

Tests for ovarian reserve: reliability and utility

Thaís S. Domingues^a, André M. Rocha^a and Paulo C. Serafini^{a,b}

^aHuntington Medicina Reprodutiva and ^bDisciplina de Ginecologia da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil

Correspondence to Paulo C. Serafini, Huntington Medicina Reprodutiva, Av. República do Líbano, 529 – São Paulo, São Paulo 04501-000, Brazil
Tel: +5511 30596122; fax: +5511 30596100; e-mail: pserafini@huntington.com.br

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Purpose of review

This review discusses ovarian reserve tests for ovulation induction and their application in determining fertility capacity, and their current applications to assess risk of natural ovarian failure and to estimate ovarian function after cancer treatment.

Recent findings

The current arsenal of ovarian reserve tests comprises hormonal markers [basal follicle stimulating hormone, estradiol, inhibin-B, antimüllerian hormone (AMH)] and ultrasonographic markers [ovarian volume, antral follicle counts (AFCs)]. These markers have limitations in terms of which test(s) should be used to reliably predict ovarian reserve with regard to accuracy, invasiveness, cost, convenience, and utility. Several studies have correlated sonographic AFCs with serum AMH levels for predicting the ovarian response to ovulation induction protocols during assisted reproduction treatments.

Summary

Serum AMH levels and AFC are reliable tests for predicting the ovarian response to ovulation induction. However, none of the currently employed tests of ovarian reserve can reliably predict pregnancy after assisted conception. Further, ovarian reserve tests cannot predict the onset of reproductive and hormonal menopause; thus, they should be used with caution for reproductive life-programming counseling. Moreover, there is no evidence to support the use of ovarian reserve tests to estimate the risk of ovarian sufficiency after cancer treatments.

Keywords

antimüllerian hormone, antral follicle count, in-vitro fertilization, ovarian reserve tests, ovulation induction

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Introduction

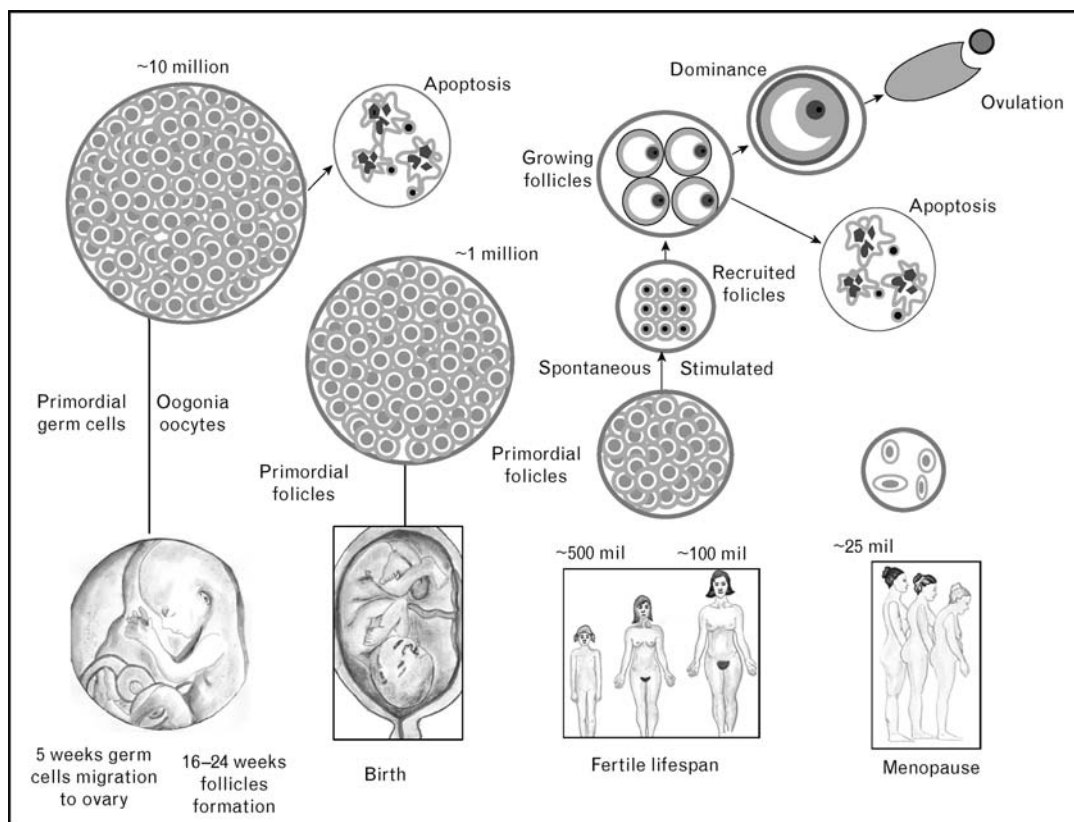
There is a growing demand to properly counsel and treat infertile women with patient-specific ovulation induction strategies for fertility enhancement and as part of the in-vitro fertilization (IVF) treatment [1–5]. The term ‘ovarian reserve’ refers to the remaining endowment of resting and primary ovarian follicles and is used to define the quantity and quality of follicles present in the ovaries at a given time [4,5]. Women who want to become mothers, but whose reproductive future is uncertain for reasons ranging from benign to oncologic causes (e.g. chemotherapy, radiation therapy) to postponement of fertility for social or intentional reasons, require a reliable and predictable evaluation of their ovarian reserve [1–3]. The most suitable markers of ovarian reserve include early follicular phase determination of serum follicle stimulating hormone (FSH), estradiol (E₂), inhibin-B, and non-cyclic dependent estimation of antimüllerian hormone (AMH) levels, and sonographic estimations of ovarian volume and antral follicle count (AFC) [6–16,17*]. Further evidence supports the use of AFC and AMH

as markers of follicle development after ovulation induction [5,11–16,17*]. Despite these recent advances in methods for predicting ovarian reserve, current tests for predicting ovarian reserve function are often limited in terms of accuracy, invasiveness, convenience, and cost. Herein, we discuss ovarian reserve tests and their application in determining fertility capacity, to manage ovulation induction and their current applications to assess risk of natural ovarian failure and to estimate ovarian function after cancer treatment.

The ovarian reserve

The population of nongrowing ovarian follicles established during the fifth month of human fetal life defines the endowed ovarian reserve. At that time, the number of primordial follicles is approximately 10 million; however, the follicle supply changes over time beginning *in utero* when the number decreases to approximately 1.5 million, and diminishing to nearly 500 000 nongrowing follicles at menarche [6–9]. Furthermore, the menarche interval is proportional to the amount of primordial follicles and

Figure 1 Lifetime span of ovarian follicles from ontogenic endowment to senescence



Modified from [18].

their recruitment rate. However, it is estimated that the 500 000 primordial follicles available at the menarche might forecast the number of ovulations [9] (Fig. 1 [18]).

Aging, medical illnesses, and surgeries, among other hazards, exert a negative impact on the size of the ovarian reserve and on oocyte quality, leading to a greater number of women who experience age-related fertility problems [5]. Substantial reduction in the ovarian reserve occurs at the age of 37 years or when it reaches the projected mark of 25 000 primordial follicles [8,10]. Therefore, the rate and speed of ovarian reserve diminishment might be inordinately variable from one person to the next [3]. This variability adds challenges when physicians try to establish general rules for reliably counseling women who are undergoing oncologic treatment or who would like to postpone childbirth.

Overview of ovarian reserve tests

Current ovarian reserve tests include hormonal markers (FSH), estradiol, inhibin-B, and AMH) ultrasonographic markers (AFC and measurement of ovarian volume), and dynamic tests. Here, we will focus on hormonal and ultrasonographic markers. The 'dynamic tests' first intro-

duced in the late 1980s [19] are still in use, most likely because they utilize various ovulation induction protocols (e.g. ovulatory agents such as clomiphene citrate, agonist analogs of gonadotropin releasing hormones, and exogenous gonadotropins). However, Maheshwari *et al.* [20] recently called for the abandonment of dynamic tests, primarily due to a lack of documented evidence of success, and an urgent need to establish a consensus on test performance and to define normality. Therefore, dynamic tests are not discussed herein.

Early follicular phase serum follicle stimulating hormone

The measurement of serum FSH levels at 2 or 3 days after the onset of full menstrual flow has been used as a marker of ovarian reserve since the 1980s, based on its association with reproductive outcome [21]. Advancement of female age has been associated with a slow and steady compensatory elevation in FSH, a decrease in ovarian response, and an increase in conceptual aneuploidy [12]. Persistent elevated basal FSH levels are consistent with diminished ovarian reserve; however, some women experience transient elevated basal FSH levels unrelated to their pool of primordial follicles [22]. Age has been identified as a better predictor of pregnancy

than baseline FSH levels in women undergoing IVF, although age and FSH levels are useful in predicting the quantitative ovarian reserve [23].

Serum FSH levels are measured easily using a relatively inexpensive assay, but FSH lacks a strong association with pregnancy outcomes [5]. Furthermore, the pulsatile and circadian release of FSH in the circulation, together with fluctuations in FSH isoforms, adds to potential errors. Variation in monthly baseline FSH levels may also occur due to a persistence of corpus luteum accompanied by elevated progesterone and low E_2 levels [24]. Overall, low FSH levels during the early follicular phase reflect the normal hypothalamic-pituitary-ovarian-uterine axis following adequate previous cycle luteolysis. A recent systematic review evaluating basal FSH levels in eumenorrheic women found that FSH assays were accurate enough to predict a poor response and nonpregnancy, but only at very high threshold levels [5]. Women over 40 years of age with repetitive elevations in day 3 (D3) FSH levels are most likely to have a compromised ovarian response, as demonstrated by low oocyte yield, high oocyte retrieval cancellation rates, impaired embryo quality, and low implantation and pregnancy rates [12].

Early follicular phase serum estradiol: E_2

Estradiol is a steroid hormone produced by granulosa cells of the ovarian follicles [12]. Estradiol levels are commonly assessed during the early follicular phase of the menstrual cycle by infertility specialists as part of the hormonal profiling component of the patient work-up, because it is a simple, inexpensive, and effective screening tool [25].

Despite advances in estradiol assessment, a systematic review of the ovarian reserve test found that basal E_2 levels had a low predictive value for IVF outcomes [5]. However, extremely high D3 E_2 levels (above 75 pg/ml) were associated with a poor response to IVF and low pregnancy rates [26–28]. Therefore, although D3 serum E_2 levels may not be useful for ovarian reserve evaluation, they may help indicate whether an ovulation induction should be cancelled; for this reason, they should be included in the female screening.

Early follicular phase serum inhibin-B

Inhibin-B is one of the β subunits of the dimeric peptide inhibin [29]. It is produced by granulosa cells of preantral and early antral follicles [13], and its levels vary during the menstrual cycle [30]. Serum concentrations of inhibin-B decrease with age and during premature ovarian failure; however, inhibin-B has largely been considered a marker of ovarian activity, rather than ovarian reserve [14]. Determination of inhibin-B levels does not predict the onset of ovarian failure, and it is less predictive of menopause than other markers (e.g. AMH levels) [31].

Serum baseline levels of inhibin-B below 45 pg/ml were associated with poor ovarian response to gonadotropins, high IVF cycle cancellation rates, low numbers of retrieved oocytes, and reduced pregnancy rates [32]. Finally, inhibin-B levels do not decline gradually with age, and it is a fairly late marker of a reduced follicle pool [33,34].

Serum concentration of antimullerian hormone

AMH is a dimeric glycoprotein of the transforming growth factor- β superfamily involved in cell growth and differentiation [35]. AMH is secreted by granulosa cells of secondary, preantral, and early antral follicles up to 6 mm in diameter [36], and its secretion ceases as follicles grow into dominance [37]. Serum levels of AMH are barely detectable at birth, reach their highest levels after puberty, decrease progressively thereafter with age, and become undetectable at menopause [31,38,39].

The increased sensitivity of follicular cells to FSH in the absence of AMH, demonstrated both *in vitro* and *in vivo*, supports the hypothesis that AMH acts as a decisive factor in permitting FSH-dependent growth of ovarian follicles [40,41]. Furthermore, it appears that AMH regulates follicular recruitment by inhibiting the initiation of primordial follicle growth and preventing the depletion of the primordial follicle pool [36]. AMH secretion is ovarian cycle-independent [39]. Whereas some studies have reported a late follicular phase peak in AMH levels during the normal menstrual cycle [42], others using larger sample sizes have failed to identify any significant changes in AMH levels throughout the menstrual cycle [43,44**].

AMH levels appear to be the best hormonal marker for ovarian reserve showing low intercycle variation while correlating strongly with the number of antral follicles and follicle depletion at an earlier stage, relative to other routinely used markers [40,45–47].

Ultrasonographic markers

The greatest advantage of ultrasonographic markers of ovarian reserve is their noninvasiveness. AFC and measurements of total ovarian volume are considered accurate tests of ovarian reserve [15,48**,49]. Both markers exhibit an age-related decline, although it may be more gradual for the AFC than for the ovarian volume [16]. Therefore, AFC and the measurement of ovarian volume are preferred indicators of reproductive potential.

Despite a relatively limited number of studies, AFC may be more effective than ovarian volume for predicting poor ovarian response after ovulation induction [50]. As AFC can be performed during routine early follicular ultrasound exams, its inclusion as a marker of ovarian reserve

is advisable for all women considering ovulation induction for fertility enhancement, IVF, postponing fertility, and before undergoing oncologic treatments.

Applications of ovarian reserve tests

Here, we address the current applications of ovarian reserve tests for evaluating follicle pool, fertility capacity to manage individualized patient ovulation induction, and to predict natural ovarian failure and fertility capacity after cancer treatment.

Are ovarian reserve tests reliable for reproductive life programming?

Reproductive life stages are controlled by several aspects including individual, genetic, ethnic, environmental, life-style factors among others [51,52]. Changes either on one or in several of these characteristic factors impair child-birth counseling to women who wish to postpone child-bearing until an unforeseen time during menopause, thereby increasing their risk of natural sterility [1,2]. Specialists often rely on basal serum FSH levels as a marker of ovarian reserve. However, FSH levels have not been shown to predict fertility, since the increase in FSH levels occurs late in the transition to menopause. Serial cycle measurements of FSH levels might be a more useful short-term predictor of ovarian age. Inhibin-B is also a limited predictor of the decline in fertility and menopause [31,33].

Serum AMH levels more reliably predict ovarian reserve as they reflect the population of preantral follicles and serum AMH levels do not vary significantly between menstrual cycles [31,39,44^{••}]. Furthermore, AMH levels exhibit an age-dependent decrease beginning after 30 years of age and those women presenting with AMH levels below 0.086 $\mu\text{g/l}$ (Diagnostic Systems Laboratories) are most likely experiencing menopause [53]. Reliable methods for measuring the rate of AMH decrease are needed. However, mathematical modeling of ovarian reserve based on histological data suggests that age alone is responsible for 81% of the variance in primordial follicle size [9].

Are ovarian reserve tests reliable for evaluating fertility in women undergoing gonadotoxic treatments?

The number of cancer survivors has increased in recent decades. Unfortunately, many patients undergoing oncologic treatments experience permanent gonadal damage from chemo-radiotherapy. Thus, fertility preservation is an emerging issue for cancer patients, oncologists, and gynecologists. However, it is often difficult to determine which patients will become sterile following cancer treatment. Therefore, female cancer patients should be enrolled in a fertility preservation program for a more comprehensive counseling [54–59]. AMH has been used

to estimate ovarian damage; however, ovarian reserve tests cannot determine individual susceptibility to cancer treatment [54–59]. Therefore, oncologists should work with infertility specialists to provide these women with future fertility options, including oocyte, embryo, and ovarian tissue cryopreservation [60].

Are ovarian reserve tests reliable for the management of ovulation induction?

Knowledge of a patient's ovarian reserve aids reproductive endocrinologists in establishing an individualized ovulation induction and reducing the likelihood of ovarian hyperstimulation syndrome (OHSS) while providing a cost-effective protocol.

AFC and ultrasonographic determination of the volume of both ovaries are noninvasive and more accurate than hormonal ovarian reserve tests [15,17[•]], despite a limited number of studies [50]. AFC has also been evaluated using transvaginal 3D ultrasound during the first IVF ovulation induction [17[•]]. Although universal AFC values have not been established, recent studies have shown good correlations between AFC values and AMH levels [44^{••}], other common serum markers [39] and between women with normal and poor responses to ovulation induction. Muttukrishna *et al.* [61] showed that AFC can identify 89% of poor responders before ovulation induction, despite a reduced specificity of 39%. Furthermore, Gibreel *et al.* [50] recently reported that AFC was useful for predicting cycle cancellation with 66.7% sensitivity and 94.7% specificity.

Numerous studies have shown that the frequency of a normal response to ovulation induction is significantly higher in patients with larger AFC values [38,62^{••}]. AFC and serum AMH levels are correlated, and they might predict ovarian response to gonadotropin stimulation during IVF or intracytoplasmic sperm injection treatment [48^{••},63]. Furthermore, several studies have shown that AFC and AMH markers are equivalent in terms of their high accuracy [64].

AMH has been reported to serve as a marker of the ovarian response [63], and a strong correlation between basal serum AMH levels and the number of retrieved oocytes has been reported recently [49,65,66]. Therefore, AMH levels might be useful for predicting the risk of OHSS [67]. However, AMH levels do not appear to correlate with pregnancy following IVF treatment [68].

Conclusion

Ovarian reserve tests provide a snapshot of the pool of primordial follicles and are useful tools for predicting the ovulation induction response. Several methodologies have been studied for estimating the primordial follicle

cohort, and AMH levels and AFC are the most reliable. Furthermore, assessment of AMH levels and AFC are reliable for forecasting the ovarian response after ovulation induction and for reducing the risk of OHSS. However, there is a lack of evidence to support the use of ovarian reserve markers to counsel reproductive life programming or to estimate the risk of infertility after oncologic treatments.

Acknowledgement

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- of special interest
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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 354).

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