Biological versus chronological ageing of oocytes, distinguishable by raised FSH levels in relation to the success of IVF treatment

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BACKGROUND: The present study addresses the issue of biological ageing of the oocyte (as indicated by basal serum FSH levels) versus chronological ageing. METHODS: 1019 infertile but ovulating women were studied in their first cycle of IVF treatment. A series of logistic regression models were developed to assess statistical significance of effects of age and FSH on implantation rates and live babies born. RESULTS: The number of oocytes retrieved and embryos available for transfer declined with increasing age and basal serum FSH concentrations. Fertilizing ability of oocytes increased with advancing age but was not affected by FSH concentrations. Although the number of oocytes or embryos available for transfer had no independent effect on implantation rates, the implanting ability of fertilized oocytes (embryos) was inversely related to increasing age and independently to FSH. The chance of a baby being born, however, was determined more by age than by serum FSH. CONCLUSIONS: Ovarian ageing affecting oocyte quality and fecundity can occur independently of chronological age. This has important practical implications whereby serum basal FSH measurement may be a valuable prognostic index, though chronological age remains important.

Key words: age/FSH/implantation/ovary/pregnancy

Introduction

Advancing age in women is associated with accelerating decline in ability to conceive and increasing risks of miscarriage or of genetic abnormality in surviving offspring. These impairments can be attributed primarily to defective oocyte quality, as indicated by the success of oocyte donation treatment (Abdalla et al., 1993; Yaron et al., 1993; Balmaceda et al., 1994; Navot et al., 1994). The present study addresses the hypothesis that biological ‘ovarian ageing’ in terms of declining reproductive competence can occur independently, and in advance of the woman’s chronological age. The findings are also of practical importance. They are likely to be of diagnostic value for women wanting to delay childbearing, especially if they have a family history of premature menopause or have suffered partial ovarian loss, as well as for those already requiring treatment for infertility. They also suggest reason to deter clinical practices that involve non-essential removal of ovarian tissue.

Current theories of the age-related effect on oocyte quality imply that it is not a simple time-related effect due to prolonged exposure to risk of accidental damage, but rather that defective oocytes were defective from the start during fetal ovarian development and are less likely to be recruited for ovulation until relatively late in life (Henderson and Edwards, 1968; Polani and Crolla, 1991; Zheng and Byers, 1992). This assertion has been partially supported by a study involving female rats (Meredith and Doolin, 1997). Thus the associated reproductive impairments may be more closely related to decline in ovarian follicle number than to age; or put another way, to the interval before the menopause rather than the age at which that occurs. Experimental evidence in rodents shows that removal of an ovary when young leads not only to shortening of the reproductive life-span, but also reduced fecundity and increased incidence of abnormality in the embryos (Brook et al., 1984; Meredith and Butcher, 1985) as well as increased circulating levels of FSH (Meredith and Butcher, 1985).

In women, reduction in follicle number with advancing age (Faddy and Gosden, 1996; Leidy et al., 1998) is reflected by rising levels of serum FSH measured during the early follicular (menstrual) phase of the ovulation cycle. The rise in FSH begins more than a decade before the menopause and several years before any rise in LH (Lee et al., 1988; Ebbiary et al., 1994; Klein et al., 1996). The number of mature follicles and oocytes that can be obtained in response to maximal stimulation using exogenous gonadotrophins is an index of total ovarian follicular capacity, and correlates (inversely) with FSH levels...
independent of age (Cahill et al., 1994). The present study explores the hypothesis that raised FSH levels occurring in relatively young ovulating women indicate not only a critical decline in follicle number but also in oocyte quality as reflected by reduced implanting ability after fertilization as typically occurs in older women.

**Materials and methods**

We studied all infertile couples in their first cycle of IVF treatment undergoing attempted oocyte recovery in the University of Bristol IVF Service at the BUPA Hospital, Bristol during the 6 years 1990–1995 if they met the following criteria. The women had a normal uterus, normal ovulatory cycles (mid-luteal serum progesterone ≥30 nmol/l), and a recent (<6 months) basal serum FSH concentration measured once during the early follicular phase (days 1–5 of the menstrual cycle). [FSH concentrations have been shown to be constant between at least days 2 and 5, although estradiol rises (Hansen et al., 1996).] Couples with a diagnosis of sperm dysfunction or tubal disease with hydrosalpinges were excluded in view of the potential chance of success with age, and about increasing risk of cancellation due to poor ovarian response: ~3% aged <40 years, 25% >40 years.

A uniform treatment protocol was used of pituitary desensitization starting in mid-luteal phase of the previous cycle followed by ovarian stimulation using FSH alone (urofollitrophin, Metrodin or Metrodin-HF, Serono) as previously described (Hull et al., 1994). To avoid other potential selective bias and to eliminate the confounding effect of multiple cycles from the same individual, the study was limited to the first cycle of treatment for each couple. Only those who had fresh embryos available for transfer were included. A maximum of three embryos was transferred as legally restricted in the UK. Transfer of two or less embryos occurred if requested by the couple following counselling with regard to the risks of multiple pregnancy, or in cases where these were the only embryos available as was the practice at the time.

Data were collected for analysis on the woman’s age at treatment, the number of oocytes collected, and the number that fertilized and cleaved normally, the number of embryos transferred, clinical pregnancy indicated by a gestation sac in which a fetal heartbeat could be seen on ultrasound examination 4 weeks after embryo transfer, the number of sacs and pregnancy outcome. Fertilization was defined as normal by the development of two pronuclei and progressive cleavage up to the time of embryo transfer after 2–3 days. Rate of fertilization (including cleavage) was defined as the proportion of oocytes that were fertilized normally. Implantation rates were defined as the proportion of individual embryos transferred resulting in a gestation sac including ectopic gestations (also including ectopic sacs without an evident fetal heartbeat). Live baby rates were similarly defined, per individual embryo transferred, whereas the pregnancy and live birth rates were per patient who had an embryo transfer, multiple births being counted as one.

Serum FSH concentration was measured using a two-site immunofluorimetric method employing two monoclonal antibodies directed first against the β subunit and then the α subunit (Delfia: Wallac Oy, Turku, Finland). Inter-assay coefficient of variation over the useful range was 4–6% and intra-assay variation <5%. The normal range in our study during the early follicular phase was 0.8–8.9 IU/l (95% limits) and these values and the assay performance were consistent with most laboratories participating in the UK Quality Assurance Scheme for Peptide Hormones.

Statistical analysis included the calculation of Kendall rank (non-parametric) correlation coefficients between the number of oocytes retrieved and fertilization rate and the woman’s age and serum FSH concentration, and respective partial correlation coefficients. The relationships between implantation rate (or live baby rate) per embryo and age and FSH were explored graphically using ‘smoothed’ logistic regression (Bowman and Azzalini, 1997). This ‘local likelihood’ approach was used to estimate the smoothed probability of implantation/live baby per embryo across a two-dimensional grid of ages and FSH values (50 equally spaced ages ranging from 20 to 50 years and 50 equally spaced FSH concentrations from 0 to 15 IU/l). Contour lines were drawn using S-plus (Venables and Ripley, 1994) to join pairs of age/FSH with the same estimated probabilities.

Logistic regressions were used to assess the statistical significance of age and FSH on: (i) the probability of pregnancy or live birth per transfer cycle of embryos; (ii) the probability of implantation or live baby per individual embryo transferred; and (iii) the probability of implantation of an embryo, once that had occurred, leading to the birth of a live baby. The relationships with age were not linear, therefore ‘fractional polynomials’ (Royston and Altman, 1994) initially were used to determine the best transformations for age. This methodology suggested powers ≥3 for (i) and (ii) but the models did not perform ‘significantly’ better than using just a linear term; we finally adopted models that had been used by other authors to model live birth rate per cycle (Templeton et al., 1996), namely including both age-squared and age-cubed. Having accounted for age in this way, the effect of FSH was assessed by adding FSH to the model. If FSH was found to be important, polynomials in FSH were considered in the same way as for age.

In the model for the probability of pregnancy (i), we included the number of oocytes which fertilized normally which was felt to be a proxy measure of oocyte and embryo quality. Finally, since results from embryos from the same woman were likely to be correlated, we checked the significance of the age terms, and FSH adjusting for age, using robust standard errors via the ‘cluster’ option in Stata (Stata Corp., 1999); Wald P-values are given for these in the text (other P-values shown are based on likelihood ratios).

**Results**

There were 1019 patients undergoing a first cycle of oocyte collection who were eligible for study. Their distribution in the woman’s age bands of 20–24, 25–29, 30–34, 35–39, 40–44, and 45–49 years was 11, 153, 379, 367, 99 and 10 respectively, and in the serum FSH bands of <3.0, 3.0–5.9, 6–8.9, 9.0–11.9, 12.0–14.9 and ≥15.0 IU/l was 109, 482, 327, 72, 24 and 5. Ungrouped ages and FSH concentration were used for the statistical analyses.

The median number of oocytes retrieved per woman was nine (range 0–41), with median fertilization rate 60% (range 0–100%). Correlation analysis revealed a reduction in the number of oocytes retrieved with advancing (ungrouped) age (τ = -0.209, P < 0.001) and rising FSH (τ = -0.135, P < 0.001). The partial correlation coefficients (τ = -0.201 and -0.121 respectively) changed very little, suggesting that both age and FSH independently affected the number of oocytes. Fertilization rates were positively but only weakly related to age (τ = 0.058, P = 0.008) and not significantly related to FSH (τ = 0.032, P = 0.142).

Of the 977 (96%) women who achieved embryos suitable for transfer, 275 (28.1%) became pregnant and 227 (23.2%)
had a live birth. There was an overall decline in both rates with age ($P < 0.025$ and $P = 0.001$ respectively) and both were significantly related to FSH after adjustment for age ($P < 0.01$ and $P < 0.025$). No significant interaction was found between age and FSH. The model formulae were:

logit (probability of pregnancy) =
\[-0.508 + 0.001208 \times \text{age}^2 - 0.00003424 \times \text{age}^3 - 0.07921 \times \text{FSH}\]

logit (probability of live birth) =
\[-1.588 + 0.003969 \times \text{age}^2 - 0.0000927 \times \text{age}^3 - 0.08309 \times \text{FSH},\]

where age is in years, FSH in IU/l. Outcome was not independently related to the number of embryos available for transfer (i.e. the number of oocytes that fertilized normally).

Table I gives the rates of implantation per individual embryo transferred, both as initial fetal sacs and resulting live babies born. Out of a total of 2684 embryos transferred, the overall rate of implantation was 14.0% and of a live baby was 10.8%. The probability of implantation (or live baby) per embryo transferred was related to age and FSH independently, being greatest in the youngest women with lowest FSH, and declining as either age or FSH increased. Contours derived from the ‘smoothed’ logistic regression (Figure 1) appeared to support this and showed trends of reduced implantation/live baby rate per embryo with both increasing age and increasing FSH. Logistic regression confirmed the significance firstly of the relationship between implantation and live baby rates and age ($P < 0.005$ and $P < 0.001$ respectively; $P = 0.010$ and $P < 0.001$ using the ‘robust’ approach) and secondly with FSH adjusting for age ($P < 0.01$ and $P < 0.025$; ‘robust’ approach $P < 0.025$ and $P < 0.05$). A cubed term for FSH further refined these models with the resulting formulae:

Figure 1. Contour diagram showing mathematically derived ‘smoothed’ empirical probabilities of implantation (thick line) or of a live baby born (thin line) per embryo according to the woman’s age and serum basal FSH level. The probabilities are presented as contour lines for each level of probability: 0.2, 0.15, 0.1 and 0.05; from a three-dimensional plot.

logit (probability of implantation) =
\[-2.2039 + 0.002564 \times \text{age}^2 - 0.00006107 \times \text{age}^3 - 0.0004645 \times \text{FSH}^3\]

logit (probability of live baby) =
\[-2.8356 + 0.004070 \times \text{age}^2 - 0.00009616 \times \text{age}^3 - 0.0004913 \times \text{FSH}^3,\]

Model estimates of the probabilities of implantation and live baby per embryo are given in Figure 2, calculated at the mid-points of bands of ages and FSH concentrations. In women aged <35 years FSH appears to be the dominant factor,
Even within the normal range, FSH levels are significantly correlated (variably) independent of age with ovarian follicular responsiveness to maximal exogenous stimulation using gonadotrophins (Cahill et al., 1994). In IVF treatment, patients with raised FSH levels produce fewer oocytes and achieve lower pregnancy rates, similar to results observed in ageing women, but also occurring independent of age (Toner et al., 1991).

The chance of pregnancy by IVF treatment depends in part on the number of embryos transferred; however, the numerical effect needs to be distinguished from the quality of individual embryos and of the oocytes giving rise to them. Furthermore, natural conception depends on uniovular cycles. For those reasons, the present study has focused on the implanting ability of individual embryos. Previous studies of IVF treatment and advancing age have shown, using different methods, that despite the decline in numbers of oocytes obtained by stimulation there is no reduction in the proportion reaching full maturity (Grimbizis et al., 1998), nor in their fertilizing and cleaving ability (Oehninger et al., 1995; Abdelmassih et al., 1996; Devroey et al., 1996; Hull et al., 1996; Grimbizis et al., 1998; Bancsi et al., 2000), nor in the proportion of embryos graded of high quality, at least at the 2-day stage when transferred to the uterus (Grimbizis et al., 1998). Nevertheless, implanting ability of individual embryos declines gradually with age (Hull et al., 1996) and by two-thirds or more after 40 years (Hull et al., 1996; Grimbizis et al., 1998).

The present study is the first to address implanting ability of fertilized oocytes (embryos) related to ovarian ‘reserve’ indicated by the basal FSH level independent of age. The findings first of all confirm those in our previous studies (Cahill et al., 1994; Hull et al., 1996) that oocyte numbers collectable after stimulation decline with both rising FSH and age but that fertilizing ability remains favourable and even increases (Hull et al., 1996). In addition, they show a decline in implanting ability of the fertilized oocytes which is related to rising FSH as well as to age, independently (Figure 1). Once pregnancy was established, however, the risk of failure due to miscarriage was determined by age rather than by FSH.

The distinction into discrete groups of observed rates of implantation could be smoothed, as depicted in Figure 1, and the probability of implantation or a live baby resulting from an embryo could be calculated from statistically modelled formulae as shown in Figure 2. The graphs show more steeply declining chance with age of successfully achieving a liveborn baby than just initial implantation, emphasizing the greater effect of age than FSH on risk of miscarriage after pregnancy is established. The findings demonstrate that biological ageing of the ovaries can occur independently of chronological age. It is indicated by rising basal serum FSH concentration, and this is associated with declining oocyte quality, as indicated by reduced implanting ability. Indeed the decline in oocyte quality is evident even as FSH levels rise while still within the normal range. As generally observed (and see Table I), pregnancy rates are markedly reduced despite transfer of multiple embryos after IVF treatment. In natural cycles, with only one embryo available, the reduction would be greater, and this has already been observed in women attending an infertility clinic (Scott et al., 1995). However, natural cycle IVF treatment may serve as an alternative form of treatment.

Discussion
Basal serum FSH levels, measured at their peak during the first 5 days of an ovulation cycle, reflect the number of early antral follicles present which can be recruited to ovulate (Chang et al., 1998), and that number in turn depends on the total ovarian follicular capacity or ‘reserve’ (Faddy and Gosden, 1995) which declines exponentially by atresia (Faddy and Gosden, 1996; Leidy et al., 1998). The levels of FSH begin to rise on average a decade or more before the menopause (Lee et al., 1988; Ebbiary et al., 1994; Klein et al., 1996), though in most individuals levels remain within the normal range at first (Lenton et al., 1988, Ebbiary et al., 1994). Even within the normal range, FSH levels are significantly correlated (inversely) independent of age with ovarian

![Figure 2. Probabilities (a) of implantation or (b) of a live baby related to the woman’s age and FSH level, calculated from statistically modelled formulae.](image-url)
in women with a previous poor ovarian response, as shown by a recent study (Bassil et al., 1999).

It could be argued that oocyte quality may be linked to the number of oocytes obtainable by stimulation and only indirectly to the woman’s age or ovarian reserve. However, after statistical adjustment, we like others (Roest et al., 1996; Van Kooij et al., 1996) could not find an independent effect of the number either of oocytes or resulting embryos obtained on the chance of an individual embryo implanting. Others have found a relationship limited to low numbers but without adjusting for age (Grimbizis et al., 1998); whereas a study of a young women with normal FSH who produced only small numbers of oocytes found no adverse effect on outcome (Lashen et al., 1999).

Heterogeneity of follicular function and oocyte quality is to be expected within a cohort of follicles after excessive stimulation in women, as occurs in naturally polyovular species (Hunter et al., 1994).

Chromosomal abnormalities of the oocyte need not affect fertilization and early embryo cleavage but can be linked to failure of implantation (Zenzes et al., 1992). Defective mechanisms that affect oocytes with increasing frequency in older women include aneuploidy before (Roberts and O’Neill, 1995) or after (Benadiva et al., 1996) fertilization due to disruption of meiotic spindle assembly affecting chromosome arrangement (Battaglia et al., 1996), and mitochondrial DNA deletions (Keefe et al., 1995). It has been proposed that such defects are not due simply to time-related exposure to risk of damage but were probably there from the start, when the oocytes were established in the fetal ovaries (Henderson and Edwards, 1968; Polani and Crolla, 1991; Zheng and Byers, 1992) and arrested during meiosis until eventually ovulated. The earliest oocytes formed in the ovary are the least likely to have suffered mutational change during the sequence of mitotic multiplication preceding oocyte formation. Possibly for that reason, they are also thought to be the earliest recruited in adulthood for growth and ovulation, resulting eventually in a relative preponderance of defective oocytes (Zheng and Byers, 1992). Thus premature reduction of ovarian follicle numbers in young women, whether by excessive atresia or accidental or iatrogenic damage (as suggested by the animal experimental evidence discussed in the introduction), could be expected to lead to advancement of all the reproductive problems. Our findings indicate that basal FSH measurement is important in prognostication and selection of treatment (whether to use own or donated oocytes) in women already troubled by infertility. A wider implication may be the importance of conserving ovarian tissue as far as possible in the surgical treatment of benign conditions.

Our study was limited to women who in many cases had FSH measured in only a single cycle up to 6 months previously. It may however, be possible to improve diagnostic accuracy by measurement in a few cycles. Women with raised levels demonstrate marked variability between cycles and a normal level in such cases can be misleading (Scott et al., 1990). A single raised FSH value, even if subsequent levels are normal, is associated with decreased ovarian responsiveness and implanting ability (Martin et al., 1996). Whether a dynamic test, which would probably have to be limited in practice to a single cycle, would be more or less valuable than measuring basal FSH in repeated cycles remains to be established. Any refinement in the diagnostic application of FSH measurement would be expected to enhance the strength of our findings, which are already clear though based on a single basal sample.

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References


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