

Basal FSH, estradiol and inhibin B concentrations in women with a previous Down's syndrome affected pregnancy

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BACKGROUND: Two recent studies reported elevated basal FSH concentrations in women with a history of aneuploid conceptions. However, it is not known whether these elevated basal FSH concentrations reflect depletion of the primordial follicle pool, or were caused by an increased secretory drive for FSH. **METHODS:** Inhibin B and estradiol concentrations were measured as indicators for depletion of the primordial follicle pool in women with a history of a Down's syndrome pregnancy and in controls. **RESULTS:** In the women with a history of a Down's syndrome pregnancy, there was a significant inverse correlation between basal FSH and inhibin B concentrations ($P < 0.0001$, 95% CI -0.26 to -0.56). In the control group, this correlation did not reach statistical significance. **CONCLUSIONS:** Our data indicate that the elevated basal FSH concentrations observed in women with a history of a Down's syndrome pregnancy are likely to reflect early depletion of the primordial follicle pool. Therefore, further research into the ovarian ageing process could provide more insight in the origination of chromosomal abnormalities during pregnancy.

Key words: Down's syndrome/FSH/inhibin B/ovarian ageing

Introduction

Trisomy-21 and other aneuploidies during pregnancy were reported to be associated with elevated FSH concentrations in the early follicular phase of the menstrual cycle (Nasseri *et al.*, 1999; van Montfrans *et al.*, 1999). Another study reported a significant relation between Down's syndrome pregnancies and a reduced ovarian reserve (Freeman *et al.*, 2000). In reproductive endocrinology, elevated basal FSH concentrations are considered a sign of depletion of the primordial follicle pool, based on reports that fertility patients with elevated basal FSH concentrations have impaired fecundity and a decreased ovarian response to exogenous gonadotrophins (Muasher *et al.*, 1988; Scott *et al.*, 1989; Toner *et al.*, 1991; Magarelli *et al.*, 1996; Martin *et al.*, 1996; Buyalos *et al.*, 1997; Gurgan *et al.*, 1997; Sharif *et al.*, 1998). However, other factors than depletion of the primordial follicle pool may also influence FSH concentrations, such as smoking, recovery from lactational amenorrhoea, the cessation of oral contraceptives, familial hereditary dizygotic twinning (FHDT) or an increased secretory drive for FSH (Lambalk and de Koning, 1998). Therefore, elevated FSH concentrations do not necessarily imply depletion of the primordial follicle pool, and the question therefore arises

whether the elevated basal FSH concentrations in women with a history of aneuploid pregnancies indeed reflect a diminished ovarian reserve.

The aim of the current study was to clarify whether the elevated basal FSH concentrations in women with a history of a Down's syndrome pregnancy were due to a diminished ovarian reserve or to an increased secretory drive, controlling for other causes of elevated FSH concentrations. We addressed this issue by measurement of basal inhibin B and estradiol concentrations in relation to Down's syndrome pregnancies, since basal state concentrations of these hormones were reported to be associated with depletion of the primordial follicle pool (Khalifa *et al.*, 1992; Klein *et al.*, 1996; Buyalos *et al.*, 1997; Danforth *et al.*, 1998; Nasseri *et al.* 1999; Seifer *et al.*, 1999).

Materials and methods

A total of 118 women with a history of a Down's syndrome pregnancy (all except one resulting in live born children) and 102 controls (mothers of at least two children without Down's syndrome) provided serum samples in the early follicular phase of three consecutive menstrual cycles (van Montfrans *et al.*, 1999). Cycle day 3 was used

Table I. Basic characteristics in women with a history of a Down's syndrome pregnancy and controls, expressed as means and (SD) unless otherwise indicated

	Patient group		P-value
	Down's syndrome mothers (n = 118)	Controls (n = 102)	
Age	33.8 (3.2)	34.2 (3.0)	NS
Smoking habits			
No. of current smokers	29 (34.2%)	26 (26.5%)	NS
Pack-years in current smokers	10.0 (8.8)	8.9 (6.1)	NS
No. of women with a family history of familiar dizygotic twinning	1 (1.2%)	2 (2.0%)	NS

NS = not significant.

for sample collections, unless this day was a weekend day, in which case cycle day 2 or 4 was used. All participants had experienced two or more spontaneous menstrual cycles after discontinuation of breastfeeding or the use of oral contraceptives. FSH and estradiol concentrations were measured in all three consecutive menstrual cycles, while inhibin B concentrations were measured during the cycle with the highest basal FSH concentration only. Participants provided information on smoking habits and family history by means of written questionnaires.

After centrifugation, samples were stored in aliquots at -80°C for a maximum of 12 months. For each analysis, a fresh aliquot was used. FSH concentrations were measured with a MEIA (micro particle enzyme immunoassay; Abbott Laboratories, Abbott Park, IL, USA), calibrated against the second International Reference Preparation for FSH (78/549). The intra- and interassay coefficients of variation were 3.7–7.6 and 0–5.9% respectively. Estradiol concentrations were measured with a competitive immunoassay (Amerlite, Kodak Clinical Diagnostics, Amersham, UK), with an intra- and interassay coefficient of variation of 13 and 11% for estradiol concentrations <500 pmol/l respectively. Inhibin concentrations were determined immunometrically by a commercially available assay (Serotec Ltd, Oxford, UK), using an immunopurified preparation of human follicular fluid to establish the standard curve. The interassay variability for the inhibin B assay was 17% at 25 ng/l, 14% at 55 ng/l and 9% at 120 ng/l; the lower limit of detection was 15 ng/l.

Data were analysed using SPSS Base 7.5 for Windows (SPSS Inc., Chicago, IL, USA). Basic characteristics were compared between Down's syndrome mothers and controls by Student's *t*-tests or χ^2 tests as appropriate. Mean concentrations of FSH, estradiol and inhibin B were compared between the two groups using a Student's *t*-test. Since basal FSH values did not have a Gaussian distribution, these data were \log_{10} -transformed before analysis. Estradiol and inhibin B concentrations were distributed in a Gaussian way and therefore no \log_{10} transformation was used on these values. The Pearson's correlation coefficient was calculated for the correlation between basal FSH and inhibin B concentrations and between basal FSH and estradiol concentrations. Statistical significance was set at $P < 0.05$.

Results

Basic characteristics are shown in Table I. There were no significant differences in age distribution, smoking habits or in the incidence of familiar dizygotic twinning between Down's syndrome mothers and controls. Other factors possibly influencing basal FSH concentrations (recent cessation of the use

of oral contraceptives or recent recovery from lactational amenorrhoea) were considered exclusion criteria.

Mean concentrations of FSH, inhibin B and estradiol are shown in Table II. Basal FSH concentrations were significantly elevated in Down's syndrome mothers. There were no immediate significant differences in mean inhibin B and estradiol concentrations between Down's syndrome mothers and controls. However, in Down's syndrome mothers, there was a statistically significant correlation between basal FSH and inhibin B concentrations; Pearson's correlation -0.42 [$P < 0.0001$, 95% confidence interval (CI): -0.26 to -0.56 , Figure 1]. In the control group, there was no relation between basal FSH concentrations and inhibin B concentrations (Pearson's correlation -0.024 , $P = 0.81$, 95% CI: -0.17 to 0.22 , Figure 2).

Both in Down's syndrome mothers and in controls, the correlation between basal FSH concentrations and estradiol concentrations was low [$r = -0.095$ ($P = 0.32$) and $r = -0.11$ ($P = 0.26$)] for Down's syndrome mothers and controls respectively.

Discussion

Our results show that there is a significant inverse correlation between early follicular phase FSH concentrations and inhibin B concentrations in women with a history of a Down's syndrome pregnancy. Although there was no difference in mean inhibin B concentrations between Down's syndrome mothers and controls, these results support the hypothesis that the elevated FSH concentrations in women with a history of a Down's syndrome pregnancy reflect a compromised primordial follicle pool, and not an increased secretory drive for FSH. The inverse correlation between basal FSH and inhibin B in Down's syndrome mothers represents an endocrine connection to decreased oocyte quality, as previously reported by Seifer *et al.* in patients undergoing assisted reproduction therapy (Seifer *et al.*, 1997, 1999).

Two mechanisms may underlie the relation between inhibin B concentrations and oocyte quality. In the first place, impaired oocyte quality leading to aneuploid conceptions may occur together with a decrease in the number of available follicles with quantitatively less granulosa cells, resulting in a decrease of hormonal feedback. This is in line with several

Table II. Endocrine parameters in women with a history of a Down's syndrome pregnancy and controls, expressed as means and (SD) unless otherwise indicated

	Patient group		P-value
	Down's syndrome mothers (n = 118)	Controls (n = 102)	
Mean basal FSH concentration (IU/l) in three menstrual cycles	6.9 (2.1)	6.3 (1.7)	0.017
No. of cases with an FSH concentration >11.5 IU/l (%) in any of the three cycles	16 (14%)	5 (5%)	0.029
Mean basal estradiol concentration (pmol/l) in three menstrual cycles	118 (51)	112 (48)	NS
Mean basal inhibin B concentration (pg/ml) corresponding to the cycle with the highest FSH concentration	86.4 (36.6)	81.6 (40.2)	NS

NS = not significant.

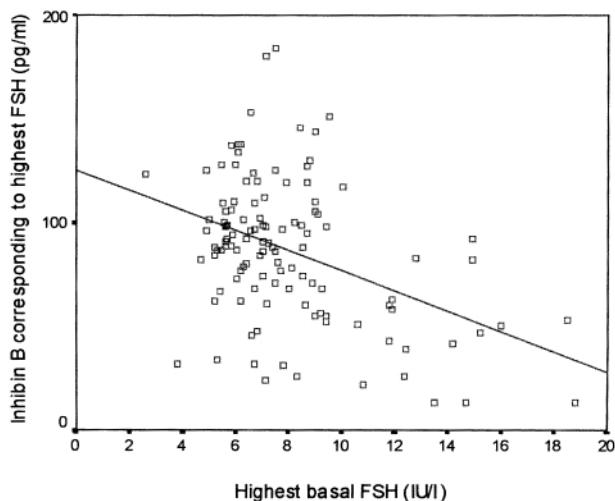


Figure 1. Inhibin B concentrations according to highest basal FSH concentrations in women with a history of a Down's syndrome pregnancy. Pearson's $r = -0.42$, $P < 0.0001$, $n = 118$.

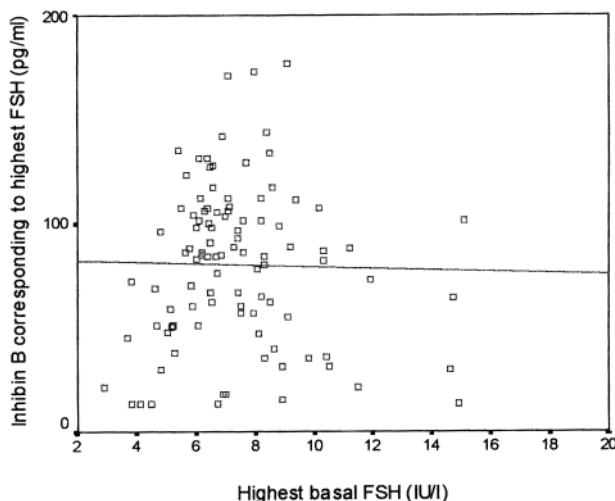


Figure 2. Inhibin B concentrations according to highest basal FSH concentrations in controls, Pearson's $r = -0.024$, $P < 0.81$, $n = 102$.

studies reporting a relation between the size of the antral follicle cohort and oocyte quality, for example patients with a decreased number of recruitable follicles during IVF ('poor responders') were also shown to have a reduced fecundity per oocyte retrieved (Muasher *et al.*, 1988; Roest *et al.*, 1996). In the second place, the granulosa cells *per se* may function suboptimally and secrete less inhibin B into the follicles with qualitatively compromised oocytes. Indeed, Vitt *et al.* and Hayashi *et al.* recently reported that the oocyte directly influences granulosa cell differentiation by production of GDF-9, a growth factor also postulated to influence inhibin B concentrations (Hayashi *et al.* 1999; Vitt *et al.*, 2000). In case of a decreased oocyte quality, impaired GDF-9 production may indirectly cause decreased inhibin B concentrations. Thus, the findings of our study are in-vivo support that a diminished oocyte quality might be directly associated with a decrease in its surrounding granulosa cell functioning.

The fact that we observed no significant correlation between basal FSH concentrations and inhibin B concentrations in the control group is likely to be caused by the smaller range of FSH concentrations in the control group. We hypothesize that a significant correlation between basal FSH concentrations and inhibin B concentrations would also be observed in the control group, had this group included more women with elevated basal FSH concentrations. Alternatively, increased basal FSH concentrations in the control group may also have been caused by an increased secretory drive for FSH and not by a diminished ovarian reserve.

Considering basal estradiol concentrations, we found no significant correlation with FSH concentrations, which is possibly due to the timing of blood sampling. Since estradiol concentrations rise predominantly in late follicular phase of the menstrual cycle, the early follicular phase of the menstrual cycle is likely to be too early to detect a relation between FSH and estradiol concentrations. Alternatively, the fact that other hormones than estradiol influence FSH concentrations as well may obscure the relation between basal FSH and estradiol concentrations on cycle day 3.

The difference in basal FSH concentrations between Down's syndrome mothers and controls was not large, and there was

considerable overlap in FSH and inhibin B concentrations between Down's syndrome mothers and controls. In our opinion, this means that basal FSH values are not a specific parameter for advanced ovarian ageing in relation to an increased risk for a Down's syndrome pregnancy. Other mechanisms than elevated basal FSH values may play a role as well in the origination of Down's syndrome (Eichenlaub-Ritter, 1996). Also, there may be other possible causes of elevated FSH concentrations then reduced ovarian reserve (Lambalk and de Koning, 1998). These causes theoretically include altered FSH activity or resistance to FSH (Anobile *et al.*, 1998; Dahl *et al.*, 1988) possibly caused by altered FSH receptor genotype (Perez Mayorga *et al.*, 2000).

In conclusion, our findings provide further evidence for the theory that accelerated ovarian ageing may lead to an increased aneuploidy rate in oocytes. Further research of the ovarian ageing process could therefore provide more insight in the origination of chromosomal abnormalities during pregnancy.

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