anovulation developed after 3–5 cycles with continued treatment. By intercalating a month of contraceptive administration, the reinitialised treatment resulted in ovulatory cycles again in each case.

We achieved unexpected positive monthly pregnancy rates with physiological FLF ($P > 23$ ng/ml) with treatment. All pregnancies took place within 1–7 months (ovulation did not occur in three patients, two patients with ovulation did not become pregnant and quit treatment after 5 and 6 months, respectively). The monthly pregnancy rate – considering

repeated pregnancies – was an average of 40.7 % during the first three months and 38.6 % during the first and seventh cycles. The monthly pregnancy rates regarding first and repeated conceptions were almost equal. The time to pregnancy (TTP) was 2.44 ± 1.3 cycles and the yearly cumulative pregnancy rate was 95.5 %. These fertility rates are even better than the most optimal statistics (see in UI). We do not have an explanation for this phenomenon. Similarly positive fertility rates were observed with physiological FLF during the treatment of other anovulatory diseases, for example, in hyperprolactinaemic patients. (It may be that permanent anovulation adversely impacts the cervical mucus, and this hinders the ascension of sperm cells and bacteria, and thus immunity against sperm cells or mild endosalpingitis cannot develop.)

Pregnancy outcomes were also very favourable with physiological FLF ($P > 23$ ng/ml): miscarriage occurred in 2.7 % of cases (95 % CI: 1–8 %), preterm birth and IUGR both in 1 % of cases (95 % CI: 0.2–5 %), and preeclampsia did not occur at all. Twins were conceived in 9.3 % of cases but no triplets were conceived. Concerning pregnancy outcome, the most optimal average *P* value at ranges appears to be 26–32 ng/ml: the average singular newborn weight is 3,716 g, length 56 cm, weight percentile 75 % and length percentile 73 % and the incidence of twin births is 5.6 % (Table 5).

## Discussion

The combined administration of oestrogen-gestagen-DEX followed by DEX treatment complemented with CC – the dosage of which is adjusted with regular *P* level control – appears suitable for inducing ovulation and for achieving physiological FLF in patients with PCOS who do not respond to CC treatment alone. We called the therapeutic method we developed hormonal wedge resection.

The effective mechanism of hormonal wedge resection can be understood as follows. The oestrogen-gestagen-DEX treatment causes adrenal and ovarian androgen secretion and the amount of oestrogens they produce to drop to the minimum, similarly to the secretion of gonadotropic hormones. When oestrogen-gestagen therapy is stopped, this releases the hypothalamo-pituitary-ovarian system from their negative feedback effect, while the concurrent DEX suppression eliminates

**Table 5. Parameters of Singular Newborns in Terms of Luteal Progesterone Value Ranges in Polycystic Ovary Syndrome.**

| | Progesterone >17–23 ng/ml *n* = 40 | Significance | Progesterone >23–29 ng/ml *n* = 66 | Significance | Progesterone >29 ng/ml *n* = 32 | Progesterone* >26–32 ng/ml *n* = 36 |
|---|---|---|---|---|---|---|
| Progesterone (ng/ml) | 21.3 ± 1.5 | $p < 0.001$ | 25.4 ± 1.8 | $p < 0.001$ | 32.7 ± 4.9 | 29.1 ± 2.0 |
| Oestradiol (pg/ml) | 355 ± 83 | $p < 0.001$ | 394 ± 96 | $p < 0.001$ | 427 ± 118 | 414 ± 82 |
| Weight | 2,993 ± 363 g | $p < 0.001$ | 3,442 ± 328 g | $p < 0.001$ | 3,722 ± 366 g | 3,716 ± 392 g |
| Length | 53 ± 1.7 cm | $p < 0.001$ | 55 ± 4 cm | $p < 0.01$ | 56 ± 3 cm | 56 ± 4 cm |
| Weight percentile | 35 ± 22 % | $p < 0.001$ | 58 ± 20 % | $p < 0.001$ | 79 ± 22 % | 75 ± 19 % |
| Length percentile | 52 ± 20 % | $p < 0.01$ | 56 ± 21 % | $p < 0.001$ | 71 ± 19 % | 73 ± 20 % |
| Twin births/birth | 2.4 % 1/41 | | 4.3 % 3/69 | | 17.9 % 7/39 | 5.6 % 2/36 |

*The apparently most optimal range: physiological average ± SD.

the negative central effects of increased CRF secretion as well as the negative feedback effects of cortisol, adrenal androgens and oestrogens converted from the latter. Enhanced CRF secretion decreases GnRH secretion mainly via the opioid system and increases prolactin secretion through the dopamine system (Barbarino et al. 1989), a mechanism which is further supported by the results obtained with opioid antagonists (Wildt et al. 1993) and by the confirmed suppressive effect of DEX on prolactin. The negative effect of elevated cortisol level on hypophysis and directly on folliculogenesis is reported by many authors (Monzani et al. 1989; Chatterton et al. 1991; Whirladge and Cidlowsky 2013). The cessation of the negative feedback – similarly to what happens during the first days of physiological cycles – causes an increase in FSH and LH secretion, folliculogenesis begins and this leads to ovulation in most (80 %) patients. However, FLI can be detected in about 50 % of cases, which is the result of the partial impairment of folliculogenesis. Impaired folliculogenesis under the above described therapy in PCOS can be explained in several ways. Permanently-enhanced LH effect can lead to the hypertrophy of androgen-producing ovarian cells (theca, hilus and stromal cells), which means they may produce excess androgens even in the case of physiological LH level that can disturb folliculogenesis by its direct ovarian influence. The extended presence of GnRH suppression may decrease the capability of the hypothalamus to respond to oestradiol (increased GnRH secretion and LH peak), which can be so pronounced in some cases that it can even impede the development of LH peak (anovulation despite of adequate preovulatory oestradiol levels).

The activity of hypertrophic androgen-producing ovarian cells (theca, hilus and stroma cells), which emerge as a result of permanently-enhanced LH, effect returns to the physiological level after 20 days of oestrogen-gestagen therapy, although the regression of hypertrophy presumably requires a longer suppression period. Our experiences also affirm these assumptions. Oestrogen-gestagen therapy administered for 3 months prior to hormonal wedge resection remarkably improved its efficacy. As a result, the CC dose required to achieve physiological FLF decreased, and in the absence of pregnancy, the continuation of the treatment successfully maintained physiological FLF over multiple cycles.

In summary, we introduced a new therapeutic method, called hormonal wedge resection, to treat PCOS and the associated infertility, based on the PCOS pathomechanism proposed by Yen et al. (1976). Its application induced ovulatory cycles and physiological luteal function in 97.6% of patients. The therapeutic protocol we developed provides a novel, simple, effective and repeatable method of treating functional infertility associated with PCOS. It is also suitable for treating clomiphene-resistant cases.

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# Sperm Retrieval Techniques

**Chak-Lam Cho, Ashok Agarwal**

## Introduction

The collection of sperm from the male genital tract was first described in 1985 [1]. But the procedures of sperm retrieval become an integral part of the management of azoospermia only after the report of a successful pregnancy by using testicular sperm extraction followed by intracytoplasmic sperm injection (ICSI) in 1993 [2]. While testicular sperm are retrieved from men with nonobstructive azoospermia (NOA), sperm may be retrieved from either the epididymis or testis in men with obstructive azoospermia (OA).

Cochrane meta-analysis has determined that there is insufficient data from trials to recommend any particular surgical sperm retrieval technique for either OA or NOA [3]. The complex interplay between male and female factors, and sperm retrieval and artificial reproductive technology (ART) means the management of infertile couples should be individualized.

In this chapter, we describe the preoperative preparation and postoperative care for patients undergoing sperm retrieval procedures. The principles of selection among different sperm retrieval techniques for patients with OA and NOA are illustrated by 2 clinical scenarios.

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## Preoperative Preparation

Patients are instructed to withhold anticoagulants and antiplatelet medications for a week before the procedure. Abstinence from ejaculation for 2–3 days prior to the procedure is advised. Blood tests including screening of infectious diseases are performed. Laboratory tests for infectious disease status may consist of hepatitis, syphilis, and human immunodeficiency virus (HIV) and may vary between centers. A quarantine cryopreservation tank may be required in case of positive test results.

The vast majority of sperm retrieval procedures are performed on an outpatient basis. Shaving or clipping of the surgical site can be performed 1 day before or on the day of surgery [4]. Intravenous antibiotic with coverage of Gram-positive organisms is administered at least 30 min before skin incision except for percutaneous procedures. A bench microscope with appropriate containers and transport media should be available in the operating room for intraoperative examination of specimens. An experienced embryologist should be present, if necessary.

General anesthesia is generally preferred if the procedure involves the use of an operative microscope. Local anesthesia may be employed for percutaneous procedures, particularly in the outpatient setting. However, many patients reported significant discomfort and anxiety during percutaneous sperm retrieval procedures with spermatic cord block alone [5]. The co-administration of intravenous sedation would offer patients the additional benefits of an anxiolytic and possibly amnesia. Patients have reported greater satisfaction with the addition of intravenous sedation especially for bilateral or longer procedures [6].

## Case Scenarios

### Case 1

A 50-year-old gentleman, who had a vasectomy 15 years ago, wishes to have another child. His wife is 38 years old with a normal evaluation. They have 1 child together but also a history of 3 miscarriages. Testes measure 20 cc in volume bilaterally. Both epididymides are mildly prominent. The couple decides for sperm retrieval and ART instead of reconstructive surgery.

Diagnostic testing for post-vasectomy patients before sperm retrieval is generally not necessary. Hormonal evaluation with serum follicle-stimulating hormone (FSH) and testosterone is performed if there is clinical suspicion of impaired spermatogenesis.

## Options of Sperm Retrieval Procedures

Sperm may be retrieved from epididymis or testis in azoospermic men after vasectomy. Epididymal sperm retrieval is the most commonly performed procedure in this situation. The procedure can be performed by percutaneous or open approaches. Percutaneous epididymal sperm aspiration (PESA) can be performed under local anesthesia, and an operative microscope is not required. It may be performed in an office setting. Patients recover quickly after the procedure with low complication rates. A needle is advanced percutaneously into the epididymal tubules. The needle is advanced in and out gently with negative suction force applied via a 30 cc or 60 cc syringe. Epididymal fluid is aspirated. Around 0.1 mL of fluid is usually obtained per aspirate [7]. The procedure is repeated at different sites from cauda to caput epididymis until an adequate number of motile sperm is retrieved. Since PESA is a blind procedure, multiple attempts may be required to obtain good quality sperm. Aspirates from cauda are often rich in senescent spermatozoa, debris, and macrophages. The phenomenon of better quality sperm at the proximal reproductive tract in men with chronic obstruction has been termed "inverted motility" [8]. It may be rational to start the procedure from corpus epididymis toward caput since vasectomy was performed 15 years ago. The concern about the detrimental effect of PESA on subsequent reconstructive microsurgery is not valid [9].

Epididymal sperm can also be acquired by an open approach with microscopic epididymal sperm aspiration (MESA). MESA involves incision of the epididymal tunica and inspection of the epididymal tubules. Dilated epididymal tubules with clear contents are punctured or incised, and the fluid is collected. A single MESA procedure usually enables retrieval of a great number of motile sperm [10], which are usually sufficient for cryopreservation for multiple subsequent in vitro fertilization (IVF) cycles [11]. The open procedure allows better hemostasis and decreased risk of hematoma formation compared to PESA [12]. Also, the

contamination of the sample by red blood cells is minimized. The risk of scarring and epididymal obstruction after MESA is likely lower compared to PESA due to targeted aspiration of individual tubules under direct microscopic vision, and incised tubules can be repaired. MESA has been modified to combine advantages of percutaneous technique with precision of microsurgical procedure. The epididymis is brought anteriorly and examined via a 1–2-cm scrotal incision during the procedure of "mini-MESA" [13].

Testicular sperm can be retrieved by testicular sperm aspiration (TESA) percutaneously from men with OA. The testicular parenchyma is aspirated by fine needle, large-diameter needles, or tissue-cutting biopsy needles. Location of sperm aspiration matters little in terms of successful sperm retrieval. Sperm in obstructed testes is found throughout the parenchyma [14]. An entry point starting at the superior testicular pole and passing inferiorly and obliquely may carry less risk of vascular injury. Conversely, testicular sperm extraction (TESE) is rarely employed in men with OA. The pros and cons of various sperm harvesting techniques for OA is summarized in Table 1.

## Selection and Results of Sperm Retrieval Technique in Post-Vasectomy Patients

The sperm retrieval rate (SRR) in patients with OA is high and ranges from 90 to 100% [15]. Successful PESA has been reported in 78.0% of the cases, and subsequent percutaneous testicular retrievals are successful in the vast majority of failed epididymal sperm retrievals. The cumulative success rate of percutaneous approaches in OA patients reaches 97.3% irrespective of the cause of obstruction [16]. A low SRR of around 20% has been reported when an epididymal cyst is present, which is a common finding after vasectomy [17, 18]. In this patient population, however, subsequent sperm retrieval by TESA or TESE still carries a high success rate.

The history of multiple miscarriages without an identifiable female factor in our clinical scenario also needs to be investigated and may have implication on the choice of sperm retrieval technique. The impact of paternal factors on reproductive outcomes is increasingly being recognized. Sperm deoxyribonucleic acid (DNA) fragmentation has been widely studied in recent years and has been increasingly associated with

**Table 1. Advantages and Disadvantages of Sperm Retrieval Techniques for Obstructive Azoospermia.**

| | Advantages | Disadvantages |
|---|---|---|
| PESA | • Fast<br>• Low cost<br>• Possibly office/outpatient procedure<br>• Minimal recovery and morbidity<br>• Repeatable<br>• No microsurgical skill and instruments required | • Few sperm retrieved<br>• May not retrieve adequate sperm for cryopreservation<br>• May cause epididymal obstruction at puncture sites<br>• Risk of hematoma formation |
| MESA | • Ample sperm retrieved<br>• Excellent chance of sperm cryopreservation<br>• Decreased risk of hematoma | • Increased cost and anesthetic/operating time<br>• Microsurgical skill and instruments required<br>• Surgical exploration required with longer postoperative recovery |
| TESA | • Fast<br>• Low cost<br>• Possibly office/outpatient procedure<br>• Minimal recovery and morbidity<br>• Repeatable<br>• No microsurgical skill and instruments required | • May not retrieve adequate sperm for cryopreservation<br>• Risk of hematoma formation<br>• Risk of testicular atrophy |
| TESE | • Fast<br>• Repeatable<br>• No microsurgical skill and instruments required | • Increased cost and operating time<br>• Surgical exploration required with longer postoperative recovery<br>• Risk of testicular atrophy |

*PESA* percutaneous epididymal sperm aspiration; *MESA* microsurgical epididymal sperm aspiration; *TESA* percutaneous testicular sperm aspiration; *TESE* conventional testicular sperm extraction

recurrent pregnancy loss particularly in the setting of ART [19]. The association between aging and loss of DNA integrity [20] is particularly worrisome in our patient. Sperm DNA fragmentation (SDF) testing may be diagnostic in identifying the etiology of recurrent miscarriage, especially after ART failure. Testing may also provide prognostic information on ART outcomes for the couple. There is evidence to show that high SDF is associated with increased risk of pregnancy loss and decreased live birth [21]. Treatment strategies, including oral antioxidants and sperm selection, can be considered in case of elevated SDF levels. Testicular sperm

retrieval with TESA or TESE may be preferable in patients with high SDF since the incidence of DNA fragmentation is markedly lower in testicular sperm [22, 23].

It is rational to start retrieval of epididymal and/or testicular sperm percutaneously (i.e., PESA ± TESA) in our patient with expected high SRR approaching 90–100% [24]. Percutaneous sperm retrieval provides the advantages of minimal invasiveness with low complication rate. The procedure can be performed in the office setting under local anesthesia without the use of operative microscope and microsurgical technique. PESA should start from corpus epididymis toward caput in view of the phenomenon of inverted motility. MESA should be considered if our patient desires a single sperm retrieval procedure and cryopreservation for multiple subsequent ART cycles [11, 25]. Retrieval of testicular sperm should be considered in the presence of epididymal cyst or high SDF.

## Artificial Reproductive Technology Outcomes in Men with Obstructive Azoospermia

Pregnancy success rates utilizing epididymal sperm from patients with OA in intrauterine insemination (IUI) [26] and in vitro fertilization [1] have been reported. Good oocyte fertilization and pregnancy rates in ICSI have been achieved with epididymal sperm. Fertilization, clinical pregnancy, and live birth rates of 60, 50, and 35%, respectively, can be achieved [27]. The use of testicular sperm from men with OA in ICSI is also associated with similar high pregnancy rates [28]. The source of sperm and retrieval modality does not affect outcome of ART in our patient [29, 30]. ICSI outcomes using fresh or frozen-thawed sperm retrieved from men with OA are also comparable [20, 31].

Sperm quality in OA patients is generally high. If sperm quality or quantity from the epididymis is poor, consideration should be given to TESE. Cryopreservation and use of frozen-thawed sperm in ART will not compromise the reproductive outcomes in our patient.

## Case 2

A 30-year-old gentleman has infertility and azoospermia on semen analysis. His 28-year-old wife has a normal evaluation. Testicular volume is 8 cc

bilaterally with palpable vasa deferentia on physical examination of the patient. A grade 3 left varicocele was revealed on physical examination. Serum FSH and testosterone levels are 30 IU/L and 200 nmol/L, respectively. Testicular sperm retrieval has been attempted previously with no sperm retrieved, and there is no further detail available.

While retrieval of good quality sperm from men with OA is very likely, sperm retrieval success rates in men with testicular failure and NOA is much lower. Donor sperm insemination and child adoption were the options left to men with NOA a few decades ago. The finding of heterogeneous "patchy" spermatogenesis within the testes of approximately one-third of men with NOA on a single diagnostic biopsy provides the rationale in the management of NOA by sperm retrieval [32]. Despite the severely impaired spermatogenesis with inadequate sperm production to reach the ejaculate, sperm can be demonstrated within the testes in at least 60% of men with NOA in a more recent study [33]. Testicular sperm retrieval combined with ICSI in our patient offers the chance for the patient to father his own biologic children.

## Preoperative Investigations and Optimization

Meticulous microscopic examination of the pellet is necessary to determine whether a semen sample is truly azoospermic. It is shown that sperm are identified in up to 35% of men who are thought to have NOA during an extended examination of a centrifuged specimen [34]. The definitive diagnosis of NOA relies on histological confirmation. However, a clinical diagnosis based on history, small testicular volume, and flat epididymides on physical examination, elevated serum FSH levels, and azoospermia on semen analysis can be made in many cases.

Diagnostic testicular biopsy remains the gold standard in differentiation between OA and NOA. However, the small samples obtained from diagnostic biopsy are unlikely to be representative since both testicular histology and sperm production are heterogeneous within the seminiferous tubules. Currently, many centers perform testicular biopsy for histology at the time of sperm acquisition. A separate procedure of testicular biopsy is not regarded as mandatory before sperm retrieval procedures by many male fertility specialists.

Karyotyping and Y-chromosome microdeletion (YCMD) testing typically identify the etiology of impaired spermatogenesis in 15–20% of NOA patients, and up to 17% of TESE candidates are found to have abnormal genetic evaluation [35]. YCMDs are more commonly detected in patients with lower sperm production. Ten percent of azoospermic men are noted to have YCMD, while no microdeletion is detected in men with sperm counts more than $5 \times 10^6$ [36]. It is now possible for the transmission of defective genetic material to offspring with advent of ICSI and sperm retrieval. It is therefore advisable to have genetic evaluation before sperm retrieval. Results of genetic tests have been shown to alter the choice of treatment in 21% of infertile couples [35]. Donor sperm, adoption, and embryo biopsy are some options elected by patients after genetic counseling. Apart from genetic evaluation, elevated serum FSH level is one of the clinical features of men with NOA. The prognostic value of hormonal and genetic evaluation on sperm retrieval will be discussed later in this chapter. Imaging modalities are generally not indicated for the management of NOA patients unless there are abnormalities on physical examination.

Spermatogenesis should be optimized for at least 3 months prior to sperm retrieval. Any reversible causes should be corrected including avoidance to gonadal toxins. The role of varicocelectomy in our patient with NOA is not well defined. Most patients have no return of sperm to the ejaculate and require sperm retrieval despite repair of varicocele [37]. Varicocelectomy does not influence subsequent SRR in men with NOA and clinical varicoceles. The beneficial effect of varicocelectomy may take 6 months or longer to appear and, therefore, may not be a sensible choice for our patient. Hormonal disturbances, including compromised serum testosterone and increased estradiol levels, are common among men with NOA [38]. Testosterone-to-estradiol ratio (TE ratio) is commonly used clinically as an expression of the overall androgen and estrogen balance. The mean TE ratio in fertile controls is significantly higher compared to men with severe infertility [38]. Increased aromatase activity of the testes may contribute to the phenomenon [39]. By directly limiting estrogen feedback to the pituitary gland, aromatase inhibitors increase production of FSH and luteinizing hormone (LH). The correction of the endocrinopathy may enhance endogenous intratesticular testosterone levels and thus spermatogenesis. Both steroidal (testolactone) and nonsteroidal

(anastrozole) aromatase inhibitors raise serum testosterone levels and correct TE ratios effectively [40]. A TE ratio of less than 10 is proposed as the cutoff to initiate treatment. Although significant improvements in the hormonal profile and semen parameters have been demonstrated in oligozoospermic men treated with testolactone, there are no studies that have demonstrated a return of sperm to the ejaculate in azoospermic men with treatment [38]. The use of aromatase inhibitors in men with NOA remains off-label. The correlation between hormone manipulation and fertility benefits remains to be defined by randomized controlled studies.

In summary, karyotyping and YCMD testing should be performed in our patient before sperm retrieval procedures. The test results carry important prognostic value. Varicocelectomy as an adjunct before sperm extraction has no evidence to improve SRR. Testing of estradiol level may be considered, and treatment initiated with TE ratio less than 10. However, the current evidence of correction of endocrinopathy with aromatase inhibitors in our patient with NOA is weak.

## Procedures in Men with Previous Failed Sperm Retrieval

Failure of previous sperm retrieval does not deter further attempts in our patient. The characteristic of patchy foci of sperm production in men with NOA renders a single biopsy inadequate for identification of sperm most of the time. Multiple biopsies are essential for the successful sperm retrieval in men with NOA. Only 23% of men have sperm identified on the first biopsy, and up to 14 biopsies may be required to locate sperm in a single procedure of open testicular biopsy from 1 or both testicles [41]. Repeating testicular biopsy or testicular sperm aspiration (TESA) may be a less favorable option in view of the low SRR. Microdissection testicular sperm extraction (mTESE) after previous failed TESA or TESE procedures is more commonly practiced and studied. Patients who had 1 or 2 prior biopsies per testis have an SRR of 50% by mTESE. SRR decreases to 22% if 3 or more previous biopsies were performed per testis. This is compared to 52% of SRR with mTESE in patients who have no prior testicular surgery [42]. The minimal impact on subsequent SRR by 1 or 2 prior testicular biopsies strongly suggests that random testicular biopsies commonly miss areas of sperm production. The chance of sperm identification on repeated mTESE is 33% even when no sperm is found on the

first mTESE [43]. Data show that mTESE achieves reasonable SRR after failed testicular biopsy, TESA, TESE, and mTESE in the hands of an experienced infertility surgeon. Repeated mTESE appears a viable option for our patient.

A 6-month interval between sperm retrieval procedures is recommended to our patient. This recommendation is based on the concept that spermatogenesis can be adversely affected by postoperative changes and sperm production may take 3 months to be fully restored. Although clinical data on the effect of the time interval between sperm retrieval procedures and SRR are lacking, the suggestion of a 6-month interval is supported by circumstantial evidence. It is found that 82% of abnormal sonographic findings of the testes at 3 months after TESE procedures resolve by 6 months [44]. The incidence of ultrasound findings suggestive of hematoma decreases from 5 to 7.5% and 12 to 2.5% at 1 and 6 months after conventional TESE and mTESE, respectively, suggesting that at least 6 months is needed for most of the testes to fully recover after sperm retrieval procedures [45]. However, the varying degree of testicular damage caused by different sperm retrieval procedures indicates that the optimal timing to repeat sperm retrieval procedures should be individualized. Serial ultrasound imaging of the testes may be helpful in defining the optimal time interval. While the majority of ultrasound abnormalities resolve by 6 months, endocrine function and serum testosterone level may take up to 18 months to recover [46]. The question remains unanswered, and the optimal time interval between sperm retrieval procedures is yet to be defined by further research.

## Sperm Retrieval Procedures for Men with Nonobstructive Azoospermia

The testis is the only sperm source for our patient. There are several options available for testicular sperm retrieval: (1) TESA, (2) conventional TESE, (3) mTESE, and (4) testicular mapping. TESA attempts to retrieve sperm by percutaneous technique. The procedure can be performed under cord block and local anesthesia when a fine needle is used. The low SRR renders percutaneous procedures uncommon [47, 48]. TESA is not recommended as the primary procedure of sperm retrieval for men with NOA except when used in conjunction with testicular mapping. It has been shown that

percutaneous procedures are less effective than open testicular biopsy in obtaining sperm [33, 49].

TESE and mTESE are open testicular biopsy techniques and are more commonly performed in men with NOA. Multiple biopsies are usually employed to locate sperm during conventional TESE [41]. Conventional multiple biopsy TESE achieves up to 50% SRR [50]. However, it carries the risk of damage to the testicular blood supply. Complete testicular devascularization has been reported after multiple biopsies.

Since the introduction of mTESE in 1999 [33], the procedure has gained popularity due to several advantages over conventional TESE. The use of a microscope allows identification of subtunical blood vessels and decreases the risk of damage to the testicular blood supply [51]. A higher SRR of 45–65% is associated with mTESE compared to 30–45% with conventional TESE [33, 47, 51]. Moreover, mTESE is more effective in recovering sperm from men with testicular volume of less than 10 mL [52]. Larger quantity of sperm is obtained during mTESE with less testicular tissue removed. An average of 160,000 spermatozoa are obtained in samples that weigh 9.4 mg during mTESE, compared to 64,000 spermatozoa yielded by 720 mg of testicular tissue from conventional TESE [33]. However, it was concluded in a systematic review that mTESE performs better than conventional TESE only in cases showing Sertoli-cell-only pattern on histology where tubules containing foci of active spermatogenesis can be identified by the microscopic appearance of larger and more opaque tubules [53].

mTESE also has the lowest complication rates compared to other sperm retrieval techniques [53]. mTESE results in less intratesticular reaction than conventional TESE despite the wide equatorial incision along the tunica albuginea and extensive dissection. The achievement of complete hemostasis during mTESE results in less acute and chronic sonographic changes on scrotal ultrasound. Less postoperative pain after mTESE has been reported due to less retraction of tunica albuginea and compression of testicular parenchyma [54].

Despite the advancement in sperm retrieval techniques, lasting effects on testicular function after testicular sperm extraction should not be overlooked. Serum testosterone levels drop by 20% of preoperative levels at 3–6 months after sperm retrieval procedures and are not completely recovered at 18 months postoperatively [46]. It also has been reported

that mTESE leads to reduction in serum testosterone levels and increase in FSH and LH levels [55]. Histologic studies of the testes after sperm extraction procedures reveal a 7 and 5% decrease in seminiferous tubule volume and germ cell density, respectively [56].

Another option for obtaining sperm from our patient with NOA is testicular mapping. It consists of systematic fine needle aspiration (FNA) following a 22-site template of bilateral testes. Further management is stratified by the test results. Patients who have no sperm identified are offered the options of adoption and donor insemination, and attempt to use a sperm retrieval procedure is generally not recommended in expert centers. On the other hand, a directed sperm retrieval procedure will be offered in the presence of sperm. The location and quantity of sperm identified on mapping guide the subsequent sperm retrieval procedures. Testicular mapping is an outpatient procedure performed under local anesthesia. The procedure is well-tolerated, and patients usually resume normal activity within a day [57]. An early study has demonstrated the potential use of FNA to identify sperm in men with NOA with 2–3 samples from each testis [58]. The role of FNA is further supported by a report of 60% SRR in men with NOA with up to 15 samples from each testes, but the quantity is insufficient to inject all a partner's ova in most cases [59]. Therefore, testicular mapping/FNA as the sole sperm retrieval procedure is not recommended. The optimal number of sites of diagnostic aspiration remains unclear. Despite the advantage in avoiding or minimizing the invasiveness of sperm retrieval procedures, the wide application of testicular mapping is hindered by the significant cytologic experience required in identifying sperm in a smear of aspirated seminiferous tubules.

Subsequent sperm retrieval is executed from the least to most technically demanding procedures in the sequence of TESA, conventional TESE, and mTESE based on the map. It has been demonstrated that sufficient sperm for injection of all available oocytes can be retrieved in 95% of cases [60]. Bilateral procedure was only required in 22% of patients. Complex sperm retrieval with mTESE was performed in 23% of men, while the majority had sperm acquired by TESA and TESE [60]. It is of note that the high SRR was reported from patients with positive FNA results to begin with. Currently, there is no head-to-head studies comparing the different strategies of mTESE and testicular mapping ± sperm retrieval. The advantages and disadvantages of various sperm retrieval techniques in men with NOA are presented in Table 2.

**Table 2. Advantages and Disadvantages of Sperm Retrieval Techniques for Nonobstructive Azoospermia.**

| | Advantages | Disadvantages | Sperm retrieval rates (%) |
|---|---|---|---|
| TESA | • Fast<br>• Low cost<br>• Possibly office/outpatient procedure<br>• Minimal recovery and morbidity<br>• No microsurgical skill and instruments required | • May not retrieve adequate sperm for injection of all retrieved oocytes<br>• Risk of hematoma formation<br>• Risk of testicular atrophy | 5–10 |
| TESE | • No microsurgical skill and instruments required | • Surgical exploration required with longer postoperative recovery<br>• Risk of testicular atrophy | 30–45 |
| mTESE | • Thorough examination of testicular parenchyma<br>• Reduced risk of damage to testicular blood supply<br>• Less testicular tissue removed<br>• Less adverse effect on testicular function | • Increased cost and operating time<br>• Surgical exploration required with longer postoperative recovery<br>• Microsurgical skill and instruments required | 45–65 |
| Testicular mapping (± sperm retrieval) | • Possibly office/outpatient procedure<br>• Minimal recovery and morbidity<br>• No microsurgical skill and instruments required<br>• Avoid morbidities associated with sperm retrieval procedures for patients with no sperm identified on testicular mapping<br>• Potentially reduce the invasiveness of the subsequent sperm retrieval procedure | • Significant cytologic experience required<br>• Some patients are subjected to 2 procedures<br>• Possible false negative despite extensive systematic fine needle aspirations | 95[a] |

*TESA* percutaneous testicular sperm aspiration; *TESE* conventional testicular sperm extraction; *mTESE* microdissection testicular sperm extraction.

[a]Patients with sperm identified on testicular mapping

The importance of intraoperative specimen handling in increasing sperm yield has been addressed. The mechanical disruption of individual tubules by aggressive mincing in the medium and repeated passage of

testicular suspension via angiocatheter increases sperm yield by up to 300-fold [61]. The procedure of sperm retrieval can be terminated once sufficient sperm are identified in the operating theater by surgeon or embryologist under microscope. The increased efficacy in sperm identification prevents unnecessary damage to the already compromised testis of our patient.

In summary, repeating sperm retrieval at least 6 months after the previous attempt is a rational approach for our patient. mTESE seems the preferred technique and has been more widely studied as the procedure after failed TESA/conventional TESE/mTESE attempts and showed promising results. The technique has also been suggested to be particularly useful for our patient with small testicular volume. mTESE may have less of a detrimental impact on testicular function. There is more rapid recovery of hormonal profile and resolution of sonographic abnormalities after mTESE. Meticulous specimen handling intraoperatively is of paramount importance in maximizing the sperm yield. The alternative of testicular mapping ± sperm retrieval can be considered if significant cytologic expertise is available.

## Prognostic Factors

The success of surgical sperm retrieval in men with NOA is variable. Early studies in identifying prognostic factors for successful sperm retrieval have been disappointing. Clinical features, including testicular volume, history of ejaculated sperm, serum FSH or inhibin levels, etiology of NOA, and biopsy histology, do not predict success of sperm retrieval procedures [62, 63]. More recent data suggest that YCMD and histopathologic diagnosis are the most promising predictive factors. The presence of AZFc in azoospermic men is considered a favorable factor associated with an SRR of 71.4% compared to 48.8% retrieval rate in patients with idiopathic azoospermia [64]. The clinical pregnancy rates per IVF cycle involving sperm retrieval from men with AZFc microdeletion are comparable to that of unaffected individuals [65]. On the other hand, sperm retrieval is universally unsuccessful in all patients with complete AZFa or AZFb deletions [66].

Histopathologic diagnosis may be helpful in predicting treatment success in case prior diagnostic biopsy has been performed. The most

advanced stage on biopsy, but not the predominant stage, is considered as the predictive factor [63]. It has been shown that sperm are identified in 81, 44, and 41% of patients with hypospermatogenesis, maturation arrest, and Sertoli-cell-only, respectively, by using mTESE [46]. Correlations between SRR and histopathologic diagnosis are also demonstrated with standard open testicular biopsy [67] and testicular FNA techniques [59]. A study suggested that the presence of Sertoli-cell-only on biopsy as the most advanced pattern in men with at least 1 prior failed sperm retrieval is associated with lower SRR [42]. Other factors have been suggested to have prognostic value on SRR as well. The role of serum FSH level as a predictive factor of successful sperm retrieval is less well-defined. One study has demonstrated that a cutoff level of serum FSH > 20 IU/L predicts successful sperm retrieval with open biopsy methods [68]. Conversely, other studies have demonstrated that serum FSH levels are less relevant for predicting success of mTESE. Patients who have serum FSH of 15–30 IU/L, 30–45 IU/L, or greater than 45 IU/L all have similar SRRs [69].

The negative effect of prior biopsies on conventional TESE is suggested by 56% SRR in men who underwent no prior biopsy compared to 23% SRR for those who had 3–4 biopsies per testis [41]. The phenomenon may be explained by scarring and parenchymal fibrosis as a result of devascularization by multiple biopsies. Prior success in sperm retrieval predicts good SRR on repeat procedures. The SRR reaches 96% on repeated mTESE following prior successful retrieval. On the other hand, the SRR drops to 33% if sperm is not found on previous mTESE [43].

It also has been proposed that the response to aromatase inhibitor in men with Klinefelter syndrome predicts the results of sperm retrieval [70]. Whether the result can be extrapolated to other non-Klinefelter syndrome men with NOA and low TE ratio is unknown. Table 3 is a summary list of the possible prognostic factors for sperm retrieval in men with NOA.

Therefore, obtaining the details of the previous sperm retrieval procedure is of paramount importance in addition to YCMD test results in our patient. Although a prior failure of sperm retrieval predicts lower success on subsequent procedures, other information such as the surgical technique of previous attempts and histopathologic diagnosis also carries prognostic value.

**Table 3. Prognostic Factors for Successful Sperm Retrieval in Men with Nonobstructive Azoospermia.**

| | |
|---|---|
| Y-chromosome microdeletion | Complete deletion of AZFa or AZFb are extremely poor prognostic factors<br>Presence of AZFc is associated with sperm retrieval rate of approximately 70% |
| Histopathologic diagnosis | Sertoli-cell-only pattern is generally associated with lower sperm retrieval rate compared to hypospermatogenesis and maturation arrest |
| Serum follicular-stimulating hormone (FSH) | FSH > 20 IU/L signifies remote chance of sperm retrieval with conventional multifocal testicular sperm extraction (TESE)<br>Serum FSH does not predict sperm retrieval rate during microdissection testicular sperm extraction (mTESE) |
| Previous testicular biopsies | More than 2 prior biopsies per testis is associated with reduced chance of sperm retrieval |
| Results of prior sperm retrieval | Prior success in mTESE is associated with 96% sperm retrieval rate in repeated procedure<br>Repeating mTESE in patients with previous failed attempt is associated with 33% sperm retrieval rate |
| Response to aromatase inhibitors | The role of aromatase inhibitor in non-Klinefelter syndrome men is unclear |

## Fresh Versus Cryopreserved Retrieved Testicular Sperm

There has been considerable debate between using fresh versus frozen testicular sperm for ART in men with NOA. A meta-analysis concludes that fertilization rates, clinical pregnancy rates, and ongoing clinical pregnancy rates do not differ between groups using fresh or cryopreserved testicular sperm from men with NOA [15]. Some authors also suggest cryopreservation of retrieved testicular sperm followed by ICSI later in order to avoid unnecessary ovarian stimulation of the female partner [71]. But there is a concern of using cryopreserved testicular sperm for ICSI based on the finding that only 33% of testicular samples from men with NOA show documentable viability after freeze-thaw [72]. Currently, many fertility specialists prefer fresh to freeze-thawed testicular sperm. Coordinated IVF cycles and sperm extraction procedures are required in order to use fresh testicular sperm. Fresh testicular

sperm has a high viability rate approaching 90% despite its low motility. Injection of nonmotile fresh testicular sperm during ICSI yields a high fertilization rate [73]. It is now recognized that the motility of retrieved testicular sperm remains stable or increases with incubation in vitro for 24–48 h [74]. This has simplified the timing of procedures on infertile couples, and testicular sperm can be retrieved 1–2 days before ICSI.

## Postoperative Care

Postoperative care differs between centers and surgeons. Ice packs may be applied intermittently to the scrotum for 24–48 h. Patients are strongly advised to wear briefs or a scrotal supporter until edema and pain subside. Scrotal swelling, wound ecchymosis, and discomfort usually subside in approximately 7 days. Normal daily activities can be resumed on the next day after percutaneous sperm retrieval and 3 days after open procedures. Men can begin showers after 24 h. Strenuous exercise should be avoided for 7–10 days. No sexual activity is recommended for 3–7 days. Antibiotic after the procedure is not necessary and not routinely prescribed [75], but some surgeons may prefer empirical oral antibiotic for 3–5 days. Pain medication is used as needed. Common prescription including narcotics or nonsteroidal anti-inflammatory medications is usually adequate for pain control.

## Conclusion

The acquisition of satisfactory surgical sperm retrieval technique is essential for all male fertility specialists. Currently, there is insufficient evidence from randomized trials to recommend any particular procedure for both obstructive and nonobstructive azoospermia. A variety of procurement procedures are available, and the choice of techniques varies among centers. The formulation of a protocol for sperm retrieval at a particular center largely depends on the expertise and equipment available. The collaboration and discussion among male fertility specialists, ART specialists, and embryologists is essential. Choosing the right surgical approach can only be made with a thorough understanding of the pros and cons of each sperm extraction technique.

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*Notes:*

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† Schindler AE. Progestational effects of dydrogesterone *in vitro, in vivo* and on the human endometrium. *Maturitas*. 2009;65(1):S3-S11. * Data on file. ‡ Internal calculations based on Quintiles IMS database, IMS Health Analytics Link MAT03 2017.

**Abbreviated Prescribing Information. Dydrogesterone Tablets IP. Duphaston® Composition:** Each white film-coated tablet contains: Dydrogesterone IP 10 mg. Excipients q.s. Colour: Titanium dioxide IP. **Indications:** Progesterone deficiencies, Treatment of progesterone deficiencies such as: • Treatment of dysmenorrhoea • Treatment of endometriosis • Treatment of secondary amenorrhoea • Treatment of irregular cycles • Treatment of dysfunctional uterine bleeding • Treatment of pre-menstrual syndrome • Treatment of threatened and habitual abortion • Treatment of infertility due to luteal insufficiency. Hormone replacement therapy - To counteract the effects of unopposed oestrogen on the endometrium in hormone replacement therapy for women with disorders due to natural or surgical induced menopause with an intact uterus. **Dosage and Administration:** Dosages, treatment schedule and duration of treatment may be adapted to the severity of the dysfunction and the clinical response. Dysmenorrhoea: 10 or 20 mg dydrogesterone per day from day 5 to day 25 of the menstrual cycle. Endometriosis: 10 to 30 mg dydrogesterone per day from day 5 to day 25 of the cycle or continuously. Dysfunctional uterine bleeding: When treatment is started to arrest a bleeding episode, 20 or 30 mg dydrogesterone per day is to be given for up to 10 days. For continuous treatment, 10 or 20 mg dydrogesterone per day should be given during the Second half of the menstrual cycle. The starting day and the number of treatment days will depend on the individual cycle length. Withdrawal bleeding occurs if the endometrium has been adequately primed with either endogenous or exogenous estrogen. Secondary amenorrhoea: 10 or 20 mg dydrogesterone per day, to be given daily for 14 days during the second half of the theoretical menstrual cycle to produce an optimum secretory transformation of an endometrium that has been adequately primed with either endogenous or exogenous estrogen. Pre-menstrual syndrome: 10 mg dydrogesterone twice daily starting with the second half of the menstrual cycle until the first day of the next cycle. The starting day and the number of treatment days will depend on the individual cycle length. Irregular cycles: 10 or 20 mg dydrogesterone per day starting with the second half of the menstrual cycle until the first day of the next cycle. The starting day and the number of treatment days will depend on the individual cycle length. Threatened abortion: An initial dose of up to 40 mg dydrogesterone may be given followed by 20 or 30mg per day until symptoms remit. Habitual abortion: 10 mg dydrogesterone twice daily until the twentieth week of pregnancy. Infertility due to luteal insufficiency: 10 or 20 mg dydrogesterone daily starting with the Second half of the menstrual cycle until the first day of the next cycle. Treatment should be maintained for at least three consecutive cycles. Hormone replacement therapy: Continuous sequential therapy: An estrogen is dosed continuously and one tablet of 10 mg dydrogesterone is added for the last 14 days of every 28 day cycle, in a sequential manner. Cyclic therapy: When an estrogen is dosed cyclically with a treatment-free interval, usually 21 days on and 7 days off. One tablet of 10 mg dydrogesterone is added for the last 12-14 days of estrogen therapy depending on the clinical response, the dosage can subsequently be adjusted to 20 mg dydrogesterone per day There is no relevant use of dydrogesterone before menarche. The safety and efficacy of dydrogesterone in adolescents aged 12-18 years has not been established. Currently available data are described in section 4.8 and 5.1, but no recommendation on a posology can be made.

**Contraindications:** Known hypersensitivity to the active substance or to any of the excipients. Known or suspected progestogen dependent neoplasms. Undiagnosed vaginal bleeding. Contraindications for the use of estrogens when used in combination with dydrogesterone. **Warnings and Precautions:** Before initiating dydrogesterone treatment for abnormal bleeding, the etiology for the bleeding should be clarified. Breakthrough bleeding and spotting may occur during the first months of treatment. If breakthrough bleeding or spotting appears after some time on therapy, or continues after treatment has been discontinued, the reason should be investigated, which may include endometrial biopsy to exclude endometrial malignancy. **Pregnancy and Lactation:** Pregnancy: It is estimated that more than 10 million pregnancies have been exposed to dydrogesterone. So far there were no indications of a harmful effect of dydrogesterone use during pregnancy. Some progestogens have been reported in the literature to be associated with an increased risk of hypospadias. However due to confounding factors during pregnancy, no definitive conclusion can be drawn regarding the contribution of progestogens to hypospadias. Clinical studies, where a limited number of women were treated with dydrogesterone early in pregnancy, have not shown any increase in risk. No other epidemiological data are hitherto available. Effects in non-clinical embryo-fetal and post-natal development studies were in line with the pharmacological profile. Untoward effects occurred only at exposures which exceeded the maximum human exposure considerably, indicating little relevance to clinical use. Dydrogesterone can be used during pregnancy if clearly indicated. Breastfeeding: No data exist on excretion of dydrogesterone in mother's milk. Experience with other progestogens indicates that progestogens and the metabolites pass to mother's milk in small quantities. Whether there is a risk to the child is not known. Therefore, dydrogesterone should not be used during the lactation period. Fertility: There is no evidence that dydrogesterone decreases fertility at therapeutic dose. **Adverse Reactions:** The most commonly reported adverse drug reactions of patients treated with dydrogesterone in clinical trials of indications without estrogen treatment are migraines/headache, nausea, menstrual disorders and breast pain/tenderness. Undesirable effects that are associated with an estrogen-progestogen treatment : Breast cancer, endometrial hyperplasia, endometrial carcinoma, ovarian cancer • Venous thromboembolism • Myocardial infarction, coronary artery disease, ischemic stroke. Issued on: 3/4/14. Source: Prepared based on full prescribing information (version 03) dated 13/03/2015.

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