Basal FSH concentrations as a marker of ovarian ageing are not related to pregnancy outcome in a general population of women over 30 years

J.M. van Montfrans1,5, M.H.A. van Hooff2, J.A. Huirne1, S.J. Tanahatoe1, S. Sadrezadeh1, F. Martens4, J.M.G. van Vugt3 and C.B. Lambalk1

1Research Institute for Endocrinology, Reproduction and Metabolism, and 3Division of Prenatal Diagnosis, Department of Obstetrics and Gynaecology; 2Division of Obstetrics and Gynecology, St Franciscus Gasthuis, Rotterdam, The Netherlands; 4Research Institute for Endocrinology, Reproduction and Metabolism, Department of Clinical Endocrinology, Vrije Universiteit Medical Centre, Amsterdam and 5To whom correspondence should be addressed. E-mail: j.vanmontfrans@zonnet.nl

BACKGROUND: Previous studies suggest that elevated basal FSH concentrations are related to aneuploid pregnancies. However, there have been no prospective studies evaluating the incidence of aneuploidies in relation to basal FSH concentrations. Since the majority of aneuploid conceptions end in early pregnancy loss or abortion of a recognized pregnancy, these determinants are appropriate intermediate end-points to study aneuploidy.

METHODS: We performed a prospective study in 129 women without a history of subfertility pursuing a spontaneous pregnancy. Basal FSH concentrations were measured during three menstrual cycles. Urinary HCG levels were measured during menstruation for a maximum of six menstrual cycles, to detect early pregnancy loss. We estimated the effect of basal FSH concentrations on pregnancy outcome, taking into account possible confounders.

RESULTS: We observed no significant effect of basal FSH concentrations on the incidence of early pregnancy loss or abortion of clinically recognized pregnancies.

CONCLUSIONS: We conclude that in a population of women without a history of subfertility, pursuing a spontaneous pregnancy, basal FSH concentrations are not related to the incidence of early pregnancy loss or abortions. This prospective study therefore fails to confirm a relationship between signs of decreased ovarian reserve and aneuploid pregnancies.

Key words: abortion/age/aneuploidy/FSH/subfertility

Introduction

The decline in female fecundity with age is caused mainly by decreased oocyte quality in older women, illustrated by the superior results of oocyte donation from younger to older women (Faber et al., 1997; Cohen et al., 1999). In women over 35 years of age, decreased oocyte quality is associated with a prolonged time to pregnancy and with an increased risk of spontaneous abortions. Since the decline in oocyte quality with age does not occur at the same rate in all women, there is a demand for clinical tests identifying women with decreased oocyte quality at an early age, in order to provide diagnostic and prognostic information.

Elevated basal FSH concentrations are a marker for decreased oocyte quality. For example, during controlled ovarian stimulation in IVF, elevated basal FSH concentrations are related to a diminished number of recruitable follicles and to a reduced pregnancy potential (Muasher et al., 1988; Sharif et al., 1998). Several studies reported a relationship between elevated basal FSH concentrations and aneuploid pregnancies; however, these studies were retrospective and may thus have been biased (Nasser et al., 1999; van Montfrans et al., 1999).

In the clinical situation, declining oocyte quality may cause aneuploid conceptions, leading to abortion of clinically recognized pregnancies. The majority of aneuploid conceptions, however, are believed to result in early pregnancy loss (EPL) (Simon et al., 1999), defined as abortion followed by menstruation before an increased cycle length is noticed. EPL is identified by a significant rise in serum or urinary HCG concentrations around the expected time of menses. The incidence of EPL was reported as between 6 and 27% per menstrual cycle with unprotected intercourse, depending on the group characteristics of the women studied, laboratory assay and definition of EPL (Whittaker et al., 1983; Ellish et al., 1996). The incidence of chromosomal abnormalities in EPL is estimated to be >60% (Strom et al., 1992; Nicolaides and Petersen, 1998). Thus, clinical studies investigating the relationship between oocyte quality and pregnancy outcome should include the incidence of EPL, together with the number of clinically recognized abortions and ongoing pregnancies.
We tested the hypothesis that elevated basal FSH concentrations, as markers of decreased oocyte quality, predict EPL and abortion in a prospective study in couples without prior history of subfertility pursuing a spontaneous pregnancy.

Subjects and methods

Study design, data collection and follow-up
We conducted a prospective study addressing the predictive value of basal FSH concentrations on pregnancy outcome in 129 women who were actively planning a spontaneous pregnancy. The study protocol was approved by the local ethics committee and written informed consent was given by all participants.

Participants were recruited by advertisements in local newspapers. Inclusion and exclusion criteria are shown in Table I. We selected women >30 years of age without known risk factors for decreased fecundity or spontaneous abortion. The age criterion was used to select for women with a relatively high a priori risk of spontaneous abortion (Hansen, 1986). Participants completed a written questionnaire addressing medical history, and were provided with conventional pregnancy tests (Abbott, UK), to be performed in the case of amenorrhoea >7 days after the expected onset of menses.

During the first three menstrual cycles of the study protocol (or until a pregnancy was confirmed with a conventional pregnancy test), participants provided one serum sample per cycle for basal FSH measurement on cycle day 3. This day was used unless it was during a weekend, in which case cycle day 2 or 4 was used. Samples were centrifuged and serum was isolated and stored at −20°C until further analysis. To detect EPL, first void morning urine samples were collected for ultrasensitive HCG measurement on days 1 and 2 of the menstrual period during the first six menstrual cycles of the study protocol (or until a pregnancy was confirmed with a conventional pregnancy test) (Qu et al., 1997). These samples were stored at −20°C without additional preparation until further analysis. This method to detect EPL was described by Weinberg et al. (1992). They performed urinary HCG analyses throughout the menstrual cycle, and concluded that measurements on cycle days 1 and 2 would only minimally lower the detection rate for EPL.

All participants were followed-up with written questionnaires for 12 months after the start of the first menstrual cycle of the study protocol. Every ongoing pregnancy that occurred during this period was followed-up until delivery.

Laboratory assays
Basal FSH values were measured using an immunoluminometric assay (ILMA; Amerlite FSH, Amersham, UK), with an interassay coefficient of variation (CV) of 8% at 5 IU/l and 5% at 15 IU/l.

Urinary HCG values were measured using an ILMA (Amerlite ILMA assay, based on the results in our IVF population. Using this cut-off level, 6% of the participants that became pregnant (5/86) in the current study were classified as having elevated FSH levels.

Ultraseensitive HCG measurement may yield false-positive pregnancy test results, since there is minimal HCG production in non-pregnant women (Wilcox et al., 1988). In order to determine the cut-off value to define pregnancy, 20 regularly menstruating women who had undergone tubal ligation provided first void urine samples on menstrual cycle days 1 and 2 for ultrasensitive HCG measurement. The highest HCG value in these women was 0.94 IU/l. We therefore considered EPL to have occurred in the case of a urinary HCG concentration during menses of >1.00 IU/l.

Definitions of pregnancy outcomes
Pregnancy outcomes were divided into the following categories: EPL, spontaneous abortion, early delivery or ongoing pregnancy. EPL was defined as a urinary HCG concentration >1.00 IU/l on days 1 or 2 of a menstrual period occurring within 5 weeks after the onset of the preceding menstrual period. A spontaneous abortion was defined as a positive result of a routine pregnancy test within 5 weeks after the onset of the preceding menstrual period, followed by a spontaneous abortion in the first 16 weeks of pregnancy. Early delivery was defined as delivery between 17 and 25 weeks of pregnancy. An ongoing pregnancy was defined as a pregnancy resulting in a delivery after 25 weeks of gestation.

Statistics
Continuous variables were compared using a Students t-test and categorical variables with χ² tests. For each participant, mean basal FSH values were calculated. Since these values had a skewed distribution towards the higher FSH concentrations, log₁₀ transformed values, showing a normal Gaussian distribution, were used subsequently.

For each patient, only the first pregnancy during the study protocol was included in the analysis. One-way ANOVA was performed to

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age 30 or older</td>
<td>Use of hormonal medication interfering with the hypothalamus–pituitary–ovarian axis</td>
</tr>
<tr>
<td>Not pregnant</td>
<td>Attempting to achieve a pregnancy &gt;6 months</td>
</tr>
<tr>
<td>Regular menstrual cycles &lt;36 days (variation in cycle length &lt;10 days)</td>
<td>Known subfertility due to a male factor</td>
</tr>
<tr>
<td>Written informed consent</td>
<td>History of pelvic inflammatory disease</td>
</tr>
<tr>
<td></td>
<td>Previous period of subfertility &gt;12 months</td>
</tr>
<tr>
<td></td>
<td>Uterine anomalies (e.g. leiomyomata, congenital anomalies)</td>
</tr>
<tr>
<td></td>
<td>History or current use of any assisted reproduction technique</td>
</tr>
</tbody>
</table>
investigate differences between pregnancy outcomes for mean FSH concentration and for the following possibly confounding factors: age, parity, time to pregnancy, percentage of abortions in earlier pregnancies, number of months of oral contraceptive use and number of cigarettes smoked per day (Cramer et al., 1994; Lambalk and de Koning, 1998).

We performed stepwise logistic regression analysis to estimate the influence of possible confounders on the relationship between FSH and pregnancy outcome. We subsequently defined as end-points ongoing pregnancy versus EPL and ongoing pregnancy versus EPL and clinical abortion.

χ² tests were performed to investigate the number of women with a basal FSH concentration >12.5 IU/l in any of the three measured cycles in relation to the incidence of EPL or abortion.

Statistics were performed using SPSS Base 9.0 for Windows (SPSS Inc., Chicago, IL). Statistical significance was set at \( P < 0.05 \).

Power calculation
A sample size of 80 pregnancies was needed to detect a relative risk of 3.0 for the relationship between elevated basal FSH levels and the incidence of EPL with a statistical power of 0.80. This calculation was made assuming an abortion rate of 20% in the unexposed women and a fraction of women with elevated basal FSH values of 5%. To account for women not becoming pregnant within 12 months, the planned sample size was 125.

Results

Participant inclusion and compliance
A total of 129 women fulfilled the inclusion and exclusion criteria and signed informed consent. Seven participants withdrew from the study before the end of the study protocol. Data of these participants were included in the analysis for the duration of their participation (5.2 ± 2.1 months).

Study outcome

Basic characteristics are reported in Table II. Fifty percent of the participants had not been pregnant before. The mean time participants were pursuing a pregnancy was 3.5 months, and mean female age was 34.2 years.

During the 12 month follow-up period, 86 of 129 women (67%) became pregnant. Outcomes of the first pregnancies occurring during the study protocol were divided up as follows (Table III). Twenty-two out of 86 women (26%) had EPL; 18 (21%) had abortion of a clinically confirmed pregnancy before 16 weeks of gestation; and in one woman (1%) the first pregnancy resulted in early delivery (between 16 and 25 weeks of gestation). In 45 out of 86 women (52%), the first pregnancy during the study protocol resulted in the delivery of a live born child after 25 weeks of gestation. In these live born children, there were no dysmorphic features that would have indicated the possible presence of chromosomal abnormalities. There were no ectopic pregnancies or induced abortions.

Single FSH values ranged from 1.3 to 16.0 IU/l, and mean FSH concentrations ranged from 3.7 to 15.6 IU/l. Table IV presents participant characteristics for each outcome category. In the one-way ANOVA, we found no significant effect of FSH concentrations between the four different outcome categories. Stepwise logistic regression analysis showed no significant confounding effects towards the relationship between basal FSH levels and pregnancy outcome by the following variables: age, parity, period pursuing a pregnancy at entry to the study, time to pregnancy, spontaneous abortion rate in earlier pregnancies, number of cigarettes smoked per day and period of oral contraceptive use. Post hoc \( t \)-test analysis showed that compared with women with ongoing pregnancies, those with EPL had a longer time to pregnancy (5.0 versus 3.4 months, \( P = 0.04 \)), and smoked more cigarettes per day (3.6 versus 0.8, \( P = 0.03 \)).

Two out of five (40%) of the women with elevated FSH levels had EPL, versus 20 out of 81 (25%) of the women with normal FSH levels [relative risk 1.62, 95% confidence interval (CI) 0.5–5.1]. Two out of five (40%) of the women with elevated FSH levels had EPL or abortion of a clinically recognized pregnancy, versus 38 out of 81 (47%) of the women with normal FSH levels [relative risk 0.85, 95% CI 0.28–2.56].

Discussion

We found no significant relationship between basal FSH concentrations and pregnancy outcome in this prospective study in women without a history of subfertility planning a spontaneous pregnancy. Since basal FSH concentrations were not related to the incidence of EPL or abortion, we conclude from these data that elevated basal FSH concentrations do not indicate decreased oocyte quality in this population. Several remarks concerning our findings are warranted, however.

Pregnancy rates and incidence of abortion and EPL were comparable with those mentioned in other studies (Hansen, 1986). A clinically recognized pregnancy was reported by 86 out of 129 women (67%) after 12 months of unprotected intercourse, which could be expected given the mean age of the participants. Spontaneous abortion was seen in 18 out of 86 pregnancies (21%). Earlier studies reported EPL rates per pregnancy of between 11 and 70% (depending on HCG assay,
The results of this study do not support the findings of earlier studies, reporting a relationship between decreased ovarian reserve and the incidence of chromosomal abnormalities (Nasseri et al., 1999; van Montfrans et al., 1999; Freeman et al., 2000). However, these studies were retrospective and may have been biased. Also, these studies measured specific population characteristics and cut-off criteria used to define EPL, while we found an EPL rate of 26%.

Participants analysed in this study did not fully represent an average group of couples without known subfertility factors planning a spontaneous pregnancy. First and most importantly, not all participants were included before their first menstrual cycle with unprotected intercourse, but after a mean period of 3.5 months. This resulted in selection bias in favour of women with lower fecundity than women who became pregnant in the first 3 months of unprotected intercourse. Secondly, women over 30 years of age were selected for their relatively high a priori risk of spontaneous abortion. It remains speculative if inclusion of women under 30 years of age, with higher fecundity, would yield a wider range of FSH values, thus possibly showing a significant relationship between FSH values and pregnancy loss. Thirdly, only 6% of participants had elevated basal FSH levels (>12.5 IU/l) in this group of women without a known history of subfertility. χ² tests showed no significant relationship between EPL or abortion and elevated FSH levels. However, since the majority of EPL or abortion cases occurred in women with normal FSH levels, it is unlikely that inclusion of more women with elevated basal FSH levels would alter the results of this study.

Possibly, a type II error (false-negative finding) may have occurred due to the use of basal FSH screening to detect decreased ovarian reserve and associated diminished oocyte quality. Basal FSH testing previously showed significant predictive value only in patients undergoing assisted reproduction treatment, but not in women from a general subfertility population (van Montfrans et al., 2000). A meta-analysis of studies investigating the performance of basal FSH concentrations as a predictor of IVF outcome showed limited clinical value of the test (Bancsi et al., 2003). Basal FSH values may actually reflect response to ovarian stimulation in IVF rather than oocyte quality, as recently suggested (van Rooy et al., 2003). Tests other than basal FSH screening may be more useful to measure oocyte quality: it was shown that the clomiphene challenge test had higher predictive values than basal FSH screening alone to detect decreased fecundity (Scott et al., 1993). The endogenous FSH ovarian reserve (EFOR) test and ultrasound assessment of ovarian reserve by antral follicle count have also been mentioned as superior tests in comparison with basal FSH screening (Fanchin et al., 1994; Bancsi et al., 2002). Recently, FSH receptor polymorphisms were shown to be related to basal FSH concentrations. FSH receptor polymorphisms may be responsible for inter-individual differences in basal FSH concentrations, apart from differences in ovarian reserve (primordial follicle store). Future research on the relationship between chromosomal aneuploidies during pregnancy and ovarian reserve should use more specific tests to assess ovarian reserve than basal FSH screening alone, such as dynamic testing or ultrasound measurement of follicle count. FSH receptor status could augment the predictive value of the clomiphene challenge test and basal FSH screening by identification of false-positive test results due to FSH receptor polymorphisms.

This study aimed to detect a relationship between FSH values and pregnancy loss, using pregnancy loss as a marker for oocyte quality. However, pregnancy loss may also occur due to other causes such as implantation errors or immunological incompatibility. Our data indicate a relationship between smoking and EPL, a relationship that previously was reported for pregnancy loss in general but not for EPL (Wilcox et al., 1990). Previous studies have shown that the percentage of aneuploidies in pregnancy loss in women over 30 years of age ranges from 69–78% for EPL to 23–66% for clinically recognized abortions (Boue et al., 1998). Even when assuming that 23% of pregnancy loss cases were caused by aneuploidies (the lowest rate of aneuploidies in pregnancy loss reported in previous studies), this study would still have the statistical power to detect a relative risk of 4.5 with a β of 0.80. Therefore, we conclude from our data that it is unlikely that there is a relationship between basal FSH concentrations and chromosomal aneuploidies in these participants. However, as the proxy variable used may be considered possibly inaccurate for assessing oocyte quality due to the fact that pregnancy loss is not uniquely aneuploidy related, a relationship between basal FSH and oocyte quality cannot be fully excluded.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Not pregnant (n = 43)</th>
<th>EPL (n = 22)</th>
<th>Abortion + early delivery (n = 19)</th>
<th>Ongoing pregnancy (n = 45)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal FSH</td>
<td>7.0 (2.3)</td>
<td>6.7 (2.4)</td>
<td>6.1 (1.4)</td>
<td>7.0 (2.1)</td>
<td>0.36</td>
</tr>
<tr>
<td>Age</td>
<td>35.1 (3.8)</td>
<td>34.0 (2.3)</td>
<td>34.0 (3.4)</td>
<td>33.6 (2.7)</td>
<td>0.17</td>
</tr>
<tr>
<td>Parity</td>
<td>0.9 (1.3)</td>
<td>1.1 (1.5)</td>
<td>1.2 (1.7)</td>
<td>0.9 (1.3)</td>
<td>0.86</td>
</tr>
<tr>
<td>Period pursuing pregnancy at entry to the study (months)</td>
<td>4.0 (2.3)</td>
<td>3.6 (1.9)</td>
<td>2.4 (2.2)</td>
<td>3.4 (1.8)</td>
<td>0.18</td>
</tr>
<tr>
<td>Cigarettes per day</td>
<td>1.5 (4.3)</td>
<td>3.6 (3.6)</td>
<td>0.7 (2.2)</td>
<td>0.8 (3.3)</td>
<td>0.13</td>
</tr>
<tr>
<td>BMI</td>
<td>0.24 (0.05)</td>
<td>0.23 (0.05)</td>
<td>0.24 (0.03)</td>
<td>0.24 (0.05)</td>
<td>0.99</td>
</tr>
<tr>
<td>Time to pregnancy (months)</td>
<td>NA</td>
<td>5.0 (3.4)</td>
<td>3.6 (3.0)</td>
<td>3.4 (3.0)</td>
<td>0.21</td>
</tr>
<tr>
<td>% abortion in earlier pregnancies</td>
<td>22 (33)</td>
<td>20 (33)</td>
<td>16 (33)</td>
<td>20 (30)</td>
<td>0.96</td>
</tr>
</tbody>
</table>

Values are mean (SD) unless indicated otherwise. EPL = early pregnancy loss; abortion = abortion of a clinically recognized pregnancy; NA = not applicable; BMI = body mass index.
aneuploidies (Down syndrome) or aneuploidies in general, and did not use EPL or abortion as end-points, which makes it difficult to compare these studies with our findings. Regarding the use of basal FSH screening in subfertility settings, our findings fail to show decreased fecundity in women with elevated FSH concentrations. Previously, we showed that basal FSH screening had limited predictive value in a general subfertility population (van Montfrans et al., 2000). Although basal FSH screening is an easy test, it has only been shown to have sufficient predictive value towards fecundity in IVF populations (Scott et al., 1989; Toner et al., 1991).

In conclusion, we found no relationship between basal FSH concentrations and pregnancy outcome in a prospective study of women planning a spontaneous pregnancy. Our data indicate that basal FSH concentrations most probably cannot be used to measure oocyte quality in these women.

Acknowledgements

The authors thank all participants for their cooperation, and gratefully acknowledge Dr C.Popp-Snijders and co-workers of the endocrinology laboratory for performing all laboratory assays. Mrs M.Rodenburg is thanked for secretarial support. This study was supported by grant number 28-2770-1 of the Dutch Praeventiefonds-Zorg Onderzoek Nederland, The Hague, The Netherlands.

References

Bancsi LF, Broekmans FJ, Mol BWJ et al. (2003) Performance of basal follicle-stimulating hormone and pregnancy outcome in a prospective study of women planning a spontaneous pregnancy. Our data indicate that basal FSH concentrations most probably cannot be used to measure oocyte quality in these women. 


Freeman SB, Yang Q, Allran K et al. (2000) Women with a reduced ovarian complement may have an increased risk for a child with Down syndrome. Am J Hum Genet 66,1680–1683.


Submitted on April 30, 2003; resubmitted on September 30, 2003; accepted on October 23, 2003