Assessment of ovarian reserve

Is there still a role for ovarian biopsy in the light of new data?

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Ovarian reserve depends on the number of primordial follicles in the ovarian cortex. It was suggested that determining the follicular density directly by obtaining ovarian biopsy might be more accurate than current indirect biochemical and ultrasonic tests, especially for women in the later stage of their reproductive life. It might also be important and beneficial for young patients having chemotherapy for malignant disease in whom the ovarian tissue should be considered for reimplantation after recovery. The advantages and pitfalls of obtaining ovarian biopsy in these cases are discussed in light of new emerging data on the natural distribution of primordial follicles in the human ovary and its implications.

Key words: cancer/ovarian biopsy/ovarian reserve/primordial follicles

Introduction

The pool of primordial follicles in the ovary or ‘ovarian reserve’ is a major factor in human fertility potential. The ageing ovary is characterized by reduction of the number of primordial follicles, and this loss accelerates in the late 30s and precedes the menopause by 10–12 years (Richardson et al., 1987; Faddy and Gosden, 1995). The definite pool of primordial follicles is relevant in two major aspects: (i) the fertility potential of women especially in the latter part of their reproductive life; and (ii) in women suffering from malignant disease who are going through ovarian biopsies collection as a potential measure to preserve their fertility capacity. For these women, not only the presence of follicles but their location and whether they exist in the preserved biopsies are important.

A few years ago, I suggested considering ovarian biopsy as part of infertility evaluation (Lass, 2001). In this article, the recent data on follicular density are reviewed and the role of ovarian biopsy for these two indications is re-assessed.

Ovarian biopsy as integral part of infertility evaluation

In recent years, intensive research has been conducted to try to predict the ovarian reserve, in particular before embarking on fertility treatment. The main avenues of research are endocrine tests and biochemical markers such as basal FSH (Scott et al., 1989; Toner et al., 1991) estradiol (E₂) (Licciardi et al., 1992; Smotrich et al., 1995), inhibin B (Seifer et al., 1999) levels and, recently, anti-Müllerian hormone levels (Van Rooij et al., 2002); ultrasound measurements such as ovarian volume (Lass et al., 1997a), antral follicle count (Tomas et al., 1997) and ovarian stroma blood flow (Engmann et al., 1999); and dynamic tests of clomiphen citrate challenge test (CCCT) (Navot et al., 1989), exogenous FSH ovarian reserve test (EFORT) (Fanchin et al., 1994; Kwee et al., 2003) and GnRH agonist stimulation test (GAST) (Winslow et al., 1991). Merely the fact that so many tests and markers are proposed indicates that we still do not have a satisfactory reliable method to assess the ovarian reserve.

Regrettfully, the predictive power of all these tests alone or in combination is quite limited. This lack of sufficient and adequate tests to predict the ovarian reserve led our team to suggest a novel method of quantifying the number of small follicles in ovarian biopsies from infertile patients (Lass et al., 1997b). The promising results of negative correlation between a patient’s age and follicular density and less follicles in unexplained infertility compared with tubal infertility led me to suggest considering ovarian biopsy as a diagnostic tool quite early in infertility investigation (Lass, 2001).

We were quite aware of major limitations of ovarian biopsy in this context. (i) This is an invasive procedure which, although performed during diagnostic laparoscopy, might cause future adhesions, decrease the ovarian reserve further and lengthen the time of operation under anaesthesia. (ii) Whether the biopsy(ies) is/are a true reflection of the follicular distribution in the ovarian cortex, a factor never investigated until then. Block’s initial histopathological studies did not address this question (Block, 1952). Randomized or ‘blind’ single biopsy is adequate if follicles are evenly spread in the ovarian cortex (in any case, they are not deeper than 2 mm from the surface; Lass et al., 1997b) This uncertainty triggered my call for further research to study the natural distribution of primordial follicles in the ovarian cortex and their dynamics.

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through reproductive age up to the menopause (Lass, 2001). (iii) The quantitative counting of primary follicles does not provide any information about the quality of the oocytes embedded in them.

**Ovarian biopsies for cancer patients**

A few investigators have demonstrated successful cryopreservation of ovarian tissue with good survival of follicles (Hovatta et al., 1996). These findings were followed by breakthrough studies which showed that frozen ovarian tissue can be successfully thawed and be reimplanted in the human, resulting in follicular growth and restoring normal hormonal function (Oktay and Karlikaya, 2000; Oktay, 2001; Radford et al., 2001); hence women who recover from their malignant disease following chemotherapy avoid the need for hormone replacement therapy and can have normal periods. While, to the best of my knowledge, no children have been born after such a procedure, it will probably be feasible in the future to grow the follicles and collect oocytes for assisted reproduction treatment by either hormonal stimulation or in vitro maturation regardless of the site of reimplantation of the ovarian tissue. Currently, the practice of collecting ovarian biopsies for fertility preservation is becoming more common due to these possible advantages, although the use of cryopreserved ovarian tissue to restore hormonal and fertility capacity is in its early experimental stage. Nevertheless, it is important to ensure that the frozen sections are the right ones or ideally the best ones for cryopreservation. Moreover, for these patients, larger sections of biopsies are affordable compared with infertile patients.

**The distribution of follicles in the ovarian cortex**

For both indications discussed above, the whole logic of taking an ovarian biopsy is based on understanding that the biopsy(ies) is/are a true reflection of the follicular distribution in the ovarian cortex, a factor only recently investigated.

Recently the challenge to study the natural distribution of primordial follicles in the ovarian cortex and their dynamics through reproductive age up to the menopause has been picked up by a few investigators. Qu et al. (2000) examined ovarian biopsies from 24 patients who underwent infertility evaluation. The distribution of follicles was extremely uneven in ovarian tissue. A large variation in follicle numbers was observed in ovarian tissue samples from patient to patient. Furthermore, even though some tissue samples were originally obtained from the same patient, the number of follicles counted in one sample of ovarian tissue did not match the number found in another tissue sample.

Kohl et al. (2000) compared samples of contralateral ovaries from five women and multiple biopsies from different sites in another two patients, all of them from women having diagnostic laparoscopy for infertility. They found large variation between the ovaries and between the sites of the biopsies. Poiriot et al. (2002) collected ovarian tissue from 31 patients having chemotherapy. They evaluated at least 10 serial sections per patient (range 10–15) and found that follicles were not homogeneously distributed within the ovarian cortex.

Recently, Schmidt et al. (2003), in very methodological and laborious research, showed in 21 patients with malignant disease a wide variation in follicular density from 1.1 to 190.6 follicles/mm² between the patients and again with significant inverse correlation between follicular density and age.

However, the new information from this work is the evaluation of whole ovary in three patients. In each ovary, numerous pieces of cortex were isolated, and the histological evaluation revealed a wide variation in the number of primordial follicles between each fragment of the same ovary. In all four studies, a significant negative correlation between follicular density and age has been established.

Although only three whole ovaries were studied, the combined results of this study and the previous ones mentioned above led the author to the conclusion that the ovarian cortex indeed varies significantly in its follicular content. There is no good explanation for the cause of this variation nor is it clear whether a specific area is more favourable for follicular nesting than another or if it is due to purely random allocation.

**Conclusions**

The presence of a sufficient number of follicles (how many is still not clear) is probably quite reassuring for adequate reserve, but empty cortex or a very few follicles might be just incidental and meaningless. However, the finding of erratic random distribution of follicles in the ovarian cortex combined with possible risks of this procedure suggest that on risk–benefit balance, this procedure is not justified based on the current available data and the author does not recommend it for this indication.

Young girls and women who are undergoing ovarian biopsy collection as a fertility preservation measure before chemotherapy in malignant diseases are expected to have normal follicular distribution according to their age. However, every effort should be made to assess the follicular quantity in real time by frozen section and, if impossible or impractical, to attempt multiple biopsies from different sites of the ovaries, to ensure that the frozen pieces of ovarian tissue have a sufficient number of follicles.

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