Assessment of ovarian reserve – is there a role for ovarian biopsy?

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The pool of primordial follicles in the ovary or ‘ovarian reserve’ is a major factor in the human fertility potential. The ageing ovary is characterized by reduction of the number of primordial follicles and this loss accelerates in the late 30’s and precedes the menopause by 10–12 years. Woman’s age alone or with a combination of biochemical markers, dynamic tests and ultrasound measurements fail to predict this loss accurately. In this manuscript, a novel approach of histopathological examination of ovarian biopsy for the evaluation of infertility, especially in unexplained infertility and in women in the later part of reproductive life, is discussed.

Key words: follicles/ovarian biopsy/ovarian reserve

Introduction
A major factor in successful IVF treatment is the ability of the ovary to respond to gonadotrophin stimulation and to develop several follicles. That response reflects the ovarian function or ‘ovarian reserve’ and depends on the pool of primordial follicles in the ovary. Ageing of an ovary is characterized by the reduction of the number of primordial follicles from about a million at birth to ~250 000 at menarche, to a very few at the end of reproductive life (Block, 1952). This loss accelerates around the age of 37 years and precedes the menopause by 10–12 years (Richardson et al., 1987; Faddy and Gosden, 1995). There is wide variation between women in the number and rate of depletion of follicles.

Age and regularity of menses alone are unreliable measures of predicting ovarian reserve. The biological age is more important than the chronological one (Marcus and Brinsden, 1996; Lass et al., 1998). In the ageing process, the ovaries become progressively less responsive to exogenous gonadotrophins, until they are totally refractory at the time of the menopause.

A few indirect tests have been developed to predict the ovarian reserve (Table I).

Endocrine tests

**Basal serum FSH concentrations**
Once the ovary is more or less exhausted, increased pituitary production of FSH follows. This event takes place a few years before the actual menopause. Currently, the FSH concentration is the best marker to assess ovarian reserve and of predicting response to superovulation with a good correlation with pregnancy rates (Scott et al., 1989; Toner et al., 1991). However, lack of a clear cut-off point, huge variations between different laboratories and monthly variations in FSH secretion means that FSH measurement is only of limited value in assessing the prognosis of IVF treatment (Scott and Hofman, 1995; Walach, 1995). Once the FSH concentrations start to fluctuate, there is already a decreased ovarian reserve and it is not clear whether starting stimulation in a later month with ‘normal’ FSH concentrations will give a better result (Scott et al., 1990; Lass et al., 2000).

**Basal oestradiol concentration**
Measurement of basal oestradiol in addition to FSH might improve the ability to predict fertility potential compared with basal FSH and chronological age alone (Licciardi et al., 1992; Smortich et al., 1995; Buyalos et al., 1997). Cycle day 3 oestradiol of <80 pg/ml with normal FSH concentration in women of 38–42 years of age gives a good prognosis of successful treatment (Buyalos et al., 1997).

**Basal inhibin-B concentration**
Inhibin-B is a heterodimeric glycoprotein released by the granulosa cells of the follicle. A recent study has shown that women with low day 3 inhibin B concentration (<45 pg/ml) had a poorer response to superovulation for IVF and were less likely to conceive a clinical pregnancy (Seifer et al., 1997). It also showed that a decrease in inhibin-B probably precedes the increase in FSH concentrations (Seifer et al., 1999). However, other investigators failed to show any added predictive value for inhibin-B as a measure of ovarian reserve (Corson et al., 1999; Hall et al., 1999).

Ultrasound markers

**Ovarian volume and number of antral follicles**
Increased age is associated with decreased ovarian volume. Transvaginal measurement of ovarian volume is quick, accurate and cost-effective. Decreased ovarian volume and low number of antral follicles of 2–10 mm are signs of ovarian ageing that may be observed earlier than a rise in FSH concentrations. Small ovaries are associated with poor response...
to superovulation and a high cancellation rate in IVF (Syrop et al., 1995, 1999; Lass et al., 1997a; Lass and Brinsden, 1999; Scheffer et al., 1999). Recently, Engmann et al. (1999) suggested measurement of ovarian stroma blood flow with colour Doppler (Engmann et al., 1999).

**Dynamic tests**

The clomiphene challenge test (CCT) was first described in 1987 (Navot et al., 1989). This simple test consists of measuring serum FSH on day 3 and again on cycle day 10 after administration of 100 mg of clomiphene from days 5–9. An abnormal test is defined by elevated FSH concentrations on cycle day 10. This provocative test unmasks patients who might not be detected by basal FSH screening alone. An abnormal test is highly predictive of diminished ovarian reserve in natural cycles, during ovulation induction and in IVF (Loumaye et al., 1990; Tanbo et al., 1992; Scott et al., 1993). It is superior to early follicular FSH screening, but has poor predictive value in women >40 years in terms of response to superovulation and pregnancy rate in ART cycles.

The gonadotrophin releasing hormone (GnRH) agonist challenge test has been proposed by a few authors. In this test a change in oestradiol concentrations from cycle day 2 to 3 after s.c. administration of leuprolide acetate (Winslow et al., 1991) or FSH increase 2 h after buserelin injection (Galtier-Dereure et al., 1996) are measured. However, although the dynamic tests are strongly predictive of stimulation outcome, it is not yet clear whether they are more useful than measurement of basal FSH in predicting IVF outcome.

In spite of the abundance of studies and recent comprehensive reviews on indirect tests for predicting ovarian reserve (Broekmans et al., 1998; Sharara et al., 1998) there are still doubts about their accuracy and interpretation (Barnhart and Osheroff, 1999).

This lack of sufficient and adequate tests to predict the ovarian reserve led researchers to develop a different strategy for assessing the ovarian reserve. As discussed above, the chief determining factor in the ovarian reserve is the non-renewable pool of primordial follicles. It makes sense therefore to try to evaluate the ovarian pool directly. Obviously, the only definite way, of slicing the ovary and counting all follicles in its entire cortex is out of question. We have suggested a novel method of quantifying the number of small follicles in ovarian biopsies from infertile patients (Lass et al., 1997b). The number of follicles per unit volume of cortical ovarian tissue can be mathematically calculated and defined as follicular density. This method is therefore applicable for any individual, regardless of size or shape of the ovarian biopsy. Because follicles are found no deeper than 2 mm from the ovarian cortex, only a shallow biopsy is required. It was demonstrated that follicular density decreases significantly with increasing age. Women >35 years of age have only one third of the concentration of follicles of younger women. Fewer primordial follicles are found in women with unexplained infertility than in women with tubal disease. The main limitation of this study was that the follicular density within the biopsy specimen may not accurately represent the density of follicles in the whole ovary. So far there are no published papers which examine the normal distribution in different sections of the ovary. Guleekli et al. (1999) performed few predictive tests in a small group of parous women >35 years old immediately before oophorectomy. They compared the results of these tests i.e. basal FSH, clomiphene citrate test (CCST) and GnRH agonist stimulation test (GAST) with the number of follicles counted by the pathologist. They concluded that none of the tests accurately reflected the ovarian reserve (Guleekli et al., 1999).

Vital et al. (2000) obtained ovarian biopsies laparoscopically from 52 patients with pathological conditions such as premature ovarian failure, chronic anovulation and low ovarian reserve and from 16 healthy controls. They found a strong correlation between the histological results and number of primordial follicles to the clinical condition (Vital et al., 2000).

Based on findings of the last three preliminary studies and in spite of the relatively small number of patients, it may be suggested that ovarian biopsy could have a place in the evaluation of infertility, especially in unexplained infertility and in women in the later part of their reproductive life. It may also be used as a predictor tool for response to superovulation in IVF with a better result than endocrine tests, as well as assisting in adjusting the optimal starting dose of gonadotrophin stimulation. However, in contrary to this promising theory, there are a few limiting factors: (i) Ovarian reserve is not a simple static anatomic number of follicles but rather a dynamic process, the mechanism of which is not yet fully understood (te Velde, 1993; Ranieri and Serhal, 2000); (ii) So far there is no known published study that evaluated the predictive value of histopathology count of primordial follicles before stimulation for IVF; (iii) Currently, the only practical way to retrieve ovarian tissue for pathology examination is through invasive procedures such as operative laparoscopy or open laparotomy, which are not without risk.

Whilst acknowledging that current implementation of ovarian biopsy retrieval for diagnostic purposes of ovarian reserve is controversial, this method could be more feasible in the future depending on two major factors; (i) a better knowledge on the natural distribution of primordial follicles in the ovarian cortex and their dynamics through the reproduct-
ive age up to the menopause (ii) development of an ovarian biopsy ultrasonic-guided needle/device which will facilitate obtaining the biopsy easily and safely as an outpatient procedure.

The author believes that it is important to conduct more clinical and histo-pathological studies on the ovarian reserve dynamics, as well as the correlation between the follicular reservoir to endocrine markers and performance in ovarian stimulation treatment cycles.

References

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