Assisted Reproductive Technologies

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Abstract

Since the first successful in vitro fertilization pregnancy (IVF), the progress of assisted reproduction has been surprising. With developments such as intracytoplasmic sperm injection (ICSI), egg and sperm donation, preimplantation diagnosis, and aneuploidy screening, there are many more couples who can benefit from assisted reproductive technologies (ART).

Endocrine dysfunctions represent the indication for treatments in 5–19.8%, and probably these figures are underestimated. The evaluation and treatment of women with poor ovarian reserve and polycystic ovarian (PCO) syndrome in ART remain a challenge. Numerous protocols and procedures have been proposed, but none has yet reached sufficient evidence.

In this chapter, the most used techniques of assisted reproduction and newer developments are discussed, as well as the possible complications associated with ART. Furthermore, the strategies employed during the procedure in patients with reduced ovarian reserve and PCO have been deepened.

Keywords

Assisted reproductive technologies · IVF · ICSI · Poor responder · PCO

Introduction

In 1978 the collaboration between Patrick Steptoe and Robert Edwards led to the birth of Louise Brown, the first baby to be born as a result of in vitro fertilization (IVF). This meaningful event revolutionized treatment options for couples with infertility.

Afterward, the introduction of assisted reproductive technology (ART) with fertilization of human oocytes outside of the body, culturing of the embryo in a laboratory, and its subsequent transfer to the uterus has led to the birth of tens of thousands of children worldwide. It has been estimated that more than seven million babies have been born worldwide since the first IVF baby, and around 1.5 million ART cycles are now reported each year worldwide. Data are probably underestimated, as registry figures are thought to represent around 70% of all ART treatments (ESHRE 2018).

ARTs comprise all treatments which include the handling of eggs and sperm and/or embryos. Some examples of ART are IVF, intracytoplasmic sperm injection (ICSI), gamete intrafallopian transfer, pronuclear stage tubal transfer, tubal embryo transfer, and zygote intrafallopian transfer (ASRM n.d.). Intrauterine insemination (IUI), IVF, and ICSI are the most common techniques.

The intent of this chapter is to provide an overview of the most used techniques of assisted reproduction and the corresponding indications. Subsequently, the strategies employed during the procedure in patients with endocrinological disorders (mainly reduced ovarian reserve and polycystic ovaries) have been deepened.

Intrauterine Insemination (IUI)

Although IUI has not been classified as an ART, it is widely used an empirical treatment, for a broad range of subfertility indications. According to the European Society of Human Reproduction and Embryology (ESHRE), it represents a "mild" ART procedure (ESHRE 2018).

IUI has evolved through innovations such as sperm preparation, monitoring for preovulatory timing, and induction of ovulation with human chorionic gonadotropin (hCG). Furthermore, IUI has been combined with ovarian stimulation using clomiphene citrate (CC) or gonadotropins.

This simple and noninvasive technique has several advantages: it can be performed without expensive infrastructure, has a good couple compliance (low dropout rate) and a very low risk for complications such as ovarian hyperstimulation syndrome (OHSS).

After the ESHRE workshop in 2009, IUI was considered a poor substitute for IVF treatment as it was associated with modest pregnancy rate (12% per cycle in treatment with ovarian stimulation) and a significant rate of multiple pregnancies (13% multiple births). Cycles with mild stimulation (1–2 follicles) might reduce the costs and multiple birth rates but would involve more cycles of treatment (ESHRE Capri Workshop Group 2009).

IUI is indicated for a broad range of pathologic conditions. The most obvious condition is male infertility with donor sperm. Other indications are unexplained infertility, mild endometriosis, and mild male factor infertility (NICE 2013).

IUI Technique

The IUI treatment aims to increase the chance that the maximum number of healthy sperms reaches the oocyte. Thus, the rationale is to bypass a possible cervical factor. However, the postcoital test is not a recommended routine test anymore (The Practice Committee of the American Society for Reproductive Medicine 2015). Prior to perform IUI, tubal patency and semen quality evaluation are recommended (ESHRE 2018).

There is general consensus that the chances of pregnancy are higher if IUI is performed after mild ovarian stimulation and the maturation of a maximum of two or three follicles. In the majority of the published studies, the insemination is done 32–36 h following hCG administration.

Prior to IUI, the seminal fluid should be processed by centrifuging spermatozoa through a culture medium or density gradients followed by re-suspension in suitable culture media. Procedure of IUI by unprocessed semen was associated with increased risk of pelvic infection (Boomsma et al. 2007). Moreover, unprocessed seminal fluid might induce uterine contractions through prostaglandins contained in the seminal plasma.

For the insemination sample, the recommended lower limit ranges from 3 to 5–10 million motile sperm (ESHRE Capri Workshop Group 2009).

Artificial inseminations can be done intravaginally, intracervically, pericervically using a cap, intrauterine (IUI), transcervical intrafallopian, or directly intraperitoneal. IUI is by far the most common method.

The majority of pregnancies occur during the first three or four IUI cycles (Ombelet et al. 2008). A double insemination did not prove more advantageous than a single procedure (ESHRE Capri Workshop Group 2009). Obviously, evaluating the duration of an IUI program, the age of the woman and her comorbidities should be considered, to guarantee timely reassignment to second-level treatments if indicated.

In Vitro Fertilization (IVF)/Intracytoplasmic Sperm Injection (ICSI)

Both IVF and ICSI involve ovarian stimulation, oocyte retrieval, and fertilization outside of the body. IVF involves combining an egg with sperm in a laboratory dish; the resulting embryo is then transferred into the woman's uterus. ICSI is a micromanipulation procedure in which a single sperm is injected directly into an egg (Fig. 1). Controlled ovarian stimulation (COS) and ultrasound monitoring (with or without estradiol levels) constitute part of IVF treatment (NICE 2013).

Daily doses of gonadotropins (follicle-stimulating hormone (FSH), luteinizing hormone (LH), human menopausal gonadotropin (hMG), corifollitropin alfa, follitropin delta) are used to induce multifollicular response in the ovaries. Although the number of eggs retrieved seems to depend on the starting/total doses of gonadotropins, individual woman's response differs. An individualized starting dose of gonadotropins is recommended, based on factors that predict ovarian response such as age, BMI, presence of polycystic ovaries, and ovarian reserve (NICE 2013).

Monitoring follicular growth usually involves a combination of hormonal assays and ultrasonic measurements of follicle size. It plays an important role in the prediction of ovarian responsiveness to exogenous gonadotropins (predictive of

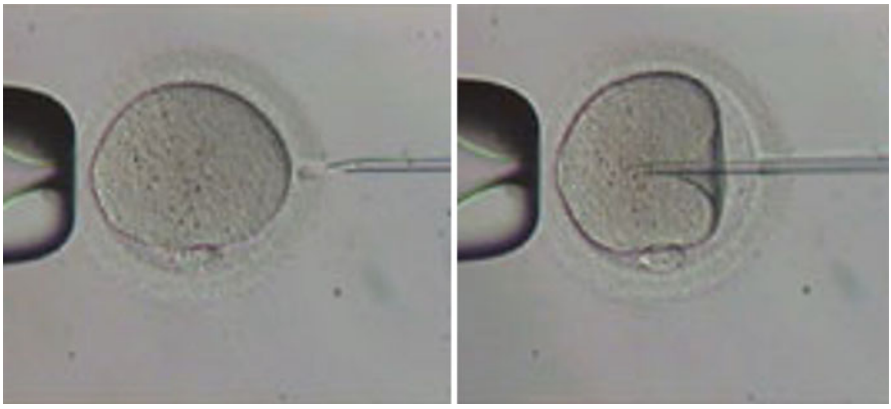

Fig. 1 Intracytoplasmic sperm injection (ICSI). A single spermatozoa is injected into each oocyte using fine micro-manipulation equipment

oocytes competence), estimation of the appropriate time to trigger the final oocyte maturation before ovum pickup, and assessment of OHSS risk.

IVF/ICSI Indications

IVF indications include doubtful tubal patency, advanced-stage endometriosis resulting in tubal disease or dysfunction, moderate alterations of semen characteristics, unexplained infertility, failure of several previous cycles of ovulation induction or IUI, and circumstances that require preimplantation screening/diagnosis to prevent genetically inherited diseases. Furthermore, IVF must be offered as a first-line treatment in women of advanced maternal age, irrespective of the cause of infertility (NICE 2013).

Originally developed for severe sperm abnormalities, ICSI was implemented in ART and applied in other conditions, such as after failed or low fertilization in previous attempts. According to *Good Clinical Treatment in Assisted Reproduction* of the ESHRE, ICSI should not represent the most suitable treatment for female conditions as poor ovarian response or previous implantation failures (Good Clinical Treatment in Assisted Reproduction 2008).

In regard to male factor, in patients with normal seminal parameters or mild male factor infertility, a number of studies demonstrated equal or higher fertilization rates with ICSI compared with IVF (Plachot et al. 2002), but once fertilization is achieved, the pregnancy rate is not enhanced if compared to IVF (NICE 2013). In patients with severe male factor infertility, the ICSI procedure results in a much higher fertilization rate and, therefore, a more beneficial outcome.

Total motile sperm concentration (TMC), calculated by multiplying the volume, the concentration (million sperm/ml), and the percentage of progressive motile spermatozoa, has been adopted as criterium to decide between IUI, conventional IVF, and ICSI. In cases of post-processing TMC $<1 \times 10^6$, IUI has no benefit, and IVF, ICSI, or a combination of both, i.e., a split setup, may be the initial suggestion to the couple (van Weert et al. 2004).

Certainly, when less than 0.5×10^6 progressively motile spermatozoa are obtained after seminal fluid processing or sperms are recovered surgically from the testis or epididymis, ICSI should be performed. In remaining cases, current strategies for choosing between IVF and ICSI are either formulated using preset cutoff values or based on the assumption that ICSI is the more robust insemination technique (Tournaye 2012).

Actually, the prevalence of complete fertilization failure after standard IVF is reported to be as high as 50%, while ICSI cycles with complete fertilization failure are less than 3% of started cycles (Tournaye 2012). Total fertilization failure during conventional IVF might be related to either oocyte, sperm, or laboratory factors. Although these events might have negative consequences, with a severe distressing impact on the couple, the aim of reproductive medicine should always be the adoption of the simplest, least expensive, and invasive procedure with the greatest chance of pregnancies and long-term prognosis of healthy children.

An alternative strategy for choosing between ICSI and IVF, in cases of moderate male factor infertility, is constituted by a combination of both, i.e., a split IVF-ICSI approach, in which sibling oocytes are either inseminated conventionally or micro-injected. Although there is still poor evidence, this method may prevent complete fertilization failure in one out of four cycles (Tournaye 2012).

Nevertheless, the crucial difference between IVF and ICSI is that, during ICSI, only mature oocytes are injected, while immature oocytes are set apart in order to complete maturation. As oocyte maturation is unpredictable, most oocytes are injected irrespective of the time when they mature. Thus, the timing of ICSI may not always be optimal, especially since research indicates that expulsion of the polar body alone is not enough to determine maturity and that the amount of time between polar body extrusion and time of insemination influences fertilization rates (Balakier et al. 2004).

Egg and Sperm Donation

Egg donation (ED) is a fertility treatment for women unable to produce their own eggs, at high risk of transmitting a genetic disease or with previous several unsuccessful IVF cycles.

Egg donation, like sperm donation, has considerably increased in recent years. From the 18th ESHRE report on ART, publishing data for 2014, a total of 56,516 cycles with ED were performed in a population of ~208 million inhabitants. These data showed a definite growing trend, 22,911 cycles more than 2012 and 15,272 more than 2013 (De Geyter et al. 2018).

Oocyte donation is associated with the highest pregnancy rate among ARTs (almost 50%) with lower delivery rates (33% with fresh eggs, 25% after frozen embryo transfers, and 21% after embryo transfers from frozen eggs) (ESHRE 2017).

Donated eggs are fertilized with partner's sperm as in a conventional IVF/ICSI treatment cycle, and one (or two) embryo(s) are transferred. Women undergoing egg donor replacement cycles need an adequate hormonal preparation of the endometrium to optimize chances of pregnancy. Many drugs and various modes of administration have been tried in order to optimize implantation rates and consequently improve the success rates of the embryo transfer procedures. Obviously, success of oocyte donation is influenced by further factors, including donor's age, quality and number of transferred embryos, and recipient's age. Donor age is clearly the most important prognostic factor (Faddy et al. 2011).

Medical and obstetrical complications are significantly increased in pregnancies obtained with oocyte donation, especially in women older than 45 years. Hypertensive disorders of pregnancy and gestational diabetes are the most frequent obstetric complications. Previous studies observed that out of 45 heterologous pregnancies of healthy women aged 50–63 years, 35% experienced pregnancy-induced hypertension, 20% developed gestational diabetes, and 78% underwent a cesarean section (Paulson et al. 2002).

Sperm donation, or therapeutic donor insemination (TDI), is an option when the male partner has severe abnormalities such as azoospermia, severe oligospermia, or other significant sperm or seminal fluid abnormalities, genetic defects, or ineradicable sexually transmissible infections. TDI is associated with a series of pregnancy complications such as preterm birth, low birth weight, preeclampsia, and increased cesarean delivery rate, but these correlations were not found in all studies that correlated sperm donation versus non-donor sperm cycles (Adams et al. 2017; Bartal et al. 2018).

Preimplantation Genetic Screening/Diagnosis (PGS/D)

Previous studies have shown that approximately 25% of oocytes in women in their early 30s and more than 75% among women older than 40 years are chromosomally abnormal (Fragouli et al. 2011). Embryonic chromosomal abnormalities could significantly decrease the proportion of successful pregnancies in women or result in early miscarriage, late abortion, or the delivery of children with chromosomal abnormalities.

Preimplantation genetic screening/diagnosis (PGS/D) is used for early identification of genetic defects of embryo. PGD aims to test the embryo for known conditions before implantation. On the other hand, PGS refers to screening embryo for aneuploidy. PGD is indicated in couples at risk of transmitting genetic abnormalities to their offspring while PGS in couples with advanced maternal age, a medical history of recurrent first-trimester pregnancy losses, or recurrent implantation failure in prior IVF/ICSI cycles.

The timing of biopsy includes polar body (PB), cleavage-stage embryos (blastomere), morula-stage embryos, and blastocyst-stage embryos (trophectoderm biopsy). PB biopsy involves the removal and subsequent analysis of the first and second PB before embryo cleavage. It avoids the removal of cells from the embryo but allows the examination only for maternal chromosomes or genes. Moreover, the small amount of genetic material might lead to loss of one allele during polymerase chain reaction (PCR) amplification of DNA (allelic dropout).

A blastomere biopsy is based on the removal of one or two blastomeres from a six- to eight-cell embryo. In this case both maternal and paternal genetic contributions to the embryo can be analyzed, but the small quantity of the genetic material represents a potential cause for misdiagnoses. Unfortunately, the removal of cells at the cleavage stage seems to slow the development of the embryo and decrease implantation and pregnancy rates. Moreover, mosaic rates have been estimated 60% at the cleavage stage.

Development of sequential culture media allowed the culture of embryos to the blastocyst stage. Then, trophectoderm biopsy was introduced in clinical practice, allowing multiple cells to be biopsied. The biopsy of trophectoderm cells and blastocyst stage transfer showed an improvement in the accuracy of PGD/PGS. However, a rate approximately 20% of mosaicism has been described at the blastocyst stage (Sullivan-Pyke and Dokras 2018).

Techniques used for genetic and chromosomal analysis comprise polymerase chain reaction (PCR); fluorescence in situ hybridization (FISH); microarray technologies, including single-nucleotide polymorphism (SNP) microarrays and array comparative genomic hybridization (aCGH); and next-generation sequencing (NGS). Platforms based on NGS are being increasingly used in PGD/PGS because of high accuracy and high throughput (Sullivan-Pyke and Dokras 2018).

A number of studies demonstrated the safety and efficacy of PGD in IVF/ICSI cycles as well as increased implantation, pregnancy, and live birth rates. The transfer of euploid blastocysts confirmed by aCGH after day 3 biopsy has shown higher implantation (52.8% vs. 27.6%) and live birth rates per transfer (64.7% vs. 27.4%) when compared with untested blastocysts (Rubio et al. 2017). Blastocyst-stage embryo biopsy analyzed with rapid qPCR, and subsequent single-embryo transfer resulted in higher ongoing pregnancy rates (55.0% vs. 41.8%) and lower miscarriage rates (10.5% vs. 24.8%) versus single-embryo transfer without PGS (Forman et al. 2012). Furthermore, data on impact of blastomere biopsy on growth parameters, birth weight, hospitalizations, or congenital malformations in children followed up to 2 years of age are reassuring (Desmyttere et al. 2012). It is important to highlight that couples should be counselled that PGD/PGS do not replace routine prenatal screening methods as these procedures have an intrinsic error rate.

ART and Female Endocrine Dysfunction

An adequate endocrine environment is imperative to maintain the reproductive function in women. Female endocrine dysfunctions affecting fertility include a heterogeneous group of disorders. ART plays an important role when the first-line treatment does not achieve the correction of the disorder. According to the Italian National Registry of ART (2016), endocrine dysfunction represents the indication for treatments in 19.8% of IUI cycles and 5.5% of ICSI/IVF (Italian National Registry of ART 2016) (Figs. 2 and 3).

These figures probably underestimate the real impact of endocrine disorders in infertile couples. Indeed, other indications as "endometriosis" also include endocrine dysfunction (3.3% in I level ARTs and 4.5% in the II level ARTs). Moreover, patients with multiple factors might include endocrine dysfunctions.

The World Health Organization (WHO) defined three categories of anovulatory disorders (Table 1):

- WHO class 1 – hypogonadotropic hypogonadal (HA) anovulation is the least common, occurring in 5–10% of cases, for example, hypothalamic amenorrhea from functional aetiologies such as excessive exercise or low body weight.
- WHO class 2 – normogonadotropic normoestrogenic anovulation is the most common, accounting for 70–85% of cases, for example, polycystic ovary syndrome (PCOS).
- WHO class 3 – Hypergonadotropic hypoestrogenic anovulation occurs in 10–30%, for example, with primary gonadal failure (POF) or gonadal dysgenesis.

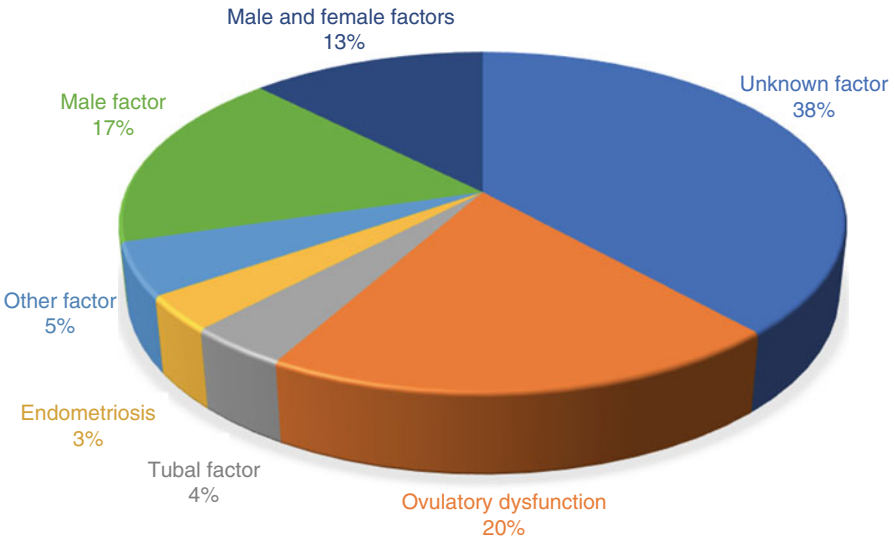

Fig. 2 Distribution of couples treated with IUI without gamete donation, according to the causes of infertility. 2016. 13,281 cycles

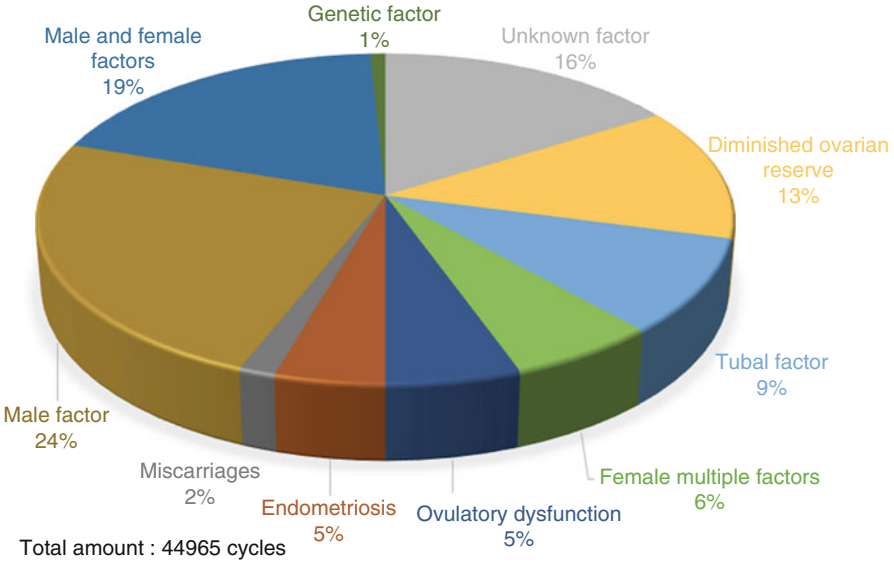

Fig. 3 Distribution of couples treated with IVF/ICSI, without gamete donation, according to the causes of infertility. 2016. 44,965 cycles

Table 1 Anovulatory disorders classified by the World Health Organization (WHO)

Main groups	Subdivision	Treatment
1. Hypothalamic dysfunction	1.a Clomiphene-resistant (hypogonadotropic, hypo-estrogenic)	Pulsatile GnRH
	1.b Clomiphene-responsive (normogonadotropicanovulation)	Gonadotropins
		Clomiphene
2. Ovarian dysfunction (PCOS)	2.a Lean patients	Clomiphene
	2.b Obese patients	Weight reduction Metformin Clomiphene
	2.c Clomiphene-resistant patients	Metformin-clomiphene Ovariandrilling HMG
3. Hyperprolactinemia		Cabergoline
4. Ovarian failure		Hormonal replacement therapy

The four most common ovulatory disorders include PCOS, poor ovarian reserve (POR), HA, and hyperprolactinemia.

POR and polycystic ovary (PCO) represent two indications to ART of particular interest, both for the frequency with which they are found and for the complexity of the management.

Poor Ovarian Reserve (POR)

Definition and Diagnosis

The standard definition of POR (or poor ovarian responder) remains uncertain, and consequently the proposed protocols adopted to manage poor responders are very difficult to compare. In a systematic review, Polyzos et al. found 41 different definitions among 47 randomized trials (Polyzos and Devroey 2011).

The Bologna criteria developed under the auspices of ESHRE in 2011 represent the first attempt to build an international consensus in the definition of POR (Ferraretti et al. 2011).

According to the Bologna criteria, at least two of the following features are required to define a POR:

1. Advanced maternal age ($\geq$40 years) or any other POR risk factor (genetic or acquired conditions, pelvic infections, ovarian endometriomas, and patients who have undergone ovarian surgery for ovarian cyst, chemotherapy, shortening of the menstrual cycle)
2. A previous cycle with POR ($\leq$3 oocytes with a conventional stimulation protocol)

3. A low ovarian reserve test in terms of anti-mullerian hormone (AMH) (<0.5–1.1 ng/ml) (<3.6–7.8 pmol/l) and antral follicle count (AFC) (<5–7 follicles)

However, two cycles with three oocytes or less after maximal stimulation are enough to classify a patient as a poor responder even in the absence of the other two criteria (Ferraretti et al. 2011).

Despite the efforts to optimize the definition of this subgroup of patients, the Bologna criteria were criticized for the heterogeneity of patients included by this definition.

Recently, the Patient-Oriented Strategies Encompassing IndividualizeD Oocyte Number (POSEIDON) group suggested a more detailed novel stratification of women with low ovarian response to stimulation. Four groups of "low prognosis patients" in ART were identified on the basis of age and the expected aneuploidy rate, biomarkers of ovarian reserve (AFC and AMH), and ovarian response in previous stimulation cycle (Table 2). The new classification introduces a new concept: the ability to achieve an adequate number of oocytes in order to give the patient the higher chance to obtain at least one euploid embryo (Humaidan et al. 2016).

The chance of pregnancy after IVF is highly dependent upon the number and quality of retrieved oocytes, as both factors determine the number of good-quality embryos.

Recently, the cumulative live birth rate (CLBR) has been suggested as indicator of quality and success in IVF. It incorporates the totality of live birth episodes following successive treatments of fresh as well as thawed frozen embryo transfer. CLBR per oocyte retrieval is a more meaningful indicator for both clinicians' and patients' perspective: the outcome of the whole IVF-ICSI cycle (including cryopreservation) allows better evaluations between different centers with different strategies for freezing and extended culture of embryos. Moreover, CLBR would be more appropriate for making economic and political decisions (Maheshwari et al. 2015).

In recent literature, several authors tried to define the ideal number of oocytes to optimize live birth rates in fresh embryo transfer cycles. Generally, a number of 10–15 oocytes has been considered adequate in order to give the patients the maximum chance of pregnancy after a fresh embryo transfer cycle: 13 according to Van der Gaast et al. (2006), 10 according to Verberg et al. (2007), 18 according to Fatemi et al. (2011), and 15 for Sunkara et al. (2011).

Table 2 Four groups of women with low prognosis in ART according to the POSEIDON's stratification. *AFC* antral follicle count, *AMH* anti-Müllerian hormone (Ferraretti et al. 2011)

Group 1: patients younger than 35 with adequate ovarian reserve (AFC ≥5, AMH ≥ 1.2 ng/ml) and with unexpected poor/suboptimal ovarian response Subgroup 1a:<4 oocytes[a] Subgroup 1b: 4–9 oocytes[a]	Group 2: patients older than 35 with adequate ovarian reserve (AFC ≥5, AMH ≥1.2 ng/ml) and with unexpected poor/suboptimal ovarian response Subgroup 2a:<4 oocytes[a] Subgroup 2b: 4–9 oocytes[a]
Group 3: patients younger than 35 with poor ovarian reserve (AFC <5, AMH <1.2 ng/ml)	Group 4: patients older than 35 with poor ovarian reserve (AFC <5, AMH <1.2 ng/ml)

[a]After standard ovarian stimulation

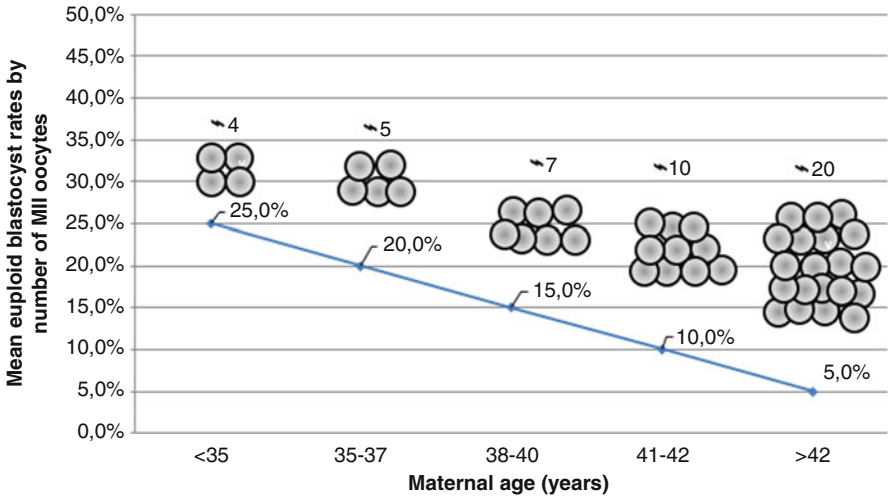

Fig. 4 Estimated number of MII oocytes needed to achieve an euploid blastocyst during IVF-ICSI cycles. (Modified from Vaiarelli et al. (2018))

Nevertheless, the prediction of a live birth cannot be based only on the oocyte yield. Indeed, the aneuploidy chromosomal constitution is strongly related to the woman's age, ranging from 25% to 30% in women younger than 35 to over 90% in women older than 42, and represents a critical element affecting embryo reproductive competence (Vaiarelli et al. 2018). Thus, women with the same number of retrieved oocytes might have opposite clinical outcomes according to the age-dependent blastocyst aneuploidy rate. Though, implantation rate of a euploid blastocyst is independent from the woman's age (Fig. 4).

In this context, the number of oocytes retrieved after COS greatly influences the clinical outcome. Thus, the optimization of the strategies of COS based on the ovarian reserve of each single patient is essential in order to maximize the age-related chances to obtain at least one euploid blastocyst.

Prevalence

Due to the lack of definition, it is difficult to determine the prevalence of POR condition. Its reported frequency in IVF cycles varies from 9% to 30% in different studies (Ferraretti et al. 2011; Keay et al. 1997). According to data published on the Italian Registry, couples treated for POR constituted the 13.1% (ART registry 2016).

Management

The management of patients with low AFC and/or AMH is still a debated issue in reproductive medicine. Certainly IVF-ICSI compared with IUI showed superior

pregnancy rates in the setting of patients with poor ovarian response after COS (Reichman et al. 2013).

IVF Protocols

The obvious and most used approach for women with POR is to increase the daily dose of gonadotropin. Actually, higher gonadotropin dosages can increase the number of transferable embryos and, therefore, cumulative pregnancy chances. The National Institute for Health and Care Excellence (NICE) guideline on fertility recommends not to use a dosage of FSH of more than 450 IU/day for COS in poor responder patients (NICE n.d.). A recent open-label, multicenter randomized controlled trial (RCT) recommended using a standard dose of 150 IU/day in women scheduled for IVF/ICSI with a predicted poor response. Five hundred eleven women were randomized in the study, 234 with an AFC $\leq$ 7 and 277 with an AFC 8–10. The cumulative live birth rate for increased versus standard dosing was 42.4% (106/250) versus 44.8% (117/261), respectively [relative risk (RR): 0.95 (95%CI, 0.78–1.15), $P = 0.58$]. The authors concluded that an increased dose strategy, despite significant higher costs, doesn't improve live birth rates. Thus, they recommended using a standard dose of 150 IU/day in these women (van Tilborg et al. 2017). Unfortunately, the authors adopted a different definition of poor responders, the study permitted small dose adjustments between cycles, and the AFC might have suffered from interobserver variation.

Among the various protocols and strategies toward optimization of management for poor responders, there is no concrete evidence on the advantage of any stimulation protocol over another. Examples of recommended protocols for poor responder patients include:

- Low-dose (or "mild") protocol
- Low-dose clomiphene/gonadotropin protocol
- Augmented natural cycle protocol
- Delayed start antagonist protocol
- Flare-up agonist protocol
- Microdose flare GnRH agonist protocol

Mild COS protocols using low doses of gonadotropins have been implemented in clinical practice, demonstrating significant advantages, including cost-effectiveness and patient-friendly regimens. This protocol optimizes the balance between outcomes and risks of treatment, although the expected number of retrieved oocytes is low, usually ranging from two to seven (Verberg et al. 2009).

Mild stimulation is based on the following evidences:

- Because of low-dose stimulation, only the healthier follicles with more competent egg(s) are encouraged to develop (Verberg et al. 2009).
- The physiologic hormonal follicular milieu might be altered when the follicles are exposed to a high dose of gonadotropins (von Wolff et al. 2014).

- Follicular AMH has been shown to be significantly higher in natural cycles compared with that with ovarian stimulation (von Wolff et al. 2014).
- A RCT found that the number of euploid embryos with conventional IVF was no higher than that with mild stimulation IVF (Baart et al. 2007).
- Supraphysiologic levels of serum E_2 could affect implantation (Fauser and Devroey 2003).

The low-dose gonadotropin protocol involves initiating 150 IU of gonadotropins daily on day 2 for 9 days. GnRH antagonist is administered when the lead follicle reaches ≥12 mm in diameter, and the ovulation trigger is suggested when the lead follicle is 16–17 mm (Gonda et al. 2018).

According to Siristatidis et al., although convincing scientific evidences, mild ovarian stimulation was shown inferior to conventional regimes when applied to poor responders undergoing IVF/ICSI, in terms of retrieved oocytes (Siristatidis et al. 2017).

Low-dose clomiphene/gonadotropin protocols may be a good option for patients who have previously responded to clomiphene, but did not have a successful cycle. This protocol is characterized by the administration of clomiphene citrate 100 mg/day for 5 days beginning on day 2. A 2016 meta-analysis suggested that mild COS protocol with CC may obtain equal pregnancy outcome in POR patients undergoing IVF treatment compared with conventional COS protocol (Song et al. 2016).

In augmented natural cycles, patients are monitored for estradiol production >20 pg/ml and/or the presence of 3–4 mm-sized basal antral follicles. Once these conditions are satisfied, ovarian stimulation is initiated with a low-dose combination of HP-hMG and rFSH (e.g., 75 IU/day of each) and continued for approximately 6 days. When the lead follicle reaches ≥12 mm, GnRH antagonist is added. Ovulation is triggered with hCG 10,000 IU or leuprolide (Gonda et al. 2018).

Delayed-start antagonist protocol is based on the observation that endogenous FSH may stimulate larger follicles in the prior luteal phase and subsequently lead to a size discrepancy in the cohort of developing follicles. According to this protocol, a GnRH antagonist is administered from day 2 and continued for 7 days, then ovarian stimulation is started with gonadotropins, and GnRH antagonist is added again if the ultrasound monitoring showed at least one follicle with diameter ≥ 14 mm and continued until the trigger day (Davar et al. 2018).

The flare-up protocols are based on the 24-h-long surge in endogenous FSH and LH released when administering GnRH agonists in the early follicular phase. GnRH agonists are initiated in the follicular phase of a stimulation cycle before commencing gonadotropin injections.

The very low-dose, "microdose" GnRH agonist flare protocol represents an attempt to decrease the suppressive effects of GnRH agonists during a flare protocol. Daily GnRH agonists are administered at the dose of 20–50 μg twice and continued until the day of hCG administration. After 2 days (on the fourth day of menstruation), the patients received rFSH 300 IU/day (Davar et al. 2018).

Synchronizing Early Follicle Development

Ovarian follicles mature over a period of approximately 2–4 months. COS can only support the cohort of follicle responsive to the stimulation without generating de novo follicles. The synchronization of earlier follicle wave with oral estrogens, contraceptive pill, or progestins, prior to traditional COS, has been suggested as a possible strategy to increase the number of responsive follicles, particularly for poor responders (McGee and Hsueh 2000).

In some patients, diminished ovarian reserve was due to an androgen deficiency state. In these women, androgen supplementation via testosterone or dehydroepiandrosterone (DHEA) may help to stimulate early follicle development and improve functional ovarian reserve (Gleicher et al. 2010).

DHEA is produced in the zona reticularis of the adrenal cortex and by ovarian theca cells. It promotes follicular development and granulosa cell proliferation and can also enhance the level of follicular insulin-like growth factor-1 (IGF-1), which promotes folliculogenesis by enhancing the effect of gonadotropin and reducing follicular arrest.

After the beginning of androgen supplementation, the follicles require 6–8 weeks to achieve synchronization and become mature enough to respond to COS. Among androgenic supplements, DHEA is the preferred method over testosterone as it is metabolized by organs as needed, whereas testosterone overflows the body with a fixed level. However, it is important to note that conclusive clinical evidence of the influence of DHEA or testosterone is limited and the use of androgen supplementation is still considered experimental. Furthermore, patients offered these supplements should be informed about the potential side effects such as acne, oily skin, deepening of the voice, hirsutism, and hair loss (Li et al. 2015).

Owen et al. (1991) observed that growth hormone (GH) co-treatment improved the ovarian response to COS in poor responders. This conclusion was supported by studies demonstrating that GH, either directly or indirectly via insulin-like growth factor 1 (IGF-1), regulates oocyte maturation by increasing the sensitivity of the ovaries to gonadotropins and promoting early follicle development. Clinical studies demonstrated contrasting effects of GH on oocyte and embryo-related outcomes; moreover studies are few in number and included small sample size. A Cochrane review on GH as adjuvant in poor responders concluded that, although the use of GH in poor responders showed a significant improvement in live birth rates, it was unable to identify which subgroup of poor responders might benefit from this co-treatment. The result needs to be interpreted with caution; the included trials were few in number and with small sample size. More recently, a study evaluating the effects of GH supplementation on oocyte maturation in vivo in aged and young mice and its effect on mitochondrial function observed a potential role of GH in improving mitochondrial function in oocytes from aged mice (Hou et al. 2019). Further research is, however, necessary to fully define GH role in IVF treatment.

Polycystic Ovary Syndrome (PCOS)

Definition and Diagnosis

According to the "Rotterdam criteria," diagnosis of PCOS requires two of the following criteria (Table 3): oligo- and/or anovulation (having an interval of >35 days between menstrual periods and/or amenorrhea, described as the absence of vaginal bleeding for at least 6 months), clinical and/or biochemical signs of hyperandrogenism (evaluated by modified Ferriman-Gallwey (mF-G) scoring method), and PCO at ultrasound (12 or more follicles in either ovary measuring 2–9 mm in diameter and/or increased ovarian volume) (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2004; Fig. 5).

Certainly, the diagnosis of PCOS is only confirmed when other conditions are excluded (e.g., oligo−/anovulation disorders and/or hyperandrogenism, such as thyroid disease, nonclassic congenital adrenal hyperplasia (NCCAH), hyperprolactinemia, and androgen-secreting tumors).

Table 3 Rotterdam criteria for diagnosis of PCOS. Total T: total testosterone. DHEA-S: deidroepiandrosterone solfato

Diagnosis confirmed by 2 of 3 criteria after exclusion of other etiologies:
1. Oligo and/or anovulation
2. Biochemical and/or clinical signs of hyperandrogenism Biochemical: Total T > 70 ng/dL, androstenedione >245 ng/dL, DHEA-S > 248 ug/dL Clinical: severe cystic acne, progressive hirsutism, acanthosis nigricans
3. Polycystic ovaries evaluated in ultrasound: ≥ 12 follicles (2–9 mm diameter) in each ovary or ovarian volume > 10 cc

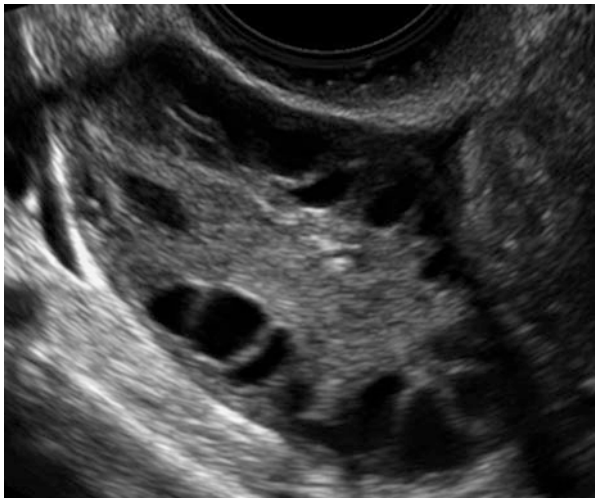

Fig. 5 Ultrasound image of human polycystic ovary: more than 12 follicles measuring 2–9 mm in diameter and surrounding an enlarged ovarian *stroma*

The aetiology of PCOS remains unclear, but evidence exists for a multifactorial origin with a genetic predisposition. In women with PCOS, we can find multiple abnormalities such as oligomenorrhea, hyperandrogenism, anovulatory infertility, and metabolic risk factors such as obesity, insulin resistance, dyslipidemia, impaired glucose tolerance, fatty liver, and obstructive sleep apnea.

Prevalence

PCOS is the commonest endocrine disorder in women of reproductive age and the leading cause of anovulatory infertility. It is estimated that around 20% of all IVF/ICSI cycles are performed in patients with PCOS (ESHRE Capri Workshop Group 2012).

Management

When lifestyle modifications are not successful in restoring ovulation, patients can be treated with ovarian stimulation. Typically, ovulation induction begins with the use of CC or letrozole. Subsequent steps are represented by administration of exogenous gonadotropins associated with timed intercourse or IUI and IVF/ICSI (ESHRE Capri Workshop Group 2012).

There is ongoing debate regarding the use of ovulation induction versus ART for the treatment of infertile women with PCOS. An RCT was conducted in order to compare the efficacy of IUI vs timed intercourse with CC as a first-line treatment for anovulatory infertility associated by Abu et al. One hundred eighty-eight women with PCOS received three consecutive cycles of ovulation induction with CC and IUI ($n = 93$, 259 cycles) or three consecutive cycles of CC with timed intercourse ($n = 95$, 266 cycles). The study showed comparable outcomes regarding the clinical pregnancy rate per cycle or per woman (8.49 vs 7.89% and 23.6 vs 22.1%; $p = 0.26$ and $p = 0.33$, respectively). Therefore, the authors concluded that ovulation induction with CC and timed intercourse is as effective as IUI in patients with PCOS and could represent the first treatment, being less invasive and less expensive than IUI (Abu et al. 2011).

A PCOS Consensus sponsored by ESHRE/American Society for Reproductive Medicine (ASRM) concluded that combining IUI with ovulation induction may be considered in anovulatory women with PCOS after failure to conceive despite successful induction of ovulation or in cases associated with male factor infertility. Such treatment has shown from 11% to 20% clinical pregnancy rate per cycle with a multiple pregnancy rate ranging from 11% to 36% (Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2008).

After failed ovulation induction or IUI, or in the presence of other infertility factors such as tubal damage, severe endometriosis, or male factor infertility, IVF/ICSI treatment in women with PCOS is recommended. At the same time, gynecologists should take into considerations other variables such as age. In fact, fertility

potential declines rapidly after 40 years of age; thus in these categories of women, clinicians need to avoid a delay in initiation of ovulation induction.

Women with PCOS are difficult to stimulate: sometimes they demonstrate resistance to stimulation, and in other cases they have exaggerated response. In PCOS women, the risk of moderate-to-severe OHSS has been evaluated of approximately 10% versus 0.5–4.0% in the general IVF population. A meta-analysis reported an OR of 6.8 (95% CI: 4.9–9.6) for the development of OHSS in ultrasound-determined PCOS patients compared with those with normal-appearing ovaries on baseline ultrasound. Moreover, although oocyte recruitment during ART is higher in these patients, quality and maturity are poor and maybe compromised (Baumgarten et al. 2013).

The goal of the ART is to find the optimal strategy to prevent OHSS and cycle cancellation. In 2016 the ASRM recommended, in women with PCOS who undergo ART, the use of "step-up" or "step-down" protocols, or a sequential scheme, where a low dose of exogenous FSH or combined gonadotropins are employed to tie up ovarian hyperstimulation (American Society for Reproductive Medicine 2016).

"Step-up" protocols consist in an initial low gonadotropin daily dose (37.5–50 IU/day), followed by a small incremental dose (augmented by 50–100%) until folliculogenesis is reached up as evidenced by a lead follicle on ultrasound and the rising of estradiol levels (Berger and Bates 2014).

"Step-down" protocol starts with a higher dose of FSH that is gradually decreased; dose reduction is progressive and based on the visualization of at least one follicle of 10 mm. Although mimicking a physiological cycle, the step-down protocol is associated with a higher risk of OHSS and cycle cancellation (Christin-Maitre et al. 2003).

Moreover, these two protocols could be combined in a "sequential protocol": FSH dose is gradually increased until a leading follicle reaches 14 mm diameter; then gonadotropin dose is decreased by 50%.

Strategies to Reduce OHSS Risk

Several interventions have been suggested in order to reduce the occurrence of OHSS while not influencing or even improving pregnancy outcomes:

- The use of GnRH antagonist protocol against conventional long GnRH agonist for pituitary suppression
- Coasting before oocyte triggering
- The use of GnRH agonist to trigger oocyte maturation
- "Freeze-all" embryo strategy
- Dopamine agonists, metformin, inositol, and aspirin

Coasting is the strategy of withholding exogenous gonadotropins at the end of ovarian stimulation: mature follicles will survive for a few days, while smaller follicles will reduce through atresia of granulosa cells. Low-quality evidence suggests that coasting reduces moderate-to-severe OHSS. Moreover, the optimal length of coasting has not been determined, with a limited number of studies suggesting 4 days (Nardo et al. 2006).

ASRM recommends the use of GnRH agonist (GnRHa) for the final oocyte maturation trigger in oocyte donation (evidence grade A). Nowadays, the use of GnRHa trigger is a well-established first-line treatment in oocyte donation and segmentation IVF/ICSI cycles (when egg collection and transfer are done through embryo cryopreservation and cryopreserved embryo transfer in a separate cycle). On the other hand, this procedure showed lower reproductive outcomes for fresh embryo transfer cycles. Essentially, this poor outcome is due to the low-circulating endogenous LH levels after the GnRHa trigger, leading to corpus luteum demise and consequently suboptimal progesterone levels at peri-implantation. This finding led to the development of modified luteal phase support protocols. Unfortunately, none of these has gained universal acceptance (American Society for Reproductive Medicine 2016).

Randomized study on infertile women with PCOS found that frozen-embryo transfer resulted in a higher rate of live births than fresh-embryo transfer, a difference that was attributed to a lower rate of pregnancy loss. Also, in the frozen-embryo group was found a lower frequency of the OHSS but a higher frequency of preeclampsia (Chen et al. 2016).

Dopamine agonists, cabergoline and quinagolide, reduce incidence of moderate or severe OHSS, based on moderate-quality evidence; administration of these treatments should start at the time of hCG trigger and continued for several days (6–8). There is no evidence that cabergoline or quinagolide influences pregnancy outcomes such as live birth rate, clinical pregnancy rate, multiple pregnancy rate, and miscarriage rate.

Another strategy being validated is the administration of aspirin during ovarian stimulation cycles; evidence about aspirin is based on a single randomized trial comparing aspirin alone to no treatment and another study comparing combined acetylsalicylic acid and steroid treatment with no treatment (American Society for Reproductive Medicine 2016).

Conclusions

Nowadays ART is available throughout most of the civilized world, and it is likely that continued enhancements will widen its appeal and applicability. The introduction of PGS/PGD allowed the screening of an IVFI-/CSI-created embryo for aneuploidy and for the testing of a genetic disorder, respectively. Moreover egg and sperm donation has allowed women with premature ovarian failure and men with intractable azoospermia to access the techniques. Although the advances in ART have opened new doors for infertile couples, treatment of patients with poor ovarian response and PCOs remains challenging and still presents many uncertainties with debated approaches. Moreover, the widespread of these techniques and the inclusion of a population of older women with comorbidities has posed the problem of managing obstetric complications following ART. Therefore more research is needed in assisted reproduction in order to optimize the chances of a safe pregnancy in certain groups of patients.

Cross-References

► The Polycystic Ovary Syndrome (PCOS)

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