Pituitary ovarian interaction in women with elevated early follicular phase FSH

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Summary

This thesis is focused on the background of elevated levels of FSH in the early follicular phase of women with regular menstrual cycles. In the introduction (*chapter 1*) we describe the characteristics of female reproductive aging which is directed by ovarian aging. We review the endocrine aspects of female reproductive aging in particular the elevation of FSH and in a later stage elevation of LH, and the changes in steroids and inhibins. Furthermore the changes in cycle length and follicle growth are discussed, as well as other substances which might be related to ovarian aging like activins, GnSIF and AMH. Other factors related to elevation of early follicular phase FSH, but not to limited ovarian reserve are also discussed.
The aims of the thesis were:

1. To gain more insight into endocrine and ultrasound events in relatively younger women with a history of elevated early follicular phase FSH levels.
2. To evaluate the neuroendocrine mechanisms responsible for elevated FSH.
3. To study the issue of ovarian reserve estimation by taking ovarian biopsies.
4. To evaluate the ovarian FSH threshold in women with elevated FSH and a regular menstrual cycle.
5. To study the distribution of FSH receptor variants in subfertile women with elevated basal FSH levels and a regular menstrual cycle.

Chapter 2 presents the endocrine and ultrasound monitoring study we performed in 22 patients with a history of elevated day 3 FSH levels and 16 controls. Eleven patients showed elevated basal FSH levels in the study cycle ("High, High"; H,H group), whereas eleven had normalized basal FSH levels ("High, Low"; H,L group). AMH was lower in both patient groups compared to controls. In the H,H group, FSH was higher in all phases of the cycle and both inhibin A and inhibin B were lower during the early follicular phase. In the H,L group, FSH was also higher in the early follicular phase and late luteal phase, and inhibin A was higher in the peri-ovulatory phase.
"Normalization" of day 3 FSH in women with previously elevated FSH was associated with inhibin B levels that became normal in the mid- and late luteal phase of the preceding cycle compared to the lower inhibin B levels when day 3 FSH values remain elevated.
The persistently low AMH levels in combination with constantly and intermittently elevated day 3 FSH levels indicate that these younger patients have diminished ovarian reserve. The endocrine cycle profile in patients with consistently elevated basal FSH resembles that of published data from older women. However, patients who present with elevated early follicular phase FSH but normal FSH in the subsequent cycle are

characterized by normalization of inhibin B in the preceding luteal phase, indicating a temporary increase of the available cohort. Peri-ovulatory inhibin A hypersecretion in the subsequent cycle could thus be a result of multiple follicle growth.

Chapter 3 describes the characteristics of episodic secretion of FSH and LH on day 3 of the menstrual cycle in a group of 13 women with elevated FSH levels (> 10 IU/l) and 16 controls. The pituitary response to gonadotrophin-releasing hormone (GnRH) was also measured. The LH and FSH pulse frequency did not differ between the groups. The FSH and LH pulse amplitudes were increased in the elevated FSH group, as well as the LH and FSH response to GnRH. Oestradiol was not different between the groups, but both inhibin A and inhibin B were lower in the patients with elevated FSH levels. We concluded that in these women the pituitary is more sensitive to GnRH.

In *chapter 4*, we studied the issue of ovarian reserve estimation by taking ovarian biopsies. In the evaluation of ovarian reserve, a test to estimate the number of follicles in the ovary has been long searched for. We examined the feasibility of ovarian biopsy for this purpose. We investigated whether any biopsy regimen is representative of the follicular reserve in a human ovary. Three whole ovaries, removed from patients of reproductive age during operations not involving ovarian pathology, were utilized to count the number and type of follicles found in multiple biopsies of 2 and 5 mm and in the whole ovary. Representative results taking into account the total number of follicles found in the whole ovary showed that predicted values based on biopsies were extremely varied. We concluded that due to the huge variation in the distribution of follicles across the surface of the ovary, there is no place for this procedure in clinical evaluation of reproductive aging in the individual patient.

In *chapter 5* the FSH threshold for monofollicular growth in patients with elevated early follicular phase FSH levels was evaluated. The sensitivity of follicles for FSH can be expressed by the FSH threshold. In six patients and thirteen controls the FSH threshold was determined with GnRH agonist desensitization and an ultra-low-dose step-up protocol. The FSH threshold in the patient group with elevated basal FSH levels was 6.75 IU/l and significantly higher than the FSH thresholds of the controls (4.65 IU/l). The FSH screening value on day 3 was 12 IU/l in the patient group and 5.0 IU/l in the controls. In the control group the basal FSH levels correlated with the FSH threshold levels ($r = 0.8$), but in the patients with elevated basal FSH this correlation was absent. In women with elevated early follicular phase FSH levels the FSH threshold is higher but not as high as their basal FSH levels. We believe that intraovarian factors are responsible for the higher FSH threshold. Basal FSH overshoots the threshold, probably because of the limited feedback of the ovary.

Chapter 6 describes three cases in which elevated early follicular phase levels were falsely elevated due to interference in the immunometricassay. If falsely elevated FSH levels are suspected the laboratory can perform serial dilution tests and PEG precipitation, cross-check with an alternative assay system, or measure additional hormones (LH, inhibin B, AMH).

In *chapter 7* the distribution of a polymorphic variant of the FSH receptor (N680S), which is associated with higher FSH levels in the early follicular phase and an increased FSH requirement to obtain follicular response in IVF patients, was described in a group of regularly menstruating subfertility patients with elevated basal FSH levels. This receptor variant is thought to be less sensitive to FSH and higher endogenous FSH levels may represent a natural compensation which is needed to enable normal follicle growth. The aim of the study was to test the hypothesis that this receptor isoform occurs more frequently in patients with elevated basal FSH levels, compared to women with normal basal FSH levels. A retrospective cohort study of 38 patients with a regular menstrual cycle and elevated (> 10 IU/l) compared to 40 patients with normal early follicular phase FSH was carried out. DNA was analysed to determine the FSH receptor genotype. The N680S variant on one or both alleles of the FSH receptor gene was significantly more prevalent in patients with elevated FSH. The conclusion of the study was that the N680S receptor variant of the FSH receptor is indeed more frequently found in women with elevated basal FSH levels and that it suggests a higher FSH threshold.

In *chapter 8* the findings of the studies described in this thesis, the clinical consequences and the future perspectives are discussed. The endocrine profile of younger women with elevated early follicular phase FSH levels and regular cycles is comparable to older reproductive aged women, however, when FSH is temporary normalized higher inhibin A levels were observed, which can be related to multiple follicle growth. The elevation of FSH is the result of relaxation of ovarian inhibin restraint of GnRH-independent pituitary FSH secretion, and relaxation of restraint by GnSIF on GnRH-dependent FSH secretion rather than changes in ovarian steroid hormones. The intercycle variability of day 3 FSH levels can be explained by 1) advanced follicular growth due to an earlier start of the growing follicle which results in a shorter cycle length, and 2) variation of cohort size with temporary normalization of the cohort. Multiple follicle growth is related to higher FSH levels as seen in spontaneous dizygotic twinning and familial dizygotic twinning. In our study there was a tendency to multiple follicle growth in the group of women with variable elevated FSH levels.
The clinical consequences of elevated FSH in young women are discussed. Since basal FSH in regularly cycling women is not a good predictor for non-pregnancy,

we should question the value of routinely screening for elevated basal FSH in counselling the subfertility patient. Clinicians should be familiar to the differential diagnosis of elevated FSH: technical errors in the immunoassay, familial dizygotic twinning and the N680S receptor variant of FSH. Follicles are unequally distributed across the ovarian cortex which makes ovarian biopsies to estimate the number of follicles in the ovary unreliable. Although the FSH threshold in women with elevated early follicular phase FSH levels is slightly higher, it should be questioned if in IVF stimulation protocols a higher FSH dose is beneficial.