Introduction

The view of deficient ovarian reserve had gained general acceptance in infertility practice. In in vitro fertilization (IVF), the linking of poor ovarian response due to deficient ovarian reserve with cycle cancellation and a significant decrease in success rates is well defined [1,2]. Proper identification of women who are at risk for poor response can help gynecologists to individualize counseling and allow women to decide whether to undergo a needed infertility management. Accurate evaluation of ovarian response potential before women enter an IVF program is, so, of an outstanding importance.

It is well known that reproductive aging is linked to both a quantitative and a qualitative decrease of the primordial follicle count. As women age, their ovarian reserve declines, and the rates of both spontaneous and treatment-induced pregnancies decrease. But, for individual predictions of ovarian response and IVF success, chronological age only is of limited importance. Basal FSH was the first used endocrine marker of ovarian response that had better potential than age alone for predicting diminished ovarian function and decreased success rates after IVF [3]. However related to this phenomenon, FSH and age appeared to be independent prognostic indicators of assisted reproduction success rate [4].

Recently, many biomarkers of ovarian reserve are suggested. Basal Estradiol (E2) is a natural estrogens produced by follicular granulosa cells. Estradiol levels (<20 or >80 pg/ml) on day 3 might indicate poor responder; if E2 level is high then even if FSH is normal we cannot predict that ovarian reserve is quite normal [5-7]. This was an outstanding finding, as increased E2 values might be able to stop FSH into the normal level in women who have substantially decreased ovarian reserve and eventually may lead to false-negative FSH test results. Also, basal inhibin B has been advocated as an endocrine prognostic indicator for assisted reproduction success, although reports were conflicting [8-10].

Many articles have been published lately on the usefulness of ovarian sonar characters in predicting diminished ovarian potential during hormone induction. The antral follicle count (AFC) as well as the volume of the ovary seemed to be indicative of diminished response in assisted reproduction [11-13].

In this retrospective study we investigated the relationship between clinical, endocrinologic, chromosomal, and immunologic parameters and intermittent ovarian activity, including follicle growth, ovulation, and pregnancy rate, of 80 POI women with desired fertility.
**Patients and Methods**

The study includes 80 women with premature ovarian insufficiency enrolled consecutively from International Fertility Centre Kingdom Saudi Arabia and studied retrospectively.

**Inclusion criteria were**

1. The age was between 18-40 years old.
2. Women with premature ovarian insufficiency. POI was defined as at least 3 months of amenorrhea, 2 serum FSH readings > 40 mIU/mL.
3. None of the patients had male factor infertility or a history of pelvic radiotherapy.

**Exclusion criteria were**

1. Known or definitive causes explaining infertility like: history of maternal hyperprolactinemia, luteal insufficiency (detected due to repeatedly decreased luteal progesterone level), hyperandrogenism, polycystic ovary syndrome or hypersecretion of luteinizing hormone (LH) and insulin resistance.
2. Acquired (antiphospholipid syndrome) or hereditary thrombophilic disorders.
3. Different forms of uterine malformation had been ruled out by ultrasound and hysteroscopy.
4. Karyotype abnormalities (as Turner syndrome).

**Women were subjected to the following procedures**

a) Full history: presumed age at the onset of POI; age at menarche; age at the initial visit; personal history of autoimmune; history of pregnancy and/or delivery; iatrogenic history, including chemotherapy or surgery on the ovary; hormonal evaluation, including determination of E2 and FSH; and systematic screening for thyroid autoimmunity. The onset of POI was presumed based on irregular menstruation or amenorrhea.

b) Detailed examination (general, abdominal and local)

c) Investigations have been collected from the cases which include mainly:
   - Lupus anticoagulant antibodies.
   - Karyotyping.
   - Anticardiolipin antibodies.
   - Semen analysis from their husbands.
   - Radiological examination in the form of pelvic ultrasonography and hysterosalpingography.

d) Pelvic ultrasound screening included the presence or absence of follicles. Follicle growth was defined as the presence of follicle(s) of any size in the ovary with a serum E2 > 25 pg/mL, or the presence of a follicle(s) in which the mean diameter was > 14 mm with or without an E2 measurement. Ovulation was defined as the disappearance of the follicle(s) and/or formation of a corpus luteum after confirmation of follicle growth with or without administration of human chorionic gonadotropin. Patients with POI desiring pregnancy with their own oocytes provided a semen specimen from their partner for analysis and underwent hysterosalpingography to rule out other causes of infertility. They were then most often treated with cyclic hormone therapy (cyclic EPT) using estrogen (conjugated equine estrogen [CEE], 1.25 - 2.5 mg [Premarin, 2 - 4 tablets]/day for 7- 28 days, representing an absolute time period) followed by estrogens in combination with progestin (0.5 mg norgestrel and 0.05 mg ethinylestradiol [Planovar, 1 tablet] or 2.00 mg clomiphene acetate and 0.05 mg mestranol [Lutedion, 1 tablet]/day for 10 -12 days, representing an absolute time period), or in some occasions, human menopausal gonadotropins with or without estrogen or gonadotropin-releasing hormone agonist. Basal serum levels of FSH and E2 were measured on cycle days 1-5 after withdrawal bleeding. While receiving cyclic EPT, follicle size and number, and ovulation were closely monitored biweekly or twice a month.

e) Hormone measurements: Blood was collected, and serum was immediately separated by centrifugation for 6 min at room temperature. Serum E2 and FSH were measured by electrochemiluminescence immunoassay (Roche Diagnostics, Basel, Switzerland). The intra- and inter-assay coefficients of variation were 1.07-3.5% and 2.03-2.55% for E2 and 0.73-1.24% and 2.10-2.40% for FSH at all ranges, respectively. The detection limits for E2 and FSH were 5 pg/mL and 0.1 mIU/mL, respectively.

**Statistical methodology**

- Analysis of data was done by IBM computer using SPSS (statistical program for social science version 12 ) as follows
  - Description of quantitative variables as mean, SD and range
  - Description of qualitative variables as number and percentage
  - Chi-square test was used to compare qualitative variables
  - Unpaired t-test was used to compare two groups as regard quantitative variable in parametric data (SD<50%mean)
  - Mann Whitney Willcoxon U test was used instead of unpaired t-test in non-parametric data (SD>50%mean)
The mean presumed age of onset of POI in patients with POI was 34.8 ± 3.1 years. During the follow-up, some patients exhibited intermittent ovarian activity; 19 POI patients (23.75%) had follicle growth. Ovulation was observed in 10 patients (14.3%). Four (5%) patients conceived and all gave birth to healthy babies. The relationship between each parameter and intermittent ovarian activities is demonstrated in Table 2. The mean presumed age of onset of POI in patients with follicle growth was not significantly different from that of patients without follicle development (31.8 ± 4.1 years vs. 32.1 ± 3.2 years, P = 0.74). The median DOD in ovulatory patients was not significantly shorter than that in an ovulatory patients (P = 0.7054; Table 2). The DOD in patients with follicle growth and pregnancy was also not comparatively shorter than that in patients without follicle growth and pregnancy (P= 0.7358; Table 2). None of the other clinical parameters were significantly different between patients with and without intermittent ovarian activity, as well as laboratory factors, including FSH levels at the time of diagnosis. But serum E2 was significantly higher in patients with intermittent ovarian activity than those without.

There was a slight correlation between the incidence of ovulation and pregnancy, although the trend was not significant (R = 0.76, P = 0.07). For further assessment, Day 3 E2 and Day 1-3 FSH averaged 27.2 ± 3.4 pg/mL and 37.1 ± 4.1 mIU/mL, respectively in those with follicular growth. The average age at the time the treatment cycle was 32.2 ± 1.1 years. Comparison of the mean value of each parameter between cycles with and without intermittent ovarian activity is shown in Table 3.

Evaluating intermittent ovarian activity, follicle development was observed in 19 women. Ovulation was confirmed in 10 women. Pregnancy occurred in 4 women. Day 3 E2 were significantly higher in women with successful follicle growth and ovulation than cycles without ovarian activity (P < 0.05). ROC curve analysis on prediction of follicle growth and ovulation revealed that an optimal cut-off value of 25 pg/mL for Day 3 E2 had sensitivities of 75.1% and 71.1%, and specificities of 81.9 and 80.4 %, respectively.

To address the relationship between Day 3 E2 and intermittent ovarian activity, the patients were divided into two groups based on the Day 3 E2 (< 25 pg/mL and ≥ 25 pg/mL). Patients with Day 3 E2 ≥ 25 pg/mL were more likely to have follicle growth and ovulation than patients with Day 3 E2 < 25 pg/mL (P < 0.05; Table 4).

### Discussion

In the current study we looked for a possible factor that might predict intermittent ovulation in premature ovarian insufficiency (POI) women. The results assumed that the cycle in which Day 3 E2 was higher than 25pg/mL had a higher rate of follicular growth and ovulation in women with premature ovarian insufficiency. The accurate mechanism underlying the linkage between resuming ovarian function and high E2 values in cycles with follicular growth or ovulation on cycle day 3 remains to be elucidated; but, a possible mechanism is that hormone replacement therapy containing estrogen and progesterone down-regulates FSH release through a negative feedback; subsequent stoppage of hormonal supplementation leads to the release of the negative feedback, and thereafter, increases FSH release, which consequently, might stimulate the follicular development and its E2 release when a FSH-responsive competent

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#### Table 1: Clinical and hormonal backgrounds of POI women (n = 80).

| Character                          | Number (%) |
|-----------------------------------|------------|
| Presumed age of POI onset (years) | 32.2 ± 4.2 |
| Age of menarche (years)           | 12.5 ± 1.1 |
| Age at the initial visit (years)   | 34.8 ± 3.1 |
| Duration of ovarian dysfunction (years) | 3.6 ± 0.2 |
| Serum E2 at the initial diagnosis (pg/mL) | 25.6 ± 1.9 |
| Serum FSH at the initial diagnosis (mIU/mL) | 41.3 ± 8.2 |
| Pregnancy history                 | 15         |
| Delivery history                  | 12         |
| Iatrogenic history                | 11         |

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Table 2: Relationship between clinical and hormonal parameters and resumption of ovarian function in POI patients.

| Character                              | Follicular growth | Ovulation | Pregnancy |
|----------------------------------------|-------------------|-----------|-----------|
|                                        | Yes (N=19)        | No (N=61) | P-value   | Yes (N=10) | No (N=70) | P-value   | Yes (N=4) | No (N=76) | P-value   |
| Presumed age of POI onset (years)      | 31.8± 4.1         | 32.1± 3.2 | 0.7400    | 32.2± 1.1  | 33.4± 3.6 | 0.3006    | 32.4± 1.2  | 32.5± 2.6 | 0.9395    |
| Age of menarche (years)                | 12.4± 1.2         | 12.1± 2.3 | 0.6878    | 11.9± 3.2  | 12.5± 2.2 | 0.4499    | 11.8± 2.9  | 12.2± 3.2 | 0.8075    |
| Age at the initial visit (years)       | 33.2± 1.2         | 34.2± 3.7 | 0.2517    | 32.9± 4.5  | 35.5± 4.2 | 0.0733    | 32.4± 2.7  | 35.2± 3.2 | 0.0903    |
| Duration of ovarian dysfunction (years)| 3.1± 1.2          | 3.2± 1.1  | 0.7358    | 3.6± 1.8   | 3.3± 2.4  | 0.7054    | 3.2± 1.3   | 3.9± 3.2  | 0.6659    |
| Serum E2 at the initial diagnosis (Pg/mL)| 27.1± 4.3     | 22.6± 6.2 | <0.05     | 30.2± 4.2  | 21.3± 5.1 | <0.05     | 30.4± 4.3  | 21.2± 7.2 | <0.05     |
| Serum FSH at the initial diagnosis (mIU/mL)| 40.2± 3.2 | 41.5± 6.7 | 0.4178    | 40.1± 2.8  | 42.2± 4.6 | 0.1648    | 39.2± 3.8  | 42.4± 5.2 | 0.2297    |
| Pregnancy history                      | 5                 | 10        | 0.333     | 4          | 11        | 0.0656    | 4          | 11        | 0.0656    |
| Delivery history                       | 3                 | 9         | 0.912     | 4          | 8         | 0.179     | 4          | 8         | 0.179     |
| Iatrogenic history                     | 3                 | 8         | 0.767     | 2          | 9         | 0.539     | 2          | 9         | 0.539     |

Table 3: Cycle-based analysis for prediction of intermittent ovarian activation.

| Character                              | Follicular growth | Ovulation | Pregnancy |
|----------------------------------------|-------------------|-----------|-----------|
|                                        | Yes (N=61)        | No (N=58) | P-value   | Yes (N=4) | No (N=22) | P-value   |
| Age during the cycle (years)           | 32.2± 1.1         | 32.5± 2.4 | 0.6003    | 32.4± 1.1  | 31.3± 3.1 | 0.0859    |
| Day 3 E2 (Pg/ml)                       | 27.2±3.4          | 24.9±3.1  | 0.0072*   | 27.6±2.1   | 24.2±1.1  | <0.05     |
| Day 1-3 FSH (U/L)                      | 37.1±4.1          | 42.2±5.7  | 0.0005*   | 31.2±4.8   | 44.2±4.5  | <0.05     |

Table 4: Relationship between Day 1-5 E2 and intermittent follicle activity.

| Character                              | Follicular growth | Ovulation | Pregnancy |
|----------------------------------------|-------------------|-----------|-----------|
|                                        | Yes (N=19)        | No (N=61) | P-value   | Yes (N=10) | No (N=70) | P-value   | Yes (N=4) | No (N=76) | P-value   |
| Day 3 E2 < 25 Pg/ml (n=58)              | 5                 | <0.05     | 2         | <0.05     | 1         | <0.05     | 1         | <0.05     | 3         |
| Day 3 E2 > 25 Pg/ml (n=22)              | 14                | <0.05     | 8         | <0.05     | 3         | <0.05     | 3         | <0.05     |

Follicle growth is growing; a high level FSH might strongly and immediately stimulate E2 release from the dominant follicle; and serum E2 might reach a high level on cycle days 1-5, that is an indicator of a dominant follicle and the subsequently intermittent ovarian activity.

A shorter POI might also be considered a favorable possible factor for ovulation. The POI also had an inverse relationship with follicular growth and pregnancy, although a significant difference was not demonstrated, presumably due to small sample size. There are various case reports in the literature involving POI women who spontaneously and unexpectedly got pregnant [6,8,14-25], but very few had demonstrated statistical analysis of this population due to the rarity of POI. Bidet et al. [26], demonstrated predictive factors for spontaneous recovery of ovarian function in POI women. Retrospective and prospective studies were done, and included 358 consecutive POI women. Multivariate analysis demonstrated that a family history of POI, amenorrhea, the follicular growth on ultrasound, and inhibin B and E2 values were significantly predictive of resumption of ovulation [26]. Interestingly, serum E2, but not serum FSH, is of value as a predictive factor for follicular growth, as demonstrated in the multivariate analysis [26], and the current study. In contrast, it had been reported that POI women with a FSH level < 15 mIU/mL before stimulation might ovulate in response to exogenous gonadotropins [27]. These results collectively demonstrate that each woman might have an optimal value of serum FSH for follicular growth in each cycle.

Table 2: Relationship between clinical and hormonal parameters and resumption of ovarian function in POI patients.

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