REVIEW ARTICLE

Assessment of Ovarian Reserve in Infertile Patients

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ABSTRACT

Reduced ovarian reserve is a condition characterized by a reduced competence of the ovary to produce oocyte due to advanced age or congenital, medical surgical and idiopathic causes. Age is considered to be the principal factor in determining the reduction of ovarian reserve, especially in woman over 40 years of age, but it's well known that a premature reduction of ovarian reserve can also occur in young patients. Management of patients with diminished ovarian reserve is challenging for fertility experts and frequently the only option to conceive is represented by assisted reproduction technologies. Here we reviewed the aetiology, presentation and diagnosis of reduced ovarian reserve in advanced and young aged women and recent advances in the management of infertility in these women.

Key Words: Reduced ovarian reserve; Diminished ovarian reserve; Premature ovarian failure

Introduction

The reduced ovarian reserve is a condition of reduced ability of the ovary to produce oocytes due to advanced age or congenital, medical, surgical and idiopathic causes. This condition, also known as diminished ovarian reserve (DOR) is often used to characterize women at risk for poor performance with assisted reproductive technologies (ART) due to egg factor[1-4]. The most extreme phenotype of DOR in young age is represented by premature ovarian failure (POF), a disorder characterized by amenorrhoea, hypooestrogenism and high gonadotrophin levels in young patients below 40 years of age. Spontaneous POF affects the 1% of women under 40 years, 0.1% of patients younger than 30 years and 0.01% of patients under the age of 20 years[5,6]. However, with the increasing of cancer cures in children and in young women the incidence of POF is quickly increasing[7,8]. Analyses performed by the Childhood Cancer Survivor Study show that the 6.3% of women who received cure for cancer suffered of acute ovarian failure[9]. In this manuscript we reviewed the aetiology, presentation and diagnosis of reduced ovarian reserve in advanced and young aged women and recent advances in the management of infertility in these women.

Normal Reproductive Aging

The probable theoretical causes of decline in reproductive potential in women beginning at the third decade of life may be classified as: a) diminished ovarian reserve (DOR) and b) diminished uterine receptivity for implantation. The DOR (diminished ovarian reserve), either by decreased quantity and/or quality of the resting follicle pool, might decrease fertility after age of 30 years. There is enough evidence for both situations.

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The primordial follicle count, which is about 20 millions at the 20th week of intrauterine life, starts to decrease with the process of apoptosis[6,7,8]. The primordial follicles left are about 1 million at birth and 300 thousands at puberty. At a mean age of 37-38 years only about 25 thousands of follicles are present in the ovaries. After this age, the disappearance of the follicles accelerates and the curve follows a biphasic pattern[7]. The time interval between the beginning of accelerated follicular disappearance and menopause is constant at about thirteen years[7]. Menstrual cycles become irregular about 6 years before menopause[9]. There is a time period of about 4 years between age 37 when fertility begins to decline and age 41 when fertility practically ends[10]. It is known that the age of menopause in the general population is under 45 in 10% of women and under 40 in 1% of women[11]. Thus, if the time interval between the beginning of accelerated follicular disappearance and menopause is constant and about thirteen years it can be speculated that about 10% of women in the general population will suffer from the clinical consequences of impaired fertility in their thirties due to early ovarian aging. The data from ART cycles with fresh and nondonor oocytes and embryos demonstrate a decrease in embryo implantation, pregnancy and live birth rates per cycle when female partner age exceeds 38[12]. In ICSI cycles of men with obstructive azoospermia, the implantation rate decreases if the female partner age is over 37; this finding also demonstrates the effect of age related decline in oocyte quality on reproductive performance[13].

Data obtained from oocyte donation clearly shows that, if oocytes are donated from young women to older women, both embryo implantation and pregnancy rates are restored to normal levels[14]. These results suggest that the effect of age on fertility is largely a result of qualitative changes within the aging oocytes, rather than senescent changes in the uterus. The high rates of pregnancy wastage in older women also indicate the age-related decrease in oocyte quality[15,16,17]. Detection of high abortion rates in oocyte donation cycles if oocytes are donated from older women demonstrates that the age-related factor responsible for pregnancy wastage is also oocyte quality[18]. An increased frequency of abnormal chromosome arrangements in human oocytes in older women is reported in several studies[19,20]. Preimplantation genetic diagnosis of embryos in women over 38 shows high rates of aneuploidy, another important evidence of a strong association between advanced maternal age and pregnancy wastage[21].

Initial Evaluation of Ovarian Reserve

An important group of patients that has to be taken into consideration for diminished ovarian reserve are infertile women of advanced age (>35). The proportion of older age infertile women is gradually increasing. Other risk factors for diminished ovarian function in infertile patients are family history of early menopause, past chemotherapy or radiotherapy, past pelvic surgery, history of pelvic infection, tubal disease or severe endometriosis, smoking etc. If 10% of patients enter menopause before the age of 45, then the same proportion of women are expected to experience signs of ovarian aging in their early thirties. Thus, it should be reasonable to test all infertile women over 30 for ovarian reserve. Ovarian surgeries of any kind, but particularly for ovarian endometriosis, might be detrimental to primordial follicle pool; thus, patients with a history of ovarian surgery need to be evaluated for ovarian reserve regardless of their age. The underlying cause of subfertility might theoretically be a subtle diminished ovarian reserve. For this reason, it will be reasonable to apply ovarian reserve tests liberally to unexplained infertile couples. The ovarian reserve tests are indicated in patients over 30 years, with a history of surgery for ovarian disorders or severe endometriosis, unexplained infertility, poor response to ovarian stimulation etc.

The effect of diminished ovarian reserve on fertility outcome has largely been evaluated in patients treated with ART. In this group of infertile patients the clinical entities associated with diminished ovarian reserve are poor response to COH, increased need for exogenous gonadotrophin, high cancellation rates, low pregnancy and live birth rates in ART. On the other, hand, data regarding the reproductive outcome of ovulatory women in a

general infertility population with an abnormal ovarian test is insufficient. Hence, the treatment alternatives to increase the chance to have a baby, especially in patients with an abnormal ovarian reserve test and younger than 35 years of age are not yet known.

Ovarian Reserve Tests

Basal follicle stimulating hormone (FSH) level

Basal or cycle day 3 FSH level is an indirect indicator of ovarian reserve. It reflects the negative feedback effects of inhibin-B and estradiol produced by a cohort of follicles at pituitary level. Most of the studies of basal FSH levels are from ART cycles. The cut-off values for basal FSH vary from 10 to 25 IU/l. The value of basal FSH as a test for ovarian reserve in ART was evaluated in a meta-analysis of 21 studies[22]. The results of receiver operating curve (ROC) analysis have shown that the performance of basal FSH in ART cycles to predict poor response was moderate, whereas to predict non-pregnancy was poor. In a systemic review, Broekmans et al.[23] found that the cut off FSH levels of >10U/ L had a specificity of 80-90% and a lower sensitivity of 10-30% for the prediction of poor ovarian response to gonadotrophin in in-vitro fertilization (IVF). The lack of a clear cut-off point with reasonable sensitivity and specificity and inter-cycle variations of FSH measurements also limits the reliability and use of basal FSH in IVF practice. The increase in basal FSH levels is a late indicator of ovarian reserve. Median FSH remained consistently low ($\leqslant 5$ U/L) in women$\leqslant$35 years of age and was 6 U/L in 35- to 40-year-olds[24]. Prediction of over reserve with only basal FSH may lead to an inappropriate strategy in infertile women, and some with a diminished ovarian reserve cannot take advantage of determining the rapidly closing window of opportunity. Although it is known that the prognosis of ART cycles will be highly negative in patients with high basal FSH levels, it is generally accepted that the predictive value of FSH levels below cut-off values are limited to reflect the outcome of ART cycles. A study evaluating the predictive value of FSH with regard to age showed that the ART performance of the patients over 40 but with normal basal FSH levels was worse than the patients below 40 but with an abnormal basal FSH level[25]. That is to say, age reflects oocyte quality whereas basal FSH reflects oocyte number and the outcome of an ART cycle will be better if oocytes can be retrieved despite high basal levels in younger patients. A normal basal FSH level does not negate the effects of chronologic age on oocyte quality, embryo implantation, and pregnancy rates, and expectations should be managed accordingly. There are only a limited number of studies in which ovarian reserve tests were used to predict fertility prognosis in a general infertility population[26,27,28]. In one of these studies, the predictive value of elevated basal FSH levels during the initial sub fertility workup with respect to fecundity has been assessed in a general infertility population[28]. Long-term follow up has shown that the pregnancy rates and time interval to pregnancy were same between patients with either normal or high basal FSH levels. It was concluded that screening for high basal FSH levels was of no additional value in a general infertile population.

Basal Serum Estradiol Levels

Early elevations in serum estradiol reflect the advanced follicular development and early selection of a dominant follicle driven by rising FSH levels. A premature estradiol elevation may suppress the FSH levels, masking elevation that might otherwise reveal a low ovarian reserve. Patients with basal estradiol levels of 80 pg/ml or higher during a cycle before IVF achieved a lower pregnancy rate per initiated cycle (14.8% vs 37.0%) and had a higher cancellation rate (18.5% vs 0.4%), compared with those with estradiol levels below 80 pg/ml. Even if FSH >15 were excluded, elevated basal estradiol levels still correlated with poor ovarian response and higher cancellation rates[29]. As an ovarian test basal estradiol level has little value but may provide additional data in basal FSH interpretation. Adding cycle day 3 estradiol measurement to FSH decreases the incidence of false-negative results based on FSH alone.

Clomiphene Citrate Challenge Test (CCC Test)

The physiological basis of the CCC test is that, in a group of patients with diminished ovarian reserve but normal FSH levels, clomiphene citrate (CC)

induced serum FSH rise cannot be suppressed by decreased inhibin secretion from a decreased primordial follicle pool and elevated levels of FSH are measured after CC administration. The test is considered abnormal if any measurement of FSH either on day 3 or on day 10 after CC administration is higher than 10 IU/l. The predictive value of an abnormal CCC test is extremely high with an overall cumulative pregnancy rate of only 1.3%, which is comparable with the 1.5% cumulative pregnancy rate among women with abnormal day 3 FSH values in ART cycles[30]. Nevertheless, among older, at risk patients, the CCC test also identified 29% of patients with compromised fecundity as compared to a rate of 6% for basal FSH screening alone[30]. The use of the CCC test for screening ovarian reserve in a general infertile population was assessed only in a large series[26]. About 10% of infertile women had an abnormal CCC test result and the fecundity of patients with an abnormal test was extremely decreased.

Basal Serum Inhibin-B Levels

Inhibin-B is a dimeric peptide that is secreted by granulosa cells of preantral and early antral follicles[31]. Therefore it is thought to have some value as an ovarian test. Inhibin- B concentrations decline before a rise in basal FSH levels and thus show the reduction of in ovarian reserve earlier than basal FSH[32,33]. As the level of inhibin-B decreases, ovarian response to gonadotrophins, the number of oocytes retrieve and pregnancy rates decrease[34]. Although there is a correlation between basal Inhibin-B levels and ovarian response, it has low sensitivity (60-90%), specificity (40-80%) and positive predictive value (19-22%) even in low threshold values (40-45 pg/mL)[35]. In various studies investigating the relationship between basal inhibin-B and ART outcomes, it was concluded that inhibin-B level was not a reliable measure of ovarian reserve and had a poor predictive value for pregnancy[36-39].

Anti-Mullerian Hormone (AMH)

The AMH is produced by granulosa cells of preantral and small antral follicles. The secretion begins from the start of primordial follicle growth and continues until the follicles have become capable of responding to FSH, which occurs when the diameter of the follicle reaches 4-6 mm[40]. AMH is not expressed in atretic follicles and theca cells[41]. The gonadotrophin independent expression of AMH results of minimal variation within and between cycles provides advantage over other ovarian reserve markers. Pregnancy, the use of gonadotrophin agonists for ovarian suppression, the day of menstrual cycle does not affect serum levels of AMH[42]. AMH expression is observed as early as the 36th gestational week, serum levels are gradually increased in the first 3-4 years of life and become stable until puberty. As the number and quality of the oocytes diminish throughout the woman's reproductive life, serum concentrations of AMH gradually decrease and fall below detectable levels in the menopause[43]. Median time of menopause can be predicted by using AMH levels more accurately than Inhibin and basal FSH[44]. The number of the residual follicular pool correlates with the number of small antral follicles and AMH levels[45-48]. The first study investigating the relation between AMH levels and ovarian response to gonadotrophin on ART cycles was performed in 2002. From that time on numerous studies have been, performed. In women undergoing ART, low AMH threshold values (0.2-0.7 ng/mL) have 40-97% sensitivity, 78-92% specificity, 22-88% positive predictive value and 97-100% negative predictive value for prediction poor response to stimulation, but do not predict pregnancy[49-52]. Almost all studies revealed that there had been a correlation between AMH levels and retrieved oocyte number and AMH seems to be a better marker than age, basal FSH, estradiol, Inhibin-B in predicting ovarian response to gonadotrophin but, when compared with antral follicle counts (AFC), it has nearly the same capacity to predict ovarian response[53].

In a recent study including 1043 IVF cycles, AMH levels were found to be significantly related with the rate of ongoing pregnancy both in fresh and frozen embryo transfer cycles[54]. In a meta-analysis, a total of 13 studies were analyzed reporting on AMH and 17 on AFC and it was shown that AMH had at least the same level of accuracy and clinical value for the prediction of poor response and non pregnancy as

AFC. Both AMH and AFC have limited accuracy for non pregnancy prediction[55]. Besides retrieved oocyte number, AMH and AFC are also found to be comparable predictors of the number of good quality embryos available for transfer and freezing[56]. However, AMH determination has some advantages over AFC: 1) it does not have to be carried out on a specific day of the cycle because of stability in serum levels throughout the menstrual cycle. 2) There is no need for a skilled ultrasound operator to count ovarian follicles 3) A possible observer bias in ultrasonographer is eliminated. In their study, Silberstein at al.[57] found that the serum AMH levels at the time of hCG administration seem to predict not only ovarian reserve, but also embryo morphology. Some studies in the literature have revealed that there is a correlation between oocyte quality and AMH levels[58-61] but other studies have defended the opposite[39,62].

GnRH Stimulation Test (GAST)

Administration of GnRH agonist on cycle day 2-3 causes an initial surge of FSH, LH and estradiol. The response of estradiol is an indirect indicator of ovarian reserve. If the follicular cohort is small, GnRH agonists may lead to less estradiol increase. In two prospective studies it is shown that the response of estradiol to GnRH agonist stimulation was highly correlated with ovarian response in ART cycles[63,64]. Exogenous FSH ovarian reserve test (EFORT) In the exogenous FSH reserve test, FSH and estradiol, inhibin levels are determined before and 24 hours after administration of 300 IU recombinant FSH on day 3 of the menstrual cycle. Basal FSH and levels and increase in estradiol levels are used to predict ovarian response in ART cycles. In a prospective study investigating the predictive value of EFORT in 52 IVF cycles it was shown that at least 30 pg/mL increases in estradiol levels is a better predictor of ovarian response than basal FSH[65]. In another prospective randomized study performed by Kwee et al.[66] CCCT and EFORT were compared in terms of ovarian response in 110 ART cycle and it was found that the inhibin B increment and estradiol increment in the EFORT are the best predictors of the total number of follicles obtained after maximal ovarian hyper stimulation in an IVF treatment; CCCT, basal FSH and estradiol, age show a much lower performance. EFFORT and GAST are more complex, expensive and time consuming and the predictive value in ovarian response or pregnancy are not so different from conventional markers. It is not advised to use these tests routinely in the evaluation of ovarian reserve[35].

Assessment of Ovarian Reserve by Ultrasonography

Comparison of an indirect assessment of ovarian reserve by sonographic measurement of ovarian volume and antral follicle counts with other ovarian reserve tests in ART cycles and their performance to predict response to COH (Controlled Ovarian Hyperstimulation) and pregnancy rates have recently been reported in many studies. The most important advantage of Ultrasonography is that it can be done in every patient without any additional cost. The sonographic assessment of ovarian reserve is also advantageous in selecting poor responders and choosing appropriate stimulation protocols at the beginning of the cycle.

i. Measurement of Ovarian Volume

The age-related decline in primordial follicle pool is supposed to cause a decrease in ovarian volume. The decrease in ovarian volume is supposed to be more pronounced after the age of 38 till menopause, a time period when the follicular depletion is accelerated. In a study population of women 14 to 45 years of age attending a family planning clinic, no correlation has been detected between age and ovarian volume[67]. In a study with healthy and fertile Chinese women it was found that the ovarian volume was not different throughout the whole reproductive period[68]. In a similar study population, but in the age group of 35 to 50 years, the mean ovarian volume was detected to be similar in three age groups of 35 to 39, 40 to 44 and 45 to 49 years and the correlation of decrease in ovarian volume was evident only in the age group of 45 to 49[69]. Interestingly it was found in the same age groups that ovarian volume was decreased in infertile women compared to age-matched fertile women[70]. Although the ovarian volume was least in unexplained infertile patients, the difference did not

reach significance. Data on the predictive value of ovarian volume measurement on IVF cycles has demonstrated that although a correlation between response to COH (Controlled Ovarian Hyperstimulation) and ovarian volume was present, the predictive value of ovarian volume measurement for pregnancy was poor[71-74]. High cancellation rates have also been reported in women with ovaries measuring less than 3 cm3[71,72].

ii. Antral Follicle Counts (AFC)

The age-related decline in the number of antral follicles less than 10 mm measured by ultrasound has been shown in several studies[68,69,74]. In a study population of fertile women a biphasic pattern has been demonstrated in age related decline in antral follicle counts[75]. A yearly decline of 4,8% before the age of 37 was accelerated thereafter to the rate of 11.7%. However, a monophasic yearly decline of 3,8% has been demonstrated in a fertile population in another study[68]. The correlation of antral follicle counts with poor response in IVF has been several studies[74,76,77]. In a recent study investigating the role of AFC in IVF outcome prediction, it has been shown that antral follicle count was predictive of ovarian response, with a 67% likelihood of poor ovarian response for AFC≤4, also there was a significant linear relationship between AFC, age and live birth which is much more marked for AFC[78]. A study comparing the effectiveness of basal and CC induced inhibin-B and FSH, ovarian volume and antral follicle counts to predict the outcome of IVF cycles, reported that ovarian volume was the best parameter to predict poor ovarian response to COH(Controlled Ovarian Hyperstimulation), whereas age and antral follicle counts were found to be better than the other test with respect to predicting pregnancy success[74]. In conclusion, it can be suggested that antral follicle counts reflect the ovarian reserve better than ovarian volume in infertile patients.

iii. Ovarian Stromal Blood Flow

There is a positive and independent correlation between ovarian stromal peak systolic velocity (PSV) measured by transvaginal pulsed Doppler Ultrasonography both in the early follicular phase and after pituitary suppression[79,80]. Engman et al.[79] showed that ovarian stromal PSV was the most important single independent predictor of ovarian response in patients with a normal basal serum FSH level, compared to age, FSH/ LH ratio, estradiol levels if the the cut-off level for PSV was taken as 10 cm/s. A study using 3D ultrasound reported that ovarian stromal vascularity was associated with a higher number of retrieved oocytes and increased pregnancy rates[81]. Contrary to this Jarvela et al.[82] reported quantification of power Doppler signal in the ovaries after pituitary suppression does not provide any additional information to predict the subsequent response to gonadotrophin stimulation during IVF. In a recent study, early follicular stromal Doppler signals are correlated with ovarian response and basal ovarian reserve parameters, but has no correlation with age or with clinical pregnancy achievement in infertile women undergoing IVF-ET(In Vitro Fertilization-Embryo Transfer) treatment[83]. Further studies are needed to clarify the effect of ovarian stromal blood flow on ART outcomes.

Ovarian Biopsy

Demonstration of primordial follicles depletion in the ovary by ovarian biopsy was studied by several authors. Lass et al.[84] in their investigation attempted to find if there had been correlation between basal estradiol levels, ovarian size and follicular density in 60 infertile women. Computerized image analysis was used to measure the number of follicles per unit volume of ovarian tissue. There was no significant difference between unexplained and tubal infertility patients. They also observed that follicular density diminished significantly with increasing age. A study assessing the accuracy of basal FSH, estradiol, CCCT, GAST in predicting the total number of follicles, which was determined by histological examination of oopherectomy materials in 22 fertile patients older than 35 years, found a positive correlation between only basal estradiol levels and follicle per unit but not with others[85]. The uneven distribution of follicles in the ovary makes a large variation even in the same ovary[86]. When the random follicular distribution and potential risks of procedure are taken into

consideration together, this procedure is not justified on current available data.

Combination of Ovarian Reserve Tests

None of the tests has 100% sensitivity and specificity used for poor ovarian response prediction. In order to increase the prognostic reliability of each test, combining the ovarian tests may be considered. A scoring system using the combination of age, AFC, basal FSH, basal AMH, delta E2 and delta inhibin developed by Muttukrishna et al.[87] predicted the ovarian response more accurately than each of the parameters alone. However, in a meta-analysis investigating the performance of the combinations of ovarian reserve tests to predict ovarian response in IVF, the combination of these tests did not perform better compared with AFC alone. According to this meta-analysis there is no advantage in using multivariate model in poor response prediction[88]. Addition of age, AFC, basal FSH, Inhibin to AMH did not make a significant difference in prognostic reliability of AMH in a recent study[89]. The high level correlation of ovarian reserve tests and the differences of chosen thresholds for each test make analysis difficult. Although ovarian reserve tests reflect oocyte quantity they do not reflect oocyte quality accurately[90]. Age was found better in predicting pregnancy than these tests[90]. Women with low ovarian reserve still have a reasonable chance to achieve pregnancy. The increased rates of spontaneous abortus and aneuploidy in young women with poor ovarian reserve suggest that oocyte quality may also contribute in some unexplained infertile women. Counseling and management of the cycle with the knowledge gained only from the ovarian reserve tests is a matter of debate. In fact, many women whose tests results were lower than the cut-off could have pregnancy after IVF.

Conclusion

Assessment of ovarian reserve should not be neglected in an infertile patient if the age of the patient is above the period when the ovarian reserve is known to be declining. At present, there is no ideal ovarian reserve test reflecting fertility potential of a woman reliably. Controversial results of the studies make it difficult to compare the efficiency of different tests of ovarian reserve. Data obtained from ART cycles are useful to form models for assessing the efficiency of various tests to predict fertility potential. None of the tests of ovarian reserve is ideal to predict pregnancy. A woman with an abnormal ovarian test may conceive either spontaneously or by ART. Although the predictive value (specificity) of an abnormal hormonal parameter (basal or CC induced FSH and Inhibin) to detect diminished ovarian reserve is high, their sensitivities are low. The CC test is relatively more sensitive than basal FSH. Among the ultrasound parameters, an antral follicle count is the most reliable. There are only a limited number of studies in which ovarian reserve tests were used to predict fertility prognosis in a general infertility population. The most reliable tests in these patients seem to be AFC and AMH, according to the existing data. The studies in this group of patients will aid in forming screening strategies for asymptomatic cases of diminished fertility due to early ovarian aging in the general population. AMH has advantages compared with other markers of ovarian reserve tests. It is the earliest marker to change with age and has least inter and intra cycle variability.

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