Royal College of Obstetricians & Gynaecologists

Reproductive Ageing

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1. Introduction

The rate of attrition of a woman's lifetime stock of oocytes is inexorable. Despite controversial research suggesting that under certain circumstances the ovary might repopulate itself by differentiation of stem cells into follicles and oocytes, the picture in clinical practice now, and in the foreseeable future, is one of managing the decline in fertility and increase in rates of miscarriage and chromosomal abnormalities in offspring that result from reproductive ageing in the female. 'Unexplained infertility' is a rare diagnosis for a couple where the woman is in her 20s, but becomes the most common cause of infertility in women over 35 years of age. This increase follows from the decline in oocyte 'quality' seen as the consequence of ageing in women. Quality in this context refers to a complex series of age-related changes in nuclear and cytoplasmic competence, affecting such fundamental processes as spindle formation and chromosome segregation, mitochondrial function and the integrity of the cytoskeleton. A poor-quality oocyte is less likely to fertilise and, if fertilised, will produce an embryo which is generally slow to divide and unlikely to implant.

This paper will assess current methods for measuring ovarian reserve, discuss the complications of reproductive ageing and their consequences and describe available strategies to ameliorate their impact on fertility, and will also touch on reproductive ageing in the male.

2. Obstetric implications

Women over 35 years of age are at increased risk of early pregnancy and obstetric and neonatal complications. The older woman is more likely to experience stillbirths, miscarriage or ectopic pregnancies.[1] In the general population, the risk of miscarriage in a woman 35–39 years of age is 24%, and doubles to 51% at 40–44 years of age if the woman conceives with her own, rather than donor, eggs.[2] Obstetric complications associated with increased maternal age include gestational diabetes, placenta praevia, placenta abruption, hypertension and caesarean section. Older women are also more likely to be nulliparous, require assisted reproductive therapy for conception and have multiple pregnancies naturally and after assisted reproductive therapy, all of which are risk factors for increased obstetric and neonatal morbidity. Advice to women using routes such as schools, media, general practice and family planning should consistently reflect that the optimal age for childbearing is between 20 and 35 years, for obstetric as well as for reproductive health reasons.

3. Demographic changes and reproductive ageing

Societal changes over the past four decades have resulted in large numbers of women deferring attempts to conceive until their mid-30s or older.[3] Most will be able to conceive naturally and deliver a healthy child at term if they conceive at 35 years of age, but the proportion who experience infertility, miscarriage or fetal abnormality increases rapidly thereafter such that only two in five of those who wish to have a child at 40 years of age will be able to do so. The average age at which women have in vitro fertilisation (IVF) treatment in the UK is rising, reflecting the increase in infertility due to advancing maternal age, but success rates of IVF treatment for women over 40 are low and have not increased substantially over the past decade.[4]

The best biological advice for those women who feel that having a family is an important part of their lifetime fulfilment is for them to plan to complete childbearing by the age of 35. However, this aspiration may not fit with their life circumstances. There are several tests available commercially to test for ovarian reserve. It is important that women who have measurements made of their ovarian reserve appreciate that the available tests assess oocyte quantity not oocyte quality.

4. Ovarian reserve testing and its relevance to ovarian ageing

The term ovarian reserve describes the quantity of oocytes remaining within the ovaries of a woman at a certain time point. Ovarian reserve will decline with ageing until menopause, when ovulation ceases, with only a few hundred primordial follicles remaining.[5] It is obviously impractical to remove ovaries for histological analysis in healthy females, so a number of surrogate markers have been developed to try and assess ovarian reserve without using invasive procedures. Since studies on young women that use age at menopause as a primary endpoint would be impractically long, the response to gonadotrophin stimulation in IVF treatment has long been used as a surrogate for ovarian ageing, as young healthy women will produce more oocytes than their older counterparts in response to a given dose of follicle-stimulating hormone (FSH).

This approach has been criticised.[6] Research on women undergoing IVF superovulation shows that the response to gonadotrophin stimulation varies from month to month in the same individual, maximally stimulating doses of gonadotrophins are often avoided clinically as prophylaxis against ovarian hyperstimulation syndrome, and transvaginal egg collection does not invariably harvest all available oocytes in a cohort. However, the many studies of markers of ovarian reserve using IVF oocyte yield as the primary endpoint have produced broadly consistent results and, accepting the above caveats, it is reasonable to compare the various clinical tools for ovarian reserve measurement using this endpoint.

4.1 Serum FSH, antimüllerian hormone (AMH) and inhibin measurements

Measurement of the concentration of FSH in serum in the early follicular phase of the cycle has been the cornerstone of assessment of ovarian reserve in IVF practice for three decades. As the size of the oocyte pool diminishes, higher levels of pituitary stimulation are needed to promote development of a dominant follicle. Hence, high concentrations of FSH reflect low ovarian reserve. Use of FSH measurements in this context is imperfect as concentrations vary due to pulsatile release from the pituitary and no cut-off level predictive of poor outcome has been agreed. Nevertheless, and notwithstanding a meta-analysis suggesting lack of efficacy,[7] almost all IVF centres still measure early follicular phase FSH routinely and rely on the results to support clinical decision making.

Recent developments in stable antibody-based assays for inhibin B and AMH have allowed evaluation of these granulosa cell products as markers of ovarian reserve. Inhibin B is produced by small antral follicles in response to FSH stimulation, while AMH is mainly derived from pre-antral follicles and is non-FSH-dependent. Studies using IVF oocyte yield have confirmed that both ligands have utility as measures of ovarian reserve, with AMH offering the best predictive value in receiver operating characteristic curve analysis. Measurement of AMH has gained rapid acceptance in IVF practice and looks likely to replace FSH as the preferred biochemical predictor of ovarian response.[8,9] AMH concentrations are fairly stable throughout the cycle and there is limited cycle-to-cycle variation; blood sampling is therefore facilitated.

4.2 Antral follicle count (AFC)

Ovarian reserve can be assessed using high-resolution transvaginal ultrasound scanning to count small antral follicles 2–10 mm in diameter. AFC correlates with oocyte yield in IVF and with the various biochemical markers. Drawbacks include the test being operator-dependent and requiring attendance at the ultrasound facility, and recent studies have not shown evidence of benefit over AMH measurement.[8,10] Combinations of these various markers have been evaluated and may offer better prediction of oocyte yield after stimulation.

4.3 Oocyte quality

All the above tests are reflective of the number of primordial follicles remaining in the ovaries rather than being measures of quality. Oocyte number clearly correlates with oocyte quality, given the well-known decline in fertility and increase in karyotypic abnormalities in embryos derived from older women with

reduced oocyte numbers; however, although this applies for older women, the young IVF patient with low oocyte yield may still exhibit good oocyte quality. Studies using pre-implantation genetic screening suggest relatively high levels of aneuploidy in IVF embryos, but there is no evidence to suggest this can be identified by measurement of AMH or AFC.

Pre-IVF assessments of ovarian reserve are poor predictors of pregnancy outcome. This is unsurprising since there are so many other variables that together determine the likelihood of live birth. IVF outcome will be influenced by the embryology laboratory environment, by sperm quality, by uterine and tubal factors including the presence of hydrosalpinx or uterine anatomical abnormalities, by endometrial receptivity and by the maternal response to pregnancy. None of these is influenced much, if at all, by ovarian reserve. However, since the biggest determinants of chance of live birth are the woman's age and oocyte yield, there is an undeniable link between ovarian reserve and fertility that extends beyond IVF egg yield to more fundamental issues of pregnancy and pregnancy outcome.

4.4 Low ovarian reserve

A low ovarian reserve measurement in a young woman may serve to prompt further specialist investigation, including high-resolution transvaginal ultrasound for AFC and investigations to rule out known causes of early ovarian failure. This may allow for successful natural conception, albeit at younger age than planned, avoiding later resort to IVF and possible lifelong infertility. At the moment, however, there is an absence of longitudinal data indicating whether these markers will be useful as a screening test to predict immediate or future fertility in the general population.

5. Oocyte storage

Technological improvements in oocyte vitrification offer young healthy women the possibility of preserving oocytes until the less fertile years of life. This science is in its infancy and many practical and ethical issues remain. Chances of healthy pregnancy resulting from a vitrified oocyte are small (4%),[11,12] so many eggs should be collected and frozen to give a realistic chance of success later on. This exposes the healthy woman to superovulation and oocyte collection with concomitant hazards, albeit small. The best age for oocyte vitrification is probably under 30 years, resulting in medicalisation of relatively young women. Unrealistic reliance on their store of vitrified oocytes may lead women to defer pregnancy for many years only to experience disappointment when the stored oocytes later fail to fertilise or implant. Also, anxieties about the effects of prolonged vitrification on the offspring remain.[13] Nevertheless, oocyte vitrification has attracted considerable interest, although this treatment is currently not widely available in the NHS.

6. Oocyte donation

Another approach to infertility resulting from ovarian ageing is to use oocytes from a younger, fertile donor. Oocyte donors are required to go through superovulation with injection of fertility drugs followed by transvaginal oocyte collection. This presents a considerable burden and many potential volunteers are discouraged by the degree of intervention required. Current UK law does not permit commercial oocyte donation and the number of available oocyte donors is small, despite locally successful egg-sharing schemes. This has caused an exodus of potential recipients of this technique to countries that permit payment to egg donors, such as Spain, Cyprus, the USA and many eastern European countries. This cross-border reproductive care carries significant disadvantages for both the recipient and the donor, and recently the debate over whether UK donors might be paid has been re-opened.

7. Pre-implantation genetic screening

A further approach to the inevitable deterioration in oocyte quality in older patients is to perform embryo biopsy and testing; that is, to screen IVF embryos for aneuploidy using pre-implantation genetic

screening (PGS), with replacement of euploid embryos only. Despite the attractiveness of the scientific and clinical rationale for PGS, recent randomised prospective clinical trials failed to observe a significantly increased live birth rate after transfer of chromosomally 'normal' embryos following PGS.[14] Recent evidence suggests that the mitotic error rate in cleavage-stage embryos is higher than the meiotic aneuploidy rate; as a consequence, the genome of a single blastomere is not representative for the genome of the other cells of the embryo, which may account for the results of the clinical trials being at odds with the scientific rationale for PGS.[15]

Cytoplasmic transfer techniques to provide better quality mitochondria from eggs collected from young donors have also been investigated. Cytoplasmic transfer[16] may improve the chances of fertilisation and implantation and avoids the drawbacks of oocyte donation, allowing the woman to have her own genetic child. However, the technique results in transfer of mitochondrial DNA from the donor to the recipient and, given the shortage of donor eggs, it is not likely to become a feasible option; moreover, currently it is not legal in the UK.

8. Reproductive ageing in males

Reproductive ageing also has an impact on male fertility and on the health of their offspring.[17] While many men remain fertile into their fifth decade and beyond, the proportion of men with disorders of spermatogenesis increases with advancing age. Men over 40 years of age contribute to reduced fertility and fecundity of a couple, especially when the female partner is also of advanced age. Increasing paternal age can be associated with decreased serum androgen concentration, decreased sexual activity, alterations of testicular morphology and deterioration of semen quality (volume, motility and morphology). Increased paternal age has an influence on the DNA integrity of sperm, and is suggested to have epigenetic effects. Suffice to say, the reproductive impact of paternal ageing, albeit significantly less pronounced than that of female ageing, is at least in part responsible for the association with reduced fertility and the increase in pregnancy-associated complications and adverse outcome in the offspring.

9. Opinion

It seems unlikely that the current trends towards pregnancy at older age will reverse in the coming decade. While some may avoid age-related infertility by early resort to oocyte vitrification and others will happily conceive after oocyte donation, many will suffer permanent infertility. Obstetricians, gynaecologists and indeed all health professionals should seize opportunities to remind young couples of the biological realities of reproductive ageing, and teaching on sexual health to school-age children should include education on fertility alongside contraception and avoidance of sexually transmitted diseases.

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