Tests for ovarian reserve: reliability and utility

Thaís S. Domingues[a], André M. Rocha[a] and Paulo C. Serafini[a,b]

[a]Huntington Medicina Reprodutiva and [b]Disciplina 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

Current Opinion in Obstetrics and Gynecology
2010, 22:271–276

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, antimullerian 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

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

Curr Opin Obstet Gynecol 22:271–276

1040-872X

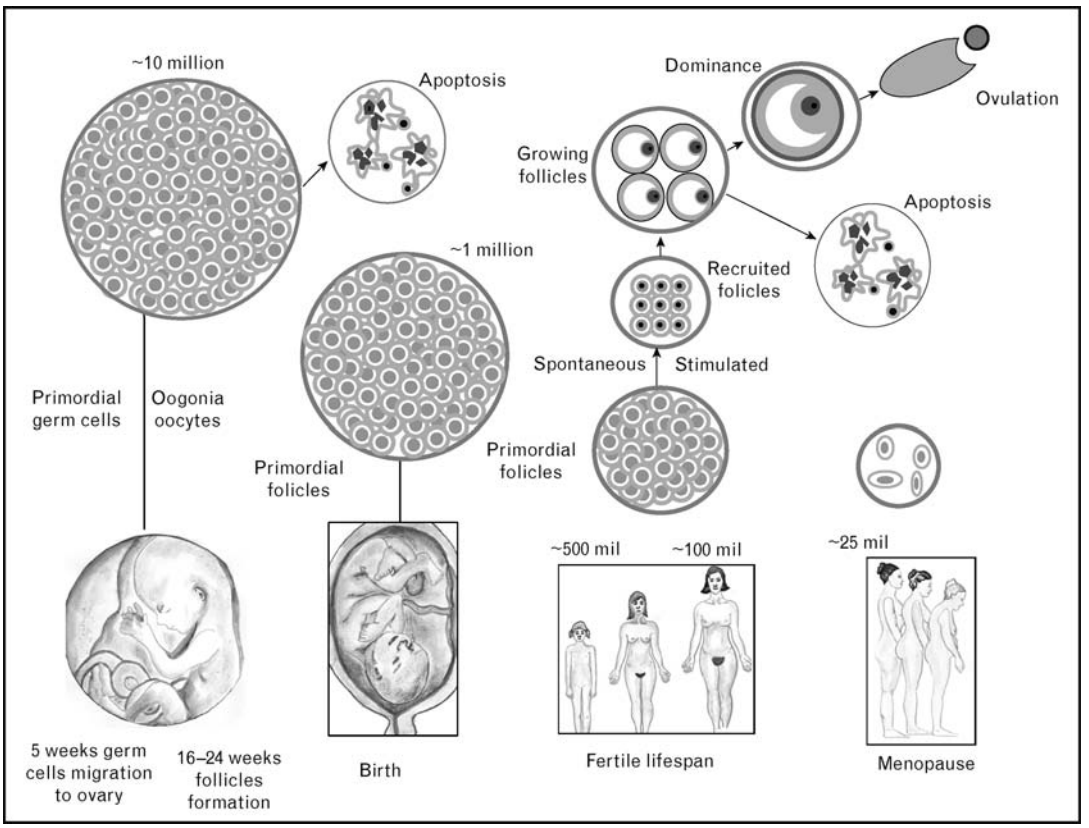
Introduction

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 (E_2), inhibin-B, and non-cyclic dependent estimation of antimullerian 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 introduced 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, lifestyle factors among others [51,52]. Changes either on one or in several of these characteristic factors impair childbirth counseling to women who wish to postpone childbearing until an unforeseen time during menacme, 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 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 μg/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

The authors would like to thank Claudia Ricci for the composition of Fig. 1.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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

1 Leridon H. Demographic effects of the introduction of steroid contraception in developed countries. Hum Reprod Update 2006; 12:603–616.

2 Leridon H, Slama R. The impact of a decline in fecundity and of pregnancy postponement on final number of children and demand for assisted reproduction technology. Hum Reprod 2008; 23:1312–1319.

3 te Velde ER, Pearson PL. The variability of female reproductive ageing. Hum Reprod Update 2002; 8:141–154.

4 Gougeon A. Regulation of ovarian follicular development in primates: facts and hypotheses. Endocr Rev 1996; 17:121–155.

5 Broekmans FJ, Kwee J, Hendriks DJ, *et al.* A systematic review of tests predicting ovarian reserve and IVF outcome. Hum Reprod Update 2006; 12:685–718.

6 Baker TG. A quantitative and cytological study of germ cells in human ovaries. Proc R Soc Lond B Biol Sci 1963; 158:417–433.

7 Faddy MJ, Gosden RG, Gougeon A, *et al.* Accelerated disappearance of ovarian follicles in mid-life: implications for forecasting menopause. Hum Reprod 1992; 7:1342–1346.

8 Faddy MJ, Gosden RG. A model conforming the decline in follicle numbers to the age of menopause in women. Hum Reprod 1996; 11:1484–1486.

9 Wallace WH, Kelsey TW. Human ovarian reserve from conception to the menopause. PLoS One 2010; 5:e8772.

10 Faddy MJ. Follicle dynamics during ovarian ageing. Mol Cell Endocrinol 2000; 163:43–48.

11 de Carvalho BR, Rosa e Silva AC, Rosa e Silva JC, *et al.* Ovarian reserve evaluation: state of the art. J Assist Reprod Genet 2008; 25:311–322.

12 Sills ES, Alper MM, Walsh AP. Ovarian reserve screening in infertility: practical applications and theoretical directions for research. Eur J Obstet Gynecol Reprod Biol 2009; 146:30–36.

13 Rosencrantz MA, Wachs DS, Coffler MS, *et al.* Comparison of inhibin B and estradiol responses to intravenous FSH in women with polycystic ovary syndrome and normal women. Hum Reprod 2010; 25:198–203.

14 Knauff EA, Eijkemans MJ, Lambalk CB, *et al.* Anti-Mullerian hormone, inhibin B, and antral follicle count in young women with ovarian failure. J Clin Endocrinol Metab 2009; 94:786–792.

15 Kwee J, Elting ME, Schats R, *et al.* Ovarian volume and antral follicle count for the prediction of low and hyper responders with in vitro fertilization. Reprod Biol Endocrinol 2007; 5:9.

16 Hendriks DJ, Kwee J, Mol BW, *et al.* Ultrasonography as a tool for the prediction of outcome in IVF patients: a comparative meta-analysis of ovarian volume and antral follicle count. Fertil Steril 2007; 87:764–775.

17 • Jayaprakasan K, Campbell B, Hopkisson J, *et al.* A prospective, comparative analysis of anti-Mullerian hormone, inhibin-B, and three-dimensional ultrasound determinants of ovarian reserve in the prediction of poor response to controlled ovarian stimulation. Fertil Steril 2008; 93:855–864.
This is the first study that related less intercycle variation of 3D AFC, compared with other 3D ultrasonographic markers or FSH.

18 Johnston RJ, Wallace WH. Normal ovarian function and assessment of ovarian reserve in the survivor of childhood cancer. Pediatr Blood Cancer 2009; 53:296–302.

19 Navot D, Rosenwaks Z, Margalioth EJ. Prognostic assessment of female fecundity. Lancet 1987; 2:645–647.

20 Maheshwari A, Gibreel A, Bhattacharya S, *et al.* Dynamic tests of ovarian reserve: a systematic review of diagnostic accuracy. Reprod Biomed Online 2009; 18:717–734.

21 Gupta S, Sharma D, Surti N, *et al.* Ovarian reserve testing: systematic review of the literature. Arch Med Sci 2009; 5:S143–S150.

22 de Koning CH, McDonnell J, Themmen AP, *et al.* The endocrine and follicular growth dynamics throughout the menstrual cycle in women with consistently or variably elevated early follicular phase FSH compared with controls. Hum Reprod 2008; 23:1416–1423.

23 Chuang CC, Chen CD, Chao KH, *et al.* Age is a better predictor of pregnancy potential than basal follicle-stimulating hormone levels in women undergoing in vitro fertilization. Fertil Steril 2003; 79:63–68.

24 Gomes CM, Serafini PC, Motta EL, *et al.* Administration of a pharmacophysiologic dose of recombinant human chorionic gonadotropin at menses promotes corpus luteum rescue. Int J Gynaecol Obstet 2010; 108:158–159.

25 Smotrich DB, Widra EA, Gindoff PR, *et al.* Prognostic value of day 3 estradiol on in vitro fertilization outcome. Fertil Steril 1995; 64:1136–1140.

26 Roberts JE, Spandorfer S, Fasouliotis SJ, *et al.* Taking a basal follicle-stimulating hormone history is essential before initiating in vitro fertilization. Fertil Steril 2005; 83:37–41.

27 Levy MJ, Smotrich DB, Widra EA, *et al.* The predictive value of serum progesterone and 17-OH progesterone levels on in vitro fertilization outcome. J Assist Reprod Genet 1995; 12:161–166.

28 Buyalos RP, Daneshmand S, Brzechffa PR. Basal estradiol and follicle-stimulating hormone predict fecundity in women of advanced reproductive age undergoing ovulation induction therapy. Fertil Steril 1997; 68:272–277.

29 Vale W, Rivier C, Hsueh A, *et al.* Chemical and biological characterization of the inhibin family of protein hormones. Recent Prog Horm Res 1988; 44:1–34.

30 Sowers M, McConnell D, Gast K, *et al.* Anti-Mullerian hormone and inhibin B variability during normal menstrual cycles. Fertil Steril (in press). doi: 10.1016/j.fertnstert.2009.07.1674.

31 Sowers MR, Eyvazzadeh AD, McConnell D, *et al.* Antimullerian hormone and inhibin B in the definition of ovarian aging and the menopause transition. J Clin Endocrinol Metab 2008; 93:3478–3483.

32 Seifer DB, Lambert-Messerlian G, Hogan JW, *et al.* Day 3 serum inhibin-B is predictive of assisted reproductive technologies outcome. Fertil Steril 1997; 67:110–114.

33 van Rooij IA, Broekmans FJ, Scheffer GJ, *et al.* Serum antimullerian hormone levels best reflect the reproductive decline with age in normal women with proven fertility: a longitudinal study. Fertil Steril 2005; 83:979–987.

34 Burger HG, Hale GE, Robertson DM, *et al.* A review of hormonal changes during the menopausal transition: focus on findings from the Melbourne Women's Midlife Health Project. Hum Reprod Update 2007; 13:559–565.

35 Penarrubia J, Fabregues F, Manau D, *et al.* Basal and stimulation day 5 anti-Mullerian hormone serum concentrations as predictors of ovarian response and pregnancy in assisted reproductive technology cycles stimulated with gonadotropin-releasing hormone agonist: gonadotropin treatment. Hum Reprod 2005; 20:915–922.

36 Weenen C, Laven JS, Von Bergh AR, *et al.* Anti-Mullerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. Mol Hum Reprod 2004; 10:77–83.

37 Broekmans FJ, Visser JA, Laven JS, *et al.* Anti-Mullerian hormone and ovarian dysfunction. Trends Endocrinol Metab 2008; 19:340–347.

38 Gnoth C, Schuring AN, Friol K, *et al.* Relevance of anti-Mullerian hormone measurement in a routine IVF program. Hum Reprod 2008; 23:1359–1365.

39 Elgindy EA, El-Haieg DO, El-Sebaey A. Anti-Mullerian hormone: correlation of early follicular, ovulatory and midluteal levels with ovarian response and cycle outcome in intracytoplasmic sperm injection patients. Fertil Steril 2008; 89:1670–1676.

40 Visser J. Role of anti-Mullerian hormone in follicle recruitment and maturation. J Gynecol Obstet Biol Reprod (Paris) 2006; 35:2S30–32S34.

41 Themmen AP. Anti-Mullerian hormone: its role in follicular growth initiation and survival and as an ovarian reserve marker. J Natl Cancer Inst Monogr 2005; 18–21.

42 Wunder DM, Bersinger NA, Yared M, *et al.* Statistically significant changes of antimullerian hormone and inhibin levels during the physiologic menstrual cycle in reproductive age women. Fertil Steril 2008; 89:927–933.

43 Tsepelidis S, Devreker F, Demeestere I, *et al.* Stable serum levels of anti-Mullerian hormone during the menstrual cycle: a prospective study in normo-ovulatory women. Hum Reprod 2007; 22:1837–1840.

44 •• van Disseldorp J, Lambalk CB, Kwee J, *et al.* Comparison of inter- and intra-cycle variability of anti-Mullerian hormone and antral follicle counts. Hum Reprod 2010; 25:221–227.
This is the first strong study, with a good number of women undergoing IVF treatment, that analyzed and compared intracycle and intercycle variation between AMH and AFC.

45 La Marca A, Sighinolfi G, Radi D, *et al.* Anti-Mullerian hormone (AMH) as a predictive marker in assisted reproductive technology (ART). Hum Reprod Update 2009; 16:113–130.

46 La Marca A, Giulini S, Tirelli A, *et al.* Anti-Mullerian hormone measurement on any day of the menstrual cycle strongly predicts ovarian response in assisted reproductive technology. Hum Reprod 2007; 22:766–771.

47 La Marca A, Stabile G, Artenisio AC, *et al.* Serum anti-Mullerian hormone throughout the human menstrual cycle. Hum Reprod 2006; 21:3103–3107.

48 •• Jayaprakasan K, Deb S, Batcha M, *et al.* The cohort of antral follicles measuring 2-6 mm reflects the quantitative status of ovarian reserve as assessed by serum levels of anti-Mullerian hormone and response to controlled ovarian stimulation. Fertil Steril 2009. doi: 10.1016/j.fertnstert.2009.10.1022.
This prospective study was the first to compare follicle size with AMH levels according to their ability in predicting ovarian response after stimulation in IVF cycles of 113 infertile women.

49 Jayaprakasan K, Campbell B, Hopkisson J, *et al.* A prospective, comparative analysis of anti-Mullerian hormone, inhibin-B, and three-dimensional ultrasound determinants of ovarian reserve in the prediction of poor response to controlled ovarian stimulation. Fertil Steril 2010; 93:855–864.

50 Gibreel A, Maheshwari A, Bhattacharya S, *et al.* Ultrasound tests of ovarian reserve; a systematic review of accuracy in predicting fertility outcomes. Hum Fertil (Camb) 2009; 12:95–106.

51 Parazzini F. Determinants of age at menopause in women attending menopause clinics in Italy. Maturitas 2007; 56:280–287.

52 Plante BJ, Cooper GS, Baird DD, *et al.* The impact of smoking on antimullerian hormone levels in women aged 38 to 50 years. Menopause 2010; 17:571–576.

53 van Disseldorp J, Faddy MJ, Themmen AP, *et al.* Relationship of serum antimullerian hormone concentration to age at menopause. J Clin Endocrinol Metab 2008; 93:2129–2134.

54 Partridge AH, Ruddy KJ, Gelber S, *et al.* Ovarian reserve in women who remain premenopausal after chemotherapy for early stage breast cancer. Fertil Steril 2009. doi: 10.1016/j.fertnstert.2009.03.045.

55 Lie Fong S, Laven JS, Hakvoort-Cammel FG, *et al.* Assessment of ovarian reserve in adult childhood cancer survivors using anti-Mullerian hormone. Hum Reprod 2009; 24:982–990.

56 Williams D, Crofton PM, Levitt G. Does ifosfamide affect gonadal function? Pediatr Blood Cancer 2008; 50:347–351.

57 Lie Fong S, Lugtenburg PJ, Schipper I, *et al.* Antimullerian hormone as a marker of ovarian function in women after chemotherapy and radiotherapy for haematological malignancies. Hum Reprod 2008; 23:674–678.

58 Oktem O, Oktay K. Quantitative assessment of the impact of chemotherapy on ovarian follicle reserve and stromal function. Cancer 2007; 110:2222–2229.

59 Su HI, Sammel MD, Green J, *et al.* Antimullerian hormone and inhibin B are hormone measures of ovarian function in late reproductive-aged breast cancer survivors. Cancer 2010; 116:592–599.

60 Maman E, Prokopis K, Levron J, *et al.* Does controlled ovarian stimulation prior to chemotherapy increase primordial follicle loss and diminish ovarian reserve? An animal study. Hum Reprod 2009; 24:206–210.

61 Muttukrishna S, McGarrigle H, Wakim R, *et al.* Antral follicle count, anti-mullerian hormone and inhibin B: predictors of ovarian response in assisted reproductive technology? BJOG 2005; 112:1384–1390.

62 •• Melo MA, Garrido N, Alvarez C, *et al.* Antral follicle count (AFC) can be used in the prediction of ovarian response but cannot predict the oocyte/embryo quality or the in vitro fertilization outcome in an egg donation program. Fertil Steril 2009; 91:148–156.
This is a lage prospective trial and the first one to compare AFC to ovarian response and embryo quality.

63 Seifer DB, Maclaughlin DT. Mullerian Inhibiting Substance is an ovarian growth factor of emerging clinical significance. Fertil Steril 2007; 88:539–546.

64 La Marca A, Sighinolfi G, Radi D, *et al.* Anti-Mullerian hormone (AMH) as a predictive marker in assisted reproductive technology (ART). Hum Reprod Update 2010; 16:113–130.

65 Nelson SM, Yates RW, Fleming R. Serum anti-Mullerian hormone and FSH: prediction of live birth and extremes of response in stimulated cycles–implications for individualization of therapy. Hum Reprod 2007; 22:2414–2421.

66 Wunder DM, Guibourdenche J, Birkhauser MH, *et al.* Anti-Mullerian hormone and inhibin B as predictors of pregnancy after treatment by in vitro fertilization/intracytoplasmic sperm injection. Fertil Steril 2008; 90:2203–2210.

67 Lee TH, Liu CH, Huang CC, *et al.* Impact of female age and male infertility on ovarian reserve markers to predict outcome of assisted reproduction technology cycles. Reprod Biol Endocrinol 2009; 7:100.

68 Silberstein T, MacLaughlin DT, Shai I, *et al.* Mullerian inhibiting substance levels at the time of HCG administration in IVF cycles predict both ovarian reserve and embryo morphology. Hum Reprod 2006; 21:159–163.