Changes of Estradiol (E2) Concentration and Sex Hormone Binding Globulin (SHBG) Concentration in Serum during Controlled Ovarian Hyperstimulation (COH) in Pregnant and Non-pregnant Women

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Abstract

Close correlation of Estradiol (E2) concentration and SHBG concentration in serum under the condition of Controlled Ovarian Hyperstimulation (COH) were investigated. E2 and SHBG coordinate each other but their secretion pattern was different. Serum E2 started to increase from D -4, became peak at D +1 then decreased rapidly next 2 days. Serum SHBG started to increase from D -1, reached peak at D +3 then kept high level during luteal phase.

Different situations of these hormones between pregnant and non-pregnant women under the condition of COH were also investigated. Though serum E2 and serum SHBG were not different at the time of hCG injection (D0) between the pregnant and the non-pregnant group, the ratio of these two was different. In the non-pregnant group SHBG/Estradiol (S/E ratio) was significantly higher (p<0.05) than pregnant group at the time of hCG injection (D0). When daily changes of S/E ratio became plateau (same value as yesterday) that was the recommend timing of hCG injection. These results suggested that S/E ratio could be the good indicator for the timing of hCG injection during COH.

If S/E ratio already started to increase at the time of hCG injection which represented the late timing of oocyte retrieval.

Keywords: Estradiol; SHBG; COH; Prognosis

Introduction

Estradiol (E2) and Sex Hormone Binding Globulin (SHBG) work together for many important physiological actions and these are affected with each other [1]. There are many reports of SHBG gene expression in ovarian cancer and breast cancer, in these neoplastic cells SHBG is thought to modulate estrogen actions [2-4]. In mammals the SHBG gene expression is primarily occurred in the liver, then SHBG transports estrogens in blood and regulate to access to different tissues [5-7]. In this study E2 concentration and SHBG concentration during Controlled Ovarian Hyperstimulation (COH) are traced. The secretion patterns of these two hormones are investigated. E2 and SHBG coordinate each other but their secretion pattern would be different. Though E2 actions during COH for the oocyte quality and success rate of pregnancy remain disputed [8,9], combined investigation of E2 and SHBG are expected to reveal the best protocol of stimulation for good prognosis. The timing of hCG injection was also searched because long stimulation and late timing of hCG injection sometimes causes premature luteinization. These late retrieval may affect to the oocyte quality [10]. At the time of hCG injection E2 and SHBG are compared between pregnant and non-pregnant group then between late retrieval and other group.

Materials and Methods

At first patients’ data base at California Fertility Associates (CFA) (Laboratory No. 9956-22901) were used for analysis. Stimulated patients previously treated with hCG (estimated as D0) were divided to pregnant and non-pregnant groups then changes of SHBG and E2 were traced according to the date of menstrual cycle.

Another study was done utilizing data base of one hundred and ninety one cycles of In Vitro Fertilization (IVF), Gamete Intrafallopian Transfer (GIFT) and Intracytoplasmic Sperm Injection (ICSI) at CFA beginning February to September, 1996. SHBG and E2 levels at hCG injection were compared with final clinical outcomes (pregnant (n=54) or not pregnant (n=137)). Then SHBG and E2 levels were compared between natural LH surge group (n=38) and others (n=153). LH concentration increased two times higher than base and LH concentration of ≥ 5 mIU/mL on the day of hCG injection was defined as premature luteinization. This group was recognized as natural LH surge group. Under the condition of COH this kind of LH rising was supposed to be caused by the late timing of hCG injection so this group was expected to represent late retrieval of oocyte.

Hormonal assay were measured with RIA (Radio Immuno Assay). Quality assurances of all immuno assay, accuracy, precision and sensitivity were controlled at Reproductive Technology Laboratories (Santa Monica, CA). Levels of E2 were determined using a double antibody RIA (Pantex, Santa Monica, CA), with a sensitive of 3 pg E2 per tube. Levels of SHBG were determined using a RIA (DSL6300, Diagnostic System Laboratories).

Statistics was done as follows. At first Bartlett test was applied to establish the homogeneity with respect to group variances. Then analysis of variances was compared. Significant differences were set when p-value was under 0.05. In Table 1 correlation was analyzed by least-squares linear regression. CRUNCH was used for all calculation.

Correlation between serum SHBG and serum E2 in stimulated patients at different date of menstrual cycle was analyzed by least-squares linear regression (Table 1). Eleven of 14 days analyzed showed the linear correlation of SHBG and E2 in stimulated patients. In the stimulated unphysiological condition SHBG and E2 were closely related at preovulatory, ovulatory and early luteal phase.

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Results

Daily Secretion of Serum Estradiol during COH in Pregnant and Non-Pregnant Patients

In both group serum E2 showed the same pattern of changes. Serum E2 started to increase from D-4 and became peak at D+1 then decreased rapidly during the following 2 days. In the luteal phase estradiol firstly increased, then decreased slowly. At preovulatory phase E2 tended to increase a little bit earlier and kept a higher peak in the pregnant group. E2 was higher in the pregnant group for all period, however, significant difference appeared only on D-2 and D+10 ~ D+13 (Figure 1).

Table 1: Correlation between serum E2 concentration and SHBG concentration in stimulated patients at different date of stimulated cycle. Correlation between serum SHBG and serum E2 was analyzed by least-squares linear regression.

| Date | R-square | P      | n  |
|------|----------|--------|----|
| -5   | 0.484    | 0.004* | 15 |
| -4   | 0.3686   | 0.004* | 20 |
| -3   | 0.2564   | 0.038* | 17 |
| -2   | 0.3922   | 0.001* | 22 |
| -1   | 0.0985   | 0.118  | 26 |
| 0    | 0.2876   | 0.000* | 73 |
| 1    | 0.1796   | 0.000* | 77 |
| 2    | 0.0682   | 0.043* | 60 |
| 3    | 0.1881   | 0.030* | 25 |
| 4    | 0.1371   | 0.044* | 30 |
| 5    | 0.0021   | 0.848  | 20 |
| 6    | 0.2486   | 0.005* | 30 |
| 7    | 0.0348   | 0.405  | 22 |
| 8    | 0.3378   | 0.002* | 24 |

Table 1: Correlation between serum E2 concentration and SHBG concentration in stimulated patients at different date of stimulated cycle. Correlation between serum SHBG and serum E2 was analyzed by least-squares linear regression.

Daily secretion of serum SHBG during COH in pregnant and non-pregnant patients

In both group serum SHBG showed the same pattern of changes. Compared with the changes of E2, SHBG started to increase at D-1 (3 days later E2 started to increase), then reached to peak at D+3 (2 days later E2 reached to peak) then kept high level during luteal phase. Between these two groups levels of SHBG were barely different at pre-ovulatory, ovulatory and luteal phase. The increase pattern was not different between pregnant and non-pregnant groups. We could only see significant differences on D+10 and D+14 at luteal phase and on D-3 at pre-ovulatory phase (Figure 2).

Daily changes of SHBG/Estradiol ratio during COH in pregnant and non-pregnant patients

SHBG/Estradiol (S/E ratio) decreased gradually to a trough on D-1. Though the S/E ratio started to increase on D0 in the non-pregnant group, it was lowest on D0 and increased beginning on D+1 in the

| Date | R-square | P      | n  |
|------|----------|--------|----|
| -5   | 0.484    | 0.004* | 15 |
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Table 1: Correlation between serum E2 concentration and SHBG concentration in stimulated patients at different date of stimulated cycle. Correlation between serum SHBG and serum E2 was analyzed by least-squares linear regression.

Figure 1: Daily secretion of serum E2 concentration during COH in pregnant (open circles) and non-pregnant (closed circles) patients. Mean ± SEM is illustrated. Numbers of serum analyzed are shown in parentheses. Symbol (*) means significant difference (p<0.05). Vertical line is Estradiol concentration in serum (pg / ml). Horizontal line is day of patient cycle. The day of hCG injection was estimated as D0.

Figure 2: Daily secretion of serum SHBG concentration during COH in pregnant (open circles) and non-pregnant (closed circles) patients. Mean ± SEM is illustrated. Symbol (*) means significant difference (p<0.05). Vertical line is SHBG concentration in serum (nmol/l). Horizontal line is day of patient cycle. The day of hCG injection was estimated as D0.

Figure 3: Daily changes of serum SHBG/Estradiol during COH in pregnant (open circles) and non-pregnant (closed circles) patients. Mean ± SEM is illustrated. Symbol (*) means significant difference (p<0.05). SHBG concentration (nmol/l) was divided by Estradiol concentration (pg/ml) and this result was calculated as SHBG/Estradiol (S/E ratio). Vertical line is SHBG/Estradiol. Horizontal line is day of patient cycle. The day of hCG injection was estimated as D0.
Table 2: Correlation between serum E2, SHBG and SHBG/Estradiol (S/E ratio) ratio at the time of hCG injection in pregnant and non-pregnant patients.

|          | n     | S/E ratio | SHBG     | Estradiol  |
|----------|-------|-----------|----------|------------|
| Pregnant | 54    | 0.029 ± 0.003* | 53.9 ± 3.2 | 2369 ± 183* |
| not Pregnant | 137 | 0.049 ± 0.005* | 50.7 ± 1.9  | 1823 ± 133*  |
| P=0.0074 | P<0.3931 | P=0.0251 |

Table 3: Correlation between serum E2, SHBG and SHBG/Estradiol (S/E ratio) at the time of hCG injection in natural LH surge and other patients.

|         | n     | S/E ratio | SHBG     | Estradiol  |
|---------|-------|-----------|----------|------------|
| Others  | 153   | 0.540 ± 0.004* | 51.3 ± 1.9  | 2123 ± 127* |
| natural LH surge | 36   | 0.058 ± 0.007* | 52.9 ± 3.5  | 1393 ± 161*  |
| P=0.0078 | P=0.6904 | P=0.0078 |

Correlation between serum SHBG, estradiol and S/E ratio (at the time of hCG injection) in pregnant and non-pregnant patients

SHBG values did not differ between outcomes. In the non-pregnant group serum E2 looked lower. In this group SHBG accumulation relative to E2 was significantly higher (because of the low accumulation of total estradiol) (Table 2).

Correlation between serum SHBG, estradiol and S/E ratio (at the time of hCG injection) in natural LH surge and other patients

Between these two groups SHBG was not different. In the natural LH surge group, serum E2 looked lower. SHBG levels relative to E2 were also significantly higher than stimulated patients (because of the low accumulation of total estradiol) (Table 3).

Discussion

In mammals the SHBG gene expression is primarily occurred in the liver [5,6], then SHBG transports estrogens in blood and regulate to access to different tissues [7]. The close correlation between serum levels of E2 and SHBG in patients at the same stage of stimulation (i.e. same day relative to hCG injection; Table 1) establishes that peripheral levels of E2 and SHBG are inter-related in patients undergoing COH with gonadotropins.

However, within patients, serum levels of E2 and SHBG were not closely correlated (Figures 1 and 2). That is, serum SHBG level did not rise until the day of hCG injection (Day 0), by each time serum E2 level had been rising for about 4 days. Furthermore, serum SHBG level continued to rise through to 3 days after hCG injection, then plateaued, during each time serum Estradiol level had fallen by about 85 %. Sustained rising of SHBG might be caused by gene expression in corpus luteum [11]. This peripheral expression of SHBG also complexly correlates with serum steroid hormone levels [12].

Because SHBG level was rising as E2 level was falling at about the time of follicle maturity, the S/E ratio was computed as a potentially sensitive index of the optimum time for hCG injection. Average values were summarized in Figure 3. SHBG accumulation relative to E2 kept the lowest level on D0 in pregnant group, but it already started to increase in non-pregnant group (Figure 3). At this time SHBG was still increasing with the same manner and same level in both groups, so it was better to say these were caused by the difference of E2. This difference of E2 might be very fine, so simple investigation of E2 could not detect the difference of this changes on D0 (Figure 1). In contrast, combined investigation of S/E ratio emphasized these changes and consequently picked up the difference on D0 (Figure 3). In non-pregnant group E2 might weaken to increase or start to decrease on D0 that affects the early increase of S/E ratio. We can expect good results of pregnancy in the case S/E ratio is at the lowest level at hCG injection.

The analysis of 191 cycles of COH at hCG injection showed the same tendencies (Table 2). Though SHBG level was not different between these two groups, E2 level was significantly higher and S/E ratio was significantly lower in pregnant group on D0. In this analysis E2 was significantly different. It might be caused by the accumulation of numbers. Natural LH surge was expected the indicator of late retrieval of oocyte [10] and others were all used as control. According to natural LH surge, E2 level was lower in this group. SHBG level was not different between these two groups, but S/E ratio was significantly higher in the natural LH surge group (Table 3). Hormonal condition of this group was similar to the non-pregnant group. Two different analysis (Tables 2 and 3) showed the the same results. If S/E ratio already started to increase on D0 this condition suggested late timing of oocyte retrieval.

In conclusion, SHBG accumulation relative to E2 (S/E ratio) could be the good indicator for the timing of hCG injection during COH. When daily changes of S/E ratio become plateau (same value as yesterday) is the recommend timing of hCG injection.

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