Individualization of treatment in controlled ovarian stimulation: myth or reality?

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ABSTRACT

Variability in the infertile population excludes the possibility of a single approach to controlled ovarian stimulation. The modern technology has led to the development of new drugs, treatment options and quantitative methods that allow an individualized approach to *in vitro* fertilization. The personalization of treatments requires a comprehensive evaluation of several important aspects. Age still remains the best predictive factor of gametes euploidy rate. It was estimated that the percentage of abnormal embryos/oocytes increased dramatically in women >35 years old. Strategies to improve the number of vital and euploid embryos in those women represents the most intriguing challenge nowadays, considering that more and more women seeking assisted reproductive technologies are in advanced age. On the other hand, ovarian reserve markers, namely follicle stimulating hormone, anti-Mullerian hormone and antral follicle count are also considered the most accurate predictor of ovarian reserve and could be successfully used to guide controlled ovarian stimulation. Finally, there is an emerging evidence in literature which suggests that the ovarian sensitivity to exogenous gonadotropins could be also influenced by specific genotypes characteristics. If these data will be confirmed, a genetic screening might allow in the future a pharmacogenomic approach to better control ovarian stimulation.

INTRODUCTION

Individualized controlled ovarian stimulation (iCOS) is becoming a reality in human reproduction field. At the beginning, controlled ovarian stimulation (COS) was carried out taking into account only demographic and anthropometric characteristics of women, such as age and body mass index (BMI). Moreover, previous response to exogenous gonadotropins still represents a useful information for pharmacological approach to infertile women. This aspect has a great importance in clinical practice in terms of both number of oocytes retrieved and cumulative dosage required during COS.

With respect to ovarian response to gonadotropin, terms as “hyper”, “normal” or “poor” response have been introduced. Essentially, those definitions are based on the number of oocytes retrieved after COS. For instance, while ‘hyper-response’ refers to the retrieval of >15 oocytes (1), the term of “poor response” (POR) reflects the retrieval of ≤3 after conventional stimulation protocol (2).

The definition of POR women still represents a debatable topic in reproductive field. A systematic review assessing the definitions for “poor ovarian responders” used among randomized trials, identified 41 different definitions of POR women (3). Only relatively recently, a consensus promoted by the European Society of Human Reproduction and Embryology (ESHRE) was reached (2). In detail, at least two of the following 3 features must be present to identify POR: a) advanced maternal age (≥40 years) or any other risk factor for POR, b) a previous POR (≤3 oocytes with a conventional stimulation protocol), c) an abnormal ovarian reserve test [i.e. antral follicle count (AFC), 5-7 follicles or anti-Mullerian hormone (AMH), 0.5-1.1 μg/L]. These criteria have subsequently been criticized for including heterogeneous populations with different characteristics and clinical prognosis. Added to this, the Bologna criteria do not address the issue of oocyte quality, which is crucial for *in vitro* fertilization (IVF) success (4). More recently, a new stratification of patients with low prognosis to assisted reproductive technology (ART) has been proposed (5). An international panel of experts has agreed on a new detailed stratification of women who show low prognosis to conventional COS [Patient-Oriented-Strategies-Encompassing-InDividualized-

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Oocyte Number ("Poseidon") classification for low prognosis women in ART], taking into account not only the number of oocytes retrieved, but also the ovarian sensitivity to exogenous gonadotropins and the age-related embryo/blastocyst aneuploidy rate.

A "normal response" is usually identified when 10-15 oocytes are collected at the end of ovarian stimulation. Recently, terms as "hyporesponse" and "suboptimal response" have been introduced to describe those women with an impaired gonadotropin response, but who do not fit POR criteria. "Suboptimal response" is based only on the number of oocytes (between 4-9) (6), whereas "hyporesponse" profile includes those patients who required higher amount of exogenous follicle stimulating hormone (FSH) to obtain an adequate number of oocytes (7) or who show a steady response in terms of both follicular growth and oestradiol level during COS (8).

The most critical issue in iCOS is the individualization of the appropriate dosage. Given that a woman has a definite number of follicles for each cycle, the optimal strategy should ideally be able to recruit all the available follicles avoiding low follicular development. At the same time, the consequence of an excessive ovarian response should be prevented.

The prediction of "poor", "hyper", "suboptimal" or "hyporesponse" to COS is necessary for a correct iCOS, allowing clinicians to provide women possible explanation of events, such as protracted cycles, cycle cancellation or interruption of treatment. Prediction of ovarian response could be explained by the interplay between demographic and anthropometric characteristic and ovarian reserve markers; however, some authors also advocate the involvement of environmental contaminants (9, 10). Emerging evidence also suggests that specific genotype characteristics could significantly affect COS (11, 12). In detail, specific polymorphisms of gonadotropins and their receptors were associated with a significant alteration in women response to gonadotropins (7, 13, 14).

The aim of the present review is to summarize the available evidence concerning iCOS predictors and pharmacological approaches retrievable in literature. The following topics will be developed: a) ovarian reserve, b) anthropometric and demographic parameters, c) ovarian reserve hormonal and functional biomarkers, d) genetic biomarkers.

MATERIAL AND METHODS

A comprehensive literature research of studies that address iCOS approaches to ART has been performed. With this purpose, a systematic research of Medline (Pubmed), Scopus and Isi Web of Science databases with no time restriction was carried out using the following search terms: "anti-Mullerian hormone", "AMH", "AFC", "antral follicle count", "individualized controlled ovarian stimulation", "iCOS", "ovarian reserve", "genetic biomarkers". English written randomized controlled trials, meta-analysis, systematic review, narrative review, prospective studies and retrospective trials were included. Case reports, books and data that were published in conference or meeting proceedings were not included. The grey literature was not considered.

RESULTS

Ovarian reserve

Ovarian reserve represents the individual reproductive potential based on both number and quality of remaining oocytes (15). The ovarian reserve pool consists in both primordial non-growing follicles and pre-antral and antral follicles recruited for the final ovulation. Primordial follicles derive from primordial germ cells migrated from hindgut to genital ridges during embryo development and arrested at the first meiotic division. Although it is believed that neo-oogenesis does not occur in adults, several lines of evidence indicate that a post-natal replenishment of follicles could not be excluded (16).

Since the prenatal period, a dramatic reduction of germ cells takes place (17). At 18 weeks of gestation, the peak number of 6-7 million of primordial follicles progressively reduces to 1-2 million at birth through apoptosis process (18). When puberty occurs, there are ~300.000 follicles. Finally, in menopause stage it was estimated that <1000 follicles remain (19). Not only the number, but also oocytes quality decreases relentlessly. As a matter of fact, an increased incidence of chromosomal anomalies (e.g. trisomy 21) is observed in older women. Some authors hypothesize that this phenomenon could be attributed to the reduced rate of anomalous oocytes apoptosis in advanced age (20).

The individual ovarian reserve and the proportion of follicles, which undergo atresia, depends on menopausal age. By the age of 45 years, a precocious decline in follicles quantity was hypothesized to be approximately at 32 years of age, although the maintenance of regular menstrual cycles would make the woman unaware. Furthermore, prenatal or post-natal exposure to nutritional, social or environmental factors may also influence ovarian reserve and age of menopause (18). For instance, elevated prenatal androgens, specific social factors and smoking habits could contribute to diminished ovarian reserve.

Anthropometric and demographic parameters

The most commonly used predictors of ovarian response in clinical practice are age and BMI. Women age is clearly linked to human fertility and significantly influences ART success. Indeed, age represents one of the key points in Bologna criteria for the identification of POR women (2). The importance of the age is even more stressed in the new stratification of low prognosis women to ART, recently proposed by Poseidon group (5). The likelihood of embryo implantation and successful live birth rate slowly decrease over the age of 35 years (21). Conversely, the outcome of women using donor eggs does not relevantly change with increasing age,

confirming the crucial role played by oocytes quality (22). Nonetheless, the chronological age alone could not reflect exhaustively the real potential fecundity. For this reason, the clear identification of biological age requires the evaluation of other parameters, such as ovarian response to FSH and specific biomarker levels (AMH, AFC). Moreover, it was reported that women with similar age showed a wide variability in baseline antral follicles (23). Age alone showed low correlation with the number of oocytes gathered (24).

Obesity is another important factor associated with ovulatory dysfunction and infertility (25). It was estimated that the relative risk (RR) of anovulatory infertility is 1.3 [95% confidence interval (CI): 1.2-1.6] among women with BMI between 24 and 31 kg/m^2 and 2.7 (95% CI: 2.0-3.7) for those with BMI ≥32 kg/m^2 (26). A large prospective including 3029 subfertile women indicated that the probability of spontaneous conception is declined by 4% per kg/m^2, when BMI is >29 kg/m^2 (27). Weight loss is significantly associated with improved reproductive outcome.

Regarding ART, an increasing body of evidence suggest that obesity could as well impair reproductive outcome. Obesity was associated with lower oocytes retrieval, cancellation rate, prolonged stimulation and reduced response to exogenous gonadotropins (25). Apparently, no evident association between obesity and oocytes quality was reported (25)

A meta-analysis by Rittenberg et al., including 47,967 treatment cycles, indicated that women with BMI ≥25 kg/m^2 showed lower clinical pregnancy rate (RR: 0.9; P <0.0001), live birth rate (RR: 0.84; P=0.0002) and significantly higher miscarriage rate (RR: 1.31; P <0.0001) (28). A subgroup analysis confirmed these findings even in women with BMI between 25 and 29.9 kg/m^2. Nevertheless, a more recent systematic review and meta-analysis did not detect any relevant detrimental effect on IVF outcome in obese oocytes donor recipients (29).

Hormonal biomarkers

FSH

Serum FSH concentrations are usually dosed on cycle day 2-5 and is commonly used as a measure of ovarian reserve (15). Elevated concentrations (10-25 IU/L) were associated with impaired ovarian stimulation and low pregnancy rate. New standardized WHO assays have shown a high specificity (83–100%), but an extremely variable sensitivity (10-80%) to identify POR women in terms of retrieved oocyte (<4 oocytes) (30). Interestingly, normal basal serum FSH with elevated concentrations of estradiol in early follicular phase was also associated with lower pregnancy rate, higher cancellation rate and poor ovarian response (31).

Inhibin B

Several studies have evaluated the role of inhibin B in predicting the ovarian response in ART (30, 32). As AMH, inhibin B belong to transforming growth factor (TGF) β family. Its concentrations rise during follicular phase, peak on 5-6 days of menstrual cycle and drop in the late follicular phase. Until the introduction in clinical practice of AMH and AFC, inhibin B has been used for the evaluation of ovarian reserve. Nevertheless, a systematic review and meta-analysis, including 9 studies, reported that the accuracy of inhibin B in the prediction of poor ovarian response and non-pregnancy was only modest (30). In addition, when compared with other ovarian reserve markers such as AMH and AFC, inhibin B showed inferior accuracy even for counselling purpose (30). Given that more reliable markers are now available, the evaluation of inhibin B is no more recommended for the evaluation of ovarian reserve (15).

AMH

AMH is a paracrine product of immature follicles, which represents one of the most valuable tool for ovarian reserve evaluation. AMH is a glycoprotein dimer composed of two 72 kDa monomers linked by disulphide bridges and is produced by the pre-antral and small antral follicles; AMH correlates with the number of primordial follicles at the gonadotropin independent stage of follicular development (33).

In a preliminary study, AMH <1.26 μg/L showed a 97% sensitivity for predicting POR (<4 oocytes retrieved). Moreover, it was estimated a 98% correct prediction of “normal” response, if AMH concentrations were above this threshold (34). In this study, this cut-off value was calculated with a ROC curve developed to discriminate between women with poor and normal response (>4 oocytes) (34). These findings indicate that circulating AMH concentrations may be a good indicator of ovarian reserve and are highly correlated with ovarian response to COS. Several studies suggest that AMH could be also a valuable marker for the identification of polycystic ovarian syndrome (35).

In a study designed to evaluate the use of AMH to determine treatment strategy, Nelson et al. observed an improved safety, tolerability and success rates (36).

Recently, new Bologna criteria and low prognosis patients introduce by Poseidon group agree on AMH <1.2 μg/L as cut-off for the identification of women with impaired reproductive prognosis (2, 5).

The main advantages of AMH determination include the minimal variability within natural cycles and the standard reference available for both adolescent and reproductive age patients (33, 37) In addition, AMH concentrations did not show the interoperator variability of other ovarian reserve markers, such as AFC (Table 1) (38). On the other hand, a relevant limiting factor in the use of AMH is the variability observed between results obtained with available commercial kits (39, 40).

Initially, two ELISA were used for the measurement of AMH, the Diagnostics Systems Laboratory (DSL) and the Immunotech assays. These assays employed different monoclonal antibodies giving different results. Subsequently, Beckman Coulter acquired both DSL and Immunotech and launched a new assay, the Beckman Coulter AMH Gen II assay (41). This new assay showed

Table 1
Advantages and disadvantages of anti-Mullerian hormone (AMH) vs. antral follicle count (AFC). Adapted from ref. 38.

AFC	AMH
Advantages	*Advantages*
- Good predictive value for stimulation response - Useful for treatment decisions - Easy to perform and personalize - Non invasive - Immediate results	- Good predictive value for stimulation response - Useful for treatment decisions - Well-characterized across adolescent and reproductive ages - Can be performed at any point during a cycle - Low intracycle, intercycle, interoperator and intercentre variability
Disadvantages	*Disadvantages*
- Must be carried out at the beginning of a cycle because of intracycle variation - Intercentre variation because of subjective estimate - Lack of standardization - Greater intercycle variation observed with overweight and obese women - High costs of ultrasound technique	- Intensive labour, requiring several hours - Requires careful sample preparation and storage - Interference with serum complement factors - No standardization across assays

~40% higher value comparing with DSL assay. After that, several other kits have been introduced in the market (42). The new fully automated systems available for AMH assay (Roche Elecsys and Beckman Coulter Access) will probably help to improve AMH measurement in terms of precision and reliability, providing faster results compared with previous assays (42, 43).

Improper storage and sampling process may dramatically interfere with the measurement of AMH (44). Furthermore, interferences by serum complement factors on AMH assays have also been observed. Smoking and use of contraceptives should also be taken into account for the evaluation of AMH levels. Decreased concentrations of AMH were observed in women with smoking habits as well as in women who underwent continuous contraceptive use (42, 45). Higher parity was associated with higher age-specific AMH values (45).

Functional biomarkers

AFC is the sum of antral follicles in both ovaries by ultrasound; it has progressively gained acceptance for the estimation of ovarian reserve and as predictive tools for ovarian response and IVF success (21). AFC is recommended by American Society for Reproductive Medicine (ASRM) for the evaluation of ovarian reserve (15). AFC is a relatively easy-to-perform technique, which is widely accessible in clinical practice and provide an immediate result (21, 38).

The main disadvantages of AFC consist in the relevant variation between sonographers and the lack of standard methodology. In addition, it depends on the operator training, technological resources as well as the ultrasound machine adopted. The presence of cysts, fibroids and other anomalies of reproductive organs could also impair the results (38, 46).

A standardization of AFC was introduced in 2010 by Broekmans et al. (46). Here the basic clinical and technical requirements proposed are reported:

1) Clinical considerations:
   a) select patients with regular menstrual cycles with no coexisting pathologic condition that could technically affect the counting of follicles, such as ovarian endometriosis or previous ovarian surgery;
   b) count follicles between days 2 and 4 of a spontaneous menstrual or oral contraceptive cycle to avoid the effect of intracycle variation;
   c) include all antral follicles of 2-10 mm diameter.
2) Technical considerations:
   a) a limited number of personnel, appropriately trained in transvaginal ultrasound should perform AFC in each unit;
   b) real-time two-dimensional imaging is adequate;
   c) use a transvaginal transducer;
   d) use a probe with a minimum frequency of 7 MHz, which is maintained in an adequate condition and able to resolve a structure of 2 mm diameter;
   e) use the following systematic process for counting antral follicles:
      1. identify the ovary;
      2. explore the dimensions in two planes (perform a scout sweep);
      3. decide on the direction of the sweep to measure and count follicles;
      4. measure the largest follicle in two dimensions:
         A. if the largest follicle is 10 mm in diameter:
            i. start to count from outer ovarian margin of the sweep to the opposite

margin;
ii. consider every round or oval transonic structure within the ovarian margins to be a follicle;
iii. repeat the procedure with the contralateral ovary;
iv. combine the number of follicles in each ovary to obtain AFC.

B. if the largest follicle is >10 mm in diameter:
i. further ascertain the size range of the follicles by measuring each sequentially smaller follicle, in turn, until a follicle with a diameter of 10 mm is found;
ii. perform a total count (as described) regardless of follicle diameter;
iii. subtract the number of follicles of >10 mm from the total follicle count.

Although this standardization have provided for the first time a guide for the evaluation of AFC, there are still unsolved issues. For instance, this recommendation excluded women with previous ovarian surgery, endometriosis cyst, with single ovary and with irregular menstrual bleeding (IMB). In addition, technical settings of the ultrasound machine (depth, gain, focus) are also missing (42).

In the last years, the use of three dimensional (3D) ultrasound calculator was evaluated for AFC (47). A preliminary study showed that the automated 3D ultrasound calculator is able to ameliorate detection of follicles by reducing intra- and interobserver variability and obtain results in a shorter time. This new ultrasound technique could also be adopted for storage and further data revaluation (47). Nevertheless, the increased costs seem not balanced by a significant improvement of clinical results. Thus, more larger trials are needed before suggesting automated 3D ultrasound for AFC (38).

Some authors have advocated the need of specific training for the operator according to the model proposed by Fetal Medicine Foundation for nuchal translucency (42). The ongoing quality control and license for AFC may ameliorate operator skills, reducing the degree of variation between centres.

Genetic biomarkers

Pharmacogenetic studies evaluate the effect of individual genotypes on medications administrated in clinical practice. In the future, treatment of women based on her genotype profile could represent an important goal for a patient-tailored approach. Although biological response to medications may be influenced by hundreds of factors, progress is being made in the identification of specific genetic profiles that could predict the safety and effectiveness of certain drugs in individual patients. In the reproductive field, the role of genetic biomarkers is still matter of debate, but, in the next years, a personalization of protocols according to specific genotype profiles may become a valuable strategy (21).

Usually, IVF/intracytoplasmic sperm injection (IVF/ICSI) protocols are optimal in ~85% of women, with an adequate number of oocytes recruited. Nonetheless, in ~12-15% of cases an initial low response is seen, leading to an increase in the daily dose of exogenous FSH. These observations led to the development of the concept of "hyporesponse" to COS. Namely, hyporesponse individuals are normogonadotrophic women who have normal estimated ovarian reserves, but require high amounts of exogenous FSH to obtain an adequate number of oocytes retrieved (48-51). On the basis of the current literature, it is possible to argue that inappropriate response to ovarian stimulation including "hyporesponse" or POR might be related to genetic characteristics (13, 52). In addition, relevant gynecological conditions, such as premature ovarian insufficiency (POI) and polycystic ovarian syndrome (PCOS) were also associated with genetic polymorphism of gonadotropins and their receptors (53-57).

The genetic variability that affects the innate activity of hormones could provide valuable predictive information and guide COS treatment choices. Mutations in the genes coding for LH (13, 58, 59), LH and FSH receptors (60-63) and FSH-29 promotor (64, 65) have been identified as possible causes of subfertility, as well as factors that may influence fertility treatment (66).

A common variant of the β subunit of the luteinizing hormone (LH) molecule (v-LH) is characterized by an additional sulphonated sugar at asparagine (Trp 8 Arg and Ile 15 Thr) (rs1800447). v-LH has elevated bioactivity *in vitro*, but significantly shorter half-life in circulation (5-9 min), when compared with the wild-type LH (wt-LH) (12-22 min) (67). The v-LH polymorphism is found in populations worldwide, but has so far been most commonly identified in northern European countries, such as Finland (68). Its incidence in Italy ranges between 12% and 13%. There is clinical evidence that the v-LH polymorphism affects FSH sensitivity and the ovarian response to COS. For example, in a group of 60 normogonadotrophic women aged 18-37 years, with normal menstruation, basal FSH ≤10 IU/L and at least 5 oocytes retrieved, one homozygote and seven v-LH heterozygotes were identified. When these women were stratified by the cumulative dose of FSH used (>3500 IU, 2000-3500 IU or <2000 IU), only one heterozygote fell in the middle range while the rest had doses >3500 IU (59). In another study, the effect of v-LH was retrospectively investigated in a larger series of women undergoing COS and, for the first time, in a Danish IVF population (13). 220 normogonadotrophic women following a long gonadotropin releasing hormone (GnRH)-agonist down-regulation protocol received an individualized dose of recombinant FSH (rFSH). Group A consisted of 196 wt/wt-LH women; group B included 24 individuals with v-LH (21 heterozygous and 3 homozygous). The latter received a significantly higher cumulative rFSH than group A (2435.9±932.8 IU vs. 1959.8±736.5 IU, P=0.048). One-way ANOVA within design showed a progressive increase from wt-LH form to that with complete v-LH genotype expression (13).

Hyposensitivity to FSH may also be caused by a genetic variant of the FSH receptor. Two FSH receptor variants that have single nucleotide polymorphism (SNP) in the coding region have been characterised (69). The SNP known as the serine680 (Ser680) variant causes the replacement of Asn with Ser at the 680 position, which is located in the intracellular domain of the FSH receptor protein (rs6166). Consistent with reduced sensitivity to endogenous FSH, carriers of this trait have higher FSH levels throughout most of the menstrual cycle and a significant increase in both total menstrual cycle length and number of antral follicles (70). One of the first studies regarding the effect of FSH receptor polymorphism rs6166 on ART detected significantly higher basal FSH levels as well as the number of FSH ampoules in carriers of the Ser680 variant (14).

The second receptor variant, known as the alanine307 (Ala307) variant, is generated through the substitution of threonine (Thr) with Ala at the 307 position, located in the extracellular domain of the FSH receptor (rs6165) (69). There is a very strong linkage disequilibrium between the two SNP. This means that women who possess Thr307 nearly always have Asn680 present on the same allele, and women who have Ala307 have Ser680 on the same allele (67). The link between FSH receptor SNP and PCOS has been studied extensively; however, there is some variation seen in the results. Some studies have demonstrated higher rates of patients with Ser680 in the PCOS population (71), whereas other investigators have shown differences in basal levels of FSH depending on the presence of the SNP (72). Interestingly, it has been demonstrated that PCOS patients with the Ser680 mutation have a natural resistance to clomiphene citrate (73). This may prove to be very important in the field of ovarian stimulation in the future, with clinicians devising their COS protocols according to factors including FSH receptor genotype. Additionally, the effect of the Ser680 SNP has been extensively studied in the general IVF population with conflicting results (74). For instance, some studies reported higher pregnancy rates in patients with Asn680 (75) and others showed higher pregnancy rates in patients with Ser680 (76).

An additional study looked at FSH-induced estradiol levels in women who were homozygous for the Ser680 variant compared with women with the wild-type (Asn/Asn). To our knowledge, this represented the first attempt to develop a pharmacogenomical approach to COS. Ser680/Ser680 carriers were randomly allocated to two subgroups to receive a daily FSH dose of 150 IU or 225 IU, respectively. Age and BMI matched Asn/Asn carriers, receiving a daily dose of 150 IU, constituted a third control group. Number of retrieved oocytes and fertilization rates were similar among groups, but women with wild-type form had higher estradiol production after treatment with 150 IU/day of FSH compared with the Ser680 group receiving the same dose; when Ser680/Ser680 carriers were treated with 225 IU/day, this difference disappeared (63).

A literature systematic review and meta-analysis published in 2011, including a total of 1421 cases, collected from 8 studies, confirmed that Ser680 carriers required a higher amount of rFSH during COS (Weighted mean difference: -268.82 IU, 95% CI: -561.28 to 23.63, P=0.07). Despite of this, no differences in terms pregnancy rate and number of retrieved oocytes were detected (62).

In addition to the SNP described above, there are other genotypic polymorphisms that have been recently related to COS and ART outcomes. An interesting polymorphism in the untranslated region (-29G/A rs1394205) of the FSH receptor gene (*FSH-R*) has been identified. As reported by Nakayama et al., this variant could influence the normal transcription activity of FSH-R and seems to be related with the development of essential hypertensions in women (77). Significantly lower estradiol levels were observed in AA carriers compared with patients with GG or GA genotypes. More interestingly, this polymorphism was associated with higher amounts of rFSH required during COS. Specifically, the subjects with AA genotype required the highest amount of rFSH with the lowest estradiol levels before the day of chorionic gonadotropin (hCG) when compared with other haplotypes (78). Moreover, AA carriers showed a significantly lower number of retrieved oocyte and preovulatory follicles. Nonetheless, the kind of protocol adopted as well as patients characteristics were not reported by the authors.

Finally, there is also evidence that specific LH/gonadotropin receptor (LHCGR) polymorphism could interfere with COS (61). Specifically, carriers of LHCGR (rs4073366) showed an increased risk to develop ovarian hyperstimulation syndrome (OHSS) (odds ratio, 2.95; 95% CI: 1.09-7.96). Moreover, another LHCGR polymorphism (rs2293275) showed a significant association with the pregnancy rate of women undergoing IVF (58). This association became even more stronger when combined with FSH Ser680 polymorphism.

In conclusion, among all identified genotypes the FSH-R 680 and the LH variant are the most studied genetic marker associated with ovarian resistance to COS (13, 64). LH supplementation may be considered in the presence of v-LH, whereas a timely identification of Ser680 FSH-R variant may represent an indication to administer higher doses of FSH (Table 2). For other polymorphisms, such as promotor 29G/A FSH-R and LHCGR polymorphisms, further studies are needed to clarify their role as genetic biomarker.

DISCUSSION

In the past, the choice of the appropriate therapy was essentially based on anamnesis, BMI, age and outcome of previous IVF. Today, the concept of iCOS is progressively raising in ART. Currently, the vast number of medications and protocols gives the opportunity to tailor stimulation on the basis of individual characteristics of women.

The most adopted pre-stimulation parameter in

Table 2
Example of interplay between genetic profiles and biomarkers in the field of controlled ovarian stimulation (COS). Adapted from ref. 79

Genetic profile	Application in clinical practice
Low AMH levels, low AFC, no relevant mutations	Suggest that even high doses of FSH would be ineffective and that LH would not improve results Patients would benefit from counselling to better understand her limited chances of success
FSH receptor variant (e.g. Ser680), good AMH levels, good AFC	Suggest a good prognosis, but it also predicts "hyporesponse" to FSH that could be compensated by increasing FSH starting dose
Variant in β subunit of LH	Suggest that patient may benefit from LH supplementation during COS

AMH, anti-Mullerian hormone; AFC, antral follicle count; FSH, follicle stimulating hormone; LH, luteinizing hormone.

Table 3
Suggested cut-off values for anti-Mullerian ormone (AMH) and antral follicle count (AFC)

Poor response

	ESHRE	La Marca-Sunkara (24)	Poseidon group (5)
AMH cut-offs	0.5-1.1 μg/L	0.7-1.3 μg/L	1.2 μg/L
AFC cut-offs	5-7	5-7	5

Hyperesponse

	Literature (see text)	La Marca-Sunkara (24)
AMH cut-offs	2.21-6.95 μg/L	3.36 μg/L
AFC cut-offs	9-16	16

ESHRE, European Society of Human Reproduction and Embryology.

clinical practice are AMH and AFC. The establishment of the right cut-off value is of great importance for a correct use of these biomarkers in clinical practice. Nonetheless, values reported in literature are widely heterogeneous across studies, so that the entire process for the identification of optimal cut-off is a challenging task. Through the analysis of literature, La Marca and Sunkara selected 66 papers that investigated the ability of AFC (n=25) and AMH (n=41) to predict response to COS (24). In Table 3 the adopted cut-off values for the prediction of both POR and hyperresponse to COS are illustrated.

More than 30 studies tried to identify the optimal cut-off for AMH to define a POR. This varies between 0.1 and 2.97 μg/L. The largest prospective trials indicate as optimal cut-off 0.7 μg/L (75% sensitivity, 91% specificity) and 1.36 μg/L (75.5% sensitivity, 74.8% specificity), respectively (80, 81). Based on those data, La Marca and Sunkara indicated a cut-off ranging from 0.7 to 1.3 μg/L (24).

AFC cut-off values proposed for the identification of poor responders vary from 3 to 12 (82, 83). La Marca and Sunkara, on the basis of the most recent papers, individuated the AFC cut-off of POR prediction between 5 and 7 (24).

It should be stressed that ovarian reserve tests (AMH/AFC) have a false positive rate of 10-20% for the identification of POR women. In other words, no women should be excluded from their first IVF techniques only based on ovarian reserve tests.

"Hyperresponse" to COS affect 7% of women who underwent IVF and 15% of women aged <30 years (24). "Hyper-response" is associated with OHSS, which represents the most critical life-threatening complication of IVF techniques. Therefore, a proper identification of a "hyper-responder" woman before COS is fundamental in order to prevent OHSS. According to the most recent data available in literature, "hyper-response" consists in the retrieval of >15 oocytes after standard COS (1, 84). 16 studies have reported the best cut-off values to identify "hyperresponders" according AMH levels, which range between 2.21 μg/L to 6.95 μg/L. Sunkara and La Marca adopted 3.36 μg/L according to DSL assay (4.67 μg/L according to Beckman Coulter AMH Gen II assay) as optimal cut-off to identify patients at risk to develop a hyperesponse during COS (24, 85). Only 7 studies reported the cut-off values for AFC, with range between 9 and 16. The largest prospective trial involving 159 women suggested an AFC cut-off value of 16, with 89% sensitivity and 92% specificity (86). In a retrospective trial involving 82 women, Ocal et al. indicate 8 as cut-off value for AFC to predict the development of OHSS (78% sensitivity, 65% specificity, 52% positive predictive value, 86% negative predictive value) (87).

Unfortunately, there are not established AFC and AMH cut-off values to identify women with hyporesponse or suboptimal response.

The number of follicles recruited during COS depends essentially on the number of antral follicles at the beginning of the cycle. When antral follicles pool is large (e.g. in PCOS) an appropriate dosage of

exogenous FSH should be offered to prevent hyper-response and/or OHSS. On the other hand, an inappropriate administration of gonadotropin could lead to a poor recruitment of follicles and cycle cancellation (24). The ideal dosage should be able to exploit women ovarian reserve as much as possible, with an optimal risk/benefit ratio. This was clearly explained by the concept of follicle output rate (FORT) introduced by Genro et al. in 2011 (88). FORT consists in the ratio between preovulatory and baseline antral follicles and is an indirect measurement of antral follicle responsiveness to FSH. Higher values of FORT mean in most cases that an adequate stimulation protocol have been adopted.

It should be stressed that low ovarian reserve in terms of antral follicles does not ameliorate only by increasing the dosage. Several studies showed that increasing FSH dosage in women with poor ovarian reserve in terms of AMH and/or AFC could be meaningless and low cost-effective (89, 90).

Several attempts to develop an algorithm to individualize starting dose were proposed. In simple models, only one parameter was adopted for gonadotropin starting dose selections. Nelson et al. for the first time evaluated AMH-based COS strategies in two IVF centres (36). In this prospective cohort study, 538 women were stratified according to their AMH concentrations prior to COS (low: <5 pmol/L, medium: 5-15 pmol/L and high: >15 pmol/L). High, medium or low doses of FSH were given to women with low, medium and high AMH concentrations, respectively. In one study centre, the choice between the long GnRH agonist protocol vs. a milder GnRH antagonist protocol was also determined based on AMH concentrations. Most patients at the first centre (regardless of AMH level) were treated with a long GnRH agonist protocol, while in the second centre, those with high or low AMH concentrations were treated with a GnRH antagonist protocol. In both centres, low AMH concentrations were associated with a reduced clinical pregnancy rate, but the cohort of patients receiving the antagonist protocol had a median duration of treatment of 10 days, with a cancellation rate of 5%, compared with a duration of 14 days and cancellation rate of 19% for the group treated with long GnRH agonist protocol. As expected, women from both centres with physiological AMH concentrations had similar good outcome with no POR and no incidence of OHSS. Interestingly, the group of patients with high AMH concentrations showed better outcome using the GnRH antagonist compared with the agonist protocol. No patients with high AMH who underwent the GnRH antagonist protocol were hospitalised due to OHSS compared with 13.9% in the high AMH level group who received the GnRH agonist long protocol. In addition, the use of the antagonist protocol did not result in any cases of "total freeze" to avoid OHSS. Furthermore, the improvements in COS safety and tolerability observed with the GnRH antagonist protocol were accompanied by higher clinical pregnancy rates in both low (11.1% vs. 18.7%) and high AMH (40.1% vs. 63.6%) groups (36). Data from this study suggested for the first time that a simple model using AMH may be used as a reliable predictive tool to determine treatment strategies, improving safety, tolerability and pregnancy outcomes.

Another retrospective large trial compared ART outcomes of 346 women using conventional stimulation protocols and 423 women treated under new AMH-tailored protocols (91). In detail, the following protocols were performed in study groups: no treatment or alternative ART in women where AMH was <2.2 pmol/L; 300 IU of human menopausal gonadotropin (hMG) plus GnRH antagonist from day 6 of stimulation in women with AMH between 2.2 pmol/L and 15.6 pmol/L; 200 IU of rFSH or 225IU hMG in long down-regulation protocol + GnRH agonist in women with AMH between 15.7 pmol/L and 28.6 pmol/L; 150 IU hMG plus GnRH antagonist from day 6 of stimulation in women with AMH >28.6 pmol/L (91). Compared with conventional stimulation protocols, AMH-tailored stimulation significantly improved embryo transfer rate and pregnancy rate per cycles. Furthermore, an amelioration in terms of OHSS syndrome and cost of fertility drug were also reported (91).

The main limitation of these two models is that they were based only on AMH parameter, measured by the "old" DSL assay, whose results are 40% lower than the new AMH Gen II assay.

Multivariate models adopting several parameters for the identification of the most appropriate starting dose were also proposed. Popovic-Todorovic et al. proposed a combination of age, AFC, ovarian volume, Doppler ovarian score and smoking status as prestimulation parameters (92). Howles et al. adopted BMI, AFC and day 3 serum FSH (93). More recently, another multivariate model was proposed by La Marca et al. (94). A cohort of women aged between 18-40 years at their IVF first cycle was enrolled with the aim of developing a model for the prediction of ovarian response. Women with irregular cycles, previous ovarian surgery or history of PCOS were excluded. A multivariate regression analysis showed that only age, AMH and serum day 3 FSH reached statistical significance to predict the number of oocytes per unit of exogenous FSH starting dose (94). Later, the same group carried out a retrospective study involving 505 IVF patients with the aim to elaborate a nomogram for the appropriate calculation of FSH starting dose (95). In this case, AFC was adopted instead of AMH as an independent variable. According to authors' findings, a model based on age, AFC and day 3 FSH was able to predict ovarian sensitivity in terms of number of oocytes retrieved (95). Comparing with previous models, the last two could be more easily applied in clinical practice; however, larger trials are needed to recommend their use.

CONCLUSIONS

After decades of IVF techniques, the availability of new markers of ovarian reserve could open a new scenario in reproductive field. While many years ago the

dosage was guided only by anthropometric characteristic (BMI, age), today the availability of more specific markers could significantly ameliorate IVF outcomes leading from a “one size fits all’ to a “patient tailored” approach. In the future, the use of specific genetic tests (such as v-LH and Ser680 variant of FSH-R) might lead to pharmacogenomic approach to COS.

CONFLICTS OF INTEREST

Alessandro Conforti reports consultancy fees from Merck. Carlo Alviggi reports honoraria from Merck.

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