Ovarian Reserve Tests in Pre-conceptual Healthy Women

Queenie Ho-Yan WONG MBBS, MRCOG, FHKCOG, FHKAM (Obstetrics and Gynaecology)
Department of Obstetrics and Gynaecology, Princess Margaret Hospital, Laichikok, Hong Kong

Women's fertility declines with age. The worldwide trend of delaying childbirth has led to more women facing fertility problems at the time they wish to conceive. There are various ultrasound and biochemical markers available for ovarian reserve testing. The common markers are antral follicle count, follicle-stimulating hormone, and anti-Mullerian hormone. Tests for these markers have their own strengths and limitations. Clinical application of ovarian reserve tests in predicting fertility in pre-conceptual healthy women remains controversial. Clinicians should be cautious when applying and interpreting these ovarian reserve tests for fertility prediction in pre-conceptual women.

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Introduction

Worldwide, women are delaying childbirth until a more advanced age because of the availability of effective contraception and desire for higher educational achievement and professional status[1,2]. In Hong Kong, the median age of women at first childbirth has increased from 25.1 years in 1981 to 31.4 years in 2015[3]. More women face reduced fertility at the time they plan to conceive, as their fertility declines with age[4]. The rate of fertility decline differs in different women, as does the process of menopause. Understanding the fertility potential in pre-conceptual women may affect their family-planning decision so as to avoid under- or over-treatment of their infertility. This article aims to review the evidence of age and ovarian reserve testing for fertility prediction in healthy pre-conceptual women.

Age and Fecundability

Increasing age is associated with decreasing fecundability. Fecundability peaks between the late 20s to early 30s, with a steady decline thereafter[5,6]. This age-related decline in fecundability is more pronounced in nulliparous women[7,8].

In a prospective fecundability study involving 782 European couples who practised natural family planning, the pregnancy rate decreased and the time to pregnancy lengthened as the woman's age increased[9]. In a prospective cohort study of fecundability in 2962 couples trying to conceive without a history of infertility, increasing female age was associated with an approximately linear decline in fecundability, with the peak at the age of 21 to 24 years; the association was stronger among nulligravid women[10].

Ovarian Reserve Markers

Ovarian reserve indicates the number of oocytes that remains in the ovaries[11], and is widely applied to predict fertility. Nonetheless, there is controversy about whether it reflects oocyte quality and helps to predict pregnancy in both natural conception and in-vitro fertilisation (IVF). Ovarian reserve markers can be classified as imaging (ultrasound) markers and biochemical markers. Ultrasound markers include antral follicle count (AFC) and ovarian volume. Biochemical markers can be subdivided into those studied in provocative tests (such as clomiphene citrate challenge test) and basal markers that include follicle-stimulating hormone (FSH), oestradiol, inhibin B, and anti-Mullerian hormone (AMH). Among them, AFC, FSH, and AMH are more commonly tested.

Antral Follicle Count

AFC is measured by transvaginal ultrasonography on day 2 to 4 of the menstrual cycle and indicates the number of follicles between 2 and 10 mm in the longitudinal and transverse planes in both ovaries. It is a reflection of the primordial follicle count. It is easy to perform and has good inter-cycle variability and inter- and intra-observer reliability if performed by experienced clinicians[12]. Nonetheless, it is limited by intra-cycle variation and needs to be measured during the early follicular phase in women with a regular cycle. Accuracy depends on the resolution of the ultrasound machine, experience of the operator, and

Correspondence to: Dr Queenie Ho-Yan Wong
Email: queeniehoyan@gmail.com

body build of the woman[13]. Both intra-cycle and inter-cycle variability increase in obese women[14].

The role of AFC as an ovarian reserve marker has been mainly studied in women undergoing IVF. AFC predicts ovarian response to gonadotrophin stimulation[15] but not pregnancy[13,16]. A low AFC count is associated with a poor response to controlled ovarian stimulation. When the AFC count is 3 to 4, the sensitivity is low (9%-73%) but the specificity is high (73%-97%) in predicting poor ovarian response[13].

Evidence for the association of AFC with natural conception is limited. The AFC is lower in infertile women than in fertile women aged 25 to 40 years[17]. Nonetheless, the absolute difference between infertile and fertile women aged 31 to 35 years is only 4. AFC was not shown to predict the time to pregnancy in 102 pregnancy planners after controlling for the women's age (hazard ratio=1.03, 95% confidence interval=1.00-1.07, p=0.08)[18].

Follicule-stimulating Hormone

FSH is produced by the anterior pituitary, which regulates the recruitment and growth of ovarian follicles from the antral stage to graafian follicles. Oestradiol is produced by the growing follicle, whereas inhibin B is produced by granulosa cells on small and large antral follicles. Both oestradiol and inhibin B inhibit pituitary secretion of FSH. FSH increases when the follicular pool is depleted owing to decreased negative feedback by inhibin B and oestradiol[19]. FSH is an indirect measure of the antral follicle pool and depends on an intact hypothalamic-pituitary ovarian axis. Its use therefore needs to be coupled with serum oestradiol measurement to avoid false negative results. In addition, FSH has significant intra-cycle and inter-cycle variability[20,21]. It should be measured in the early follicular phase, on days 2 to 5 of the menstrual cycle.

Although a high FSH level indicates decreased oocyte number, it cannot be translated into decreased fecundibility. There is no study to compare FSH levels between infertile and fertile women. In a retrospective cohort study of women with an elevated FSH level of >12 IU/L, younger women had a more favourable natural fertility prognosis than their older counterparts in terms of clinical pregnancy rate[22]. This finding suggests that FSH can reflect the quantity of ovarian reserve but not necessarily the quality of the oocytes.

Anti-Mullerian Hormone

AMH is a glycoprotein produced by granulosa cells of secondary, prenatal, and small antral follicles[23]. As it is produced by follicles that are not gonadotrophin sensitive, the AMH level has minimal fluctuation throughout the menstrual cycle[24] and little inter-cycle variation[25]. High AMH is associated with high AFC and a high number of resting primodial follicles. Its level declines with age[26]; it peaks at 25 to 30 years followed by a decline until it becomes undetectable prior to menopause[27,28].

There are different AMH assays available: AMH Gen II ELISA (Beckman Coulter Diagnostics, CA, US), Ultra-Sensitive AMH/MIS ELISA kit (Ansh Labs, TX, US), the automated Access AMH assay (Beckman-Coulter Diagnostics, CA, US), and the Elecsys AMH Immunoassay (Roche Diagnostics International, IN, US). There is a lack of correlation between different assays, making interpretation of AMH results and comparison of results from various studies difficult. AMH values measured by the Ansh Labs assay are significantly higher, and by the Roche assay are significantly lower ($p<0.05$), compared with the Gen II and Beckman Coulter automated assays[29]. In addition, the pre-assayed storage conditions of the serum affect AMH assay results[29-31]. There is no universally accepted diagnostic AMH level for decreased ovarian reserve. Levels can be affected by a number of factors (Table)[19], and the clinician should be aware of these when interpreting AMH levels.

The role of AMH in predicting natural fecundability

Table. Factors affecting serum Anti-Mullerian hormone (AMH) levels[19]

Factors
Factors increasing serum AMH level
Caucasian (higher than Chinese and Black)
Parity
Polycystic ovarian syndrome
Granulosa cell tumour
Factors decreasing serum AMH level
Smoking
Systemic illness
Breast cancer gene-1 carrier
Fragile X mental retardation 1 premutation
Ovarian suppression (oral contraceptive pills, gonadotrophin releasing-hormone agonist)
Pregnancy
Endometriosis
History of ovarian surgery
History of chemotherapy

is inconclusive; more data suggest that it does not predict short-term natural fertility. In a cross-sectional study of ovarian reserve markers in 277 women with unexplained infertility and 226 reproductive healthy controls, the two groups were comparable in terms of AFC and AMH levels[32]. AFC, AMH, and FSH do not affect the time to pregnancy in women without a history of infertility[18]. In women with a history of one or two miscarriages, AMH is not associated with fecundability in natural conception[33].

In IVF, AMH has been shown to predict ovarian response. An AMH level of 0.1-1.66 ng/ml has a sensitivity of 44%-97% and specificity of 41%-100% in predicting poor response, whereas an AMH level of 3.36-5.0 ng/ml has a sensitivity of 53%-90.5% and specificity of 60%-94.9% in predicting ovarian hyperstimulation[19].

There is some evidence that AMH can help predict the age of menopause. Its prediction is more accurate in women of late reproductive age, and the precision range in younger age women (21-46 years) is broad[34,35].

Ovarian Reserve Testing in Pre-conceptual Women

Although ovarian reserve markers are indicators of oocyte quantity, there is controversy about whether ovarian reserve tests reflect oocyte quality. Should these tests be performed in general reproductive-age women without any history or risk factor of infertility in the era of delaying childbirth? It is important to educate women on the issue; women are often unaware of the age-related decline in fertility and may overestimate the success of IVF[36-38].

Opponents of ovarian reserve screening argue that there is no solid evidence that a decreased ovarian reserve has any implication for immediate fertility potential. Low ovarian reserve can create unnecessary anxiety for women[39] and potentially lead to adverse consequences such as premature termination of education or a career or seeking parenthood outside of a stable relationship. Conversely, a result of satisfactory ovarian reserve can give false reassurance to a woman who may then delay her pregnancy planning. In addition, age affects the fertility potential on top of ovarian reserve.

Proponents of ovarian reserve screening argue that a proportion of women with low ovarian reserve will require IVF. Current evidence only shows that fecundability is not affected in the short term (6 to 12 months)[18,40-42]. A low AMH level and AFC are associated with decreased ovarian response to gonadotrophin stimulation and thus adversely affect IVF outcome. Women with low AMH levels for their age have an earlier menopause[43-45]. This information is useful for both women and clinicians. Early initiation of fertility treatment can be planned if natural conception does not occur. Personalised risk assessment may facilitate a more informed decision; 80% of women will advance their fertility planning if they know they have a low ovarian reserve[46,47]. Anticipation of oocyte exhaustion does not influence a woman's future relationship and life-choices[48], although preventive oocyte banking can provide psychological reassurance.

Conclusion

There are limited studies on the use of ovarian reserve markers to predict fecundability in generally healthy pre-conceptual women. Limitations of ovarian reserve makers should be explained to women before performing these tests. Clinicians should be prepared to provide an evidence-based explanation of the results and their clinical application. Age remains the most reliable predictor of fecundibility in healthy pre-conceptual women.

Declaration

The author has disclosed no conflicts of interest.

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