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Short Communication

Pilot Study to Investigate Select Haemostasis, Endocrine and Biochemical Parameters in Women who Undergo in-Vitro Fertilisation (IVF) before and after Stimulation with Follicle Stimulating Hormone (FSH)

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Abstract

Synopsis: Before planning an assisted reproductive technology (ART), individual thromboembolic risk should be assessed.

Objective: A pilot study designed to investigate select haemostasis, endocrine and biochemical parameters in women who undergo IVF before and after stimulation with FSH. Blood was collected and analysed before and after stimulation.

Study Design: Tests performed included Luteinising hormone (LH), estradiol (E2), progesterone, testosterone, sex hormone-binding globulin (SHBG), free androgen index (FAI), FSH, thyroid stimulating hormone (TSH), free thyroxin (FT4), protein S, protein C, antithrombin, lupus antibodies, anticardiolipin antibody (IgG and IgM), von Willebrand factor antigen, collagen binding and the following factors (F); F II, F V, F VII, F VIII, F IX, F X, F XI, F XII . Also, urinary iodine was assessed to screen for thyroid disorders.

Results: After stimulation, there were statistically significant difference in the levels of FSH, LH, E2, Testosterone, FAI, and F XI. Also, there was a significant negative correlation between E2 and FXII.

Conclusion: Before planning an ART, individual thromboembolic risk should be assessed. Our findings warrant a more comprehensive investigation in this group of patients to examine the long term effects of observed changes and their relation to a possible ovarian hyperstimulation syndrome (OHSS).

INTRODUCTION

There are three very commonly used ovarian stimulation protocols for in vitro fertilization; luteal Lupron protocol (also called "long Lupron", or agonist "down regulation"), antagonist protocols that involve use of the gonadotropin releasing hormone (GnRH) antagonist medications, and flare and micro-flare protocols (also called short Lupron protocols, or short protocols) which are used for patients expected to have a low response to ovarian stimulation. Early thrombosis could be favoured by high endogenous plasma oestrogen concentrations subsequent to ovarian stimulation when associated with another risk factor [1]. In vitro fertilisation is also associated with an increased risk of pulmonary embolism and venous thromboembolism during the first trimester.

The risk of pulmonary embolism is low in absolute terms but because the condition is a leading cause of maternal mortality and clinical suspicion is critical for diagnosis, an awareness of this risk is important [2]. It was shown in another study that screening for Factor V Leiden (FVL) and prothrombin gene G20210A mutation (PGM) does not appear to be justified to identify the patients at the risk for IVF failure, and/or for OHSS, and/or for thrombotic complications [3]. It is also reported that thrombosis could be associated with the use of gonadotropins during controlled ovarian hyperstimulation. In these cases arm swelling should

be promptly evaluated and treated [4]. Aetiological factors for bleeding in IVF still remain to be investigated [5].

Accordingly, this pilot study was designed to investigate haemostasis in women undergoing IVF before and after stimulation. We also investigated thyroid function and iodine deficiency because spontaneous OHSS can occur in pregnant women with severe hypothyroidism or extremely elevated hCG [6].

MATERIALS AND METHODS

In this pilot study, women are stimulated using gonadotropin releasing hormone (GnRH) antagonist medications. The women were given oral contraceptive to regulate the menstrual cycle. As some women undergoing IVF may have unexplained bleeding, this pilot study was designed to investigate haemostasis before and after stimulation. Also investigating thyroid function and iodine deficiency because spontaneous OHSS can occur in pregnant women with severe hypothyroidism or extremely elevated hCG [6].

A blood sample was collected from women who underwent in-vitro fertilisation (IVF) before and after stimulation with FSH. Plasma and serum were separated and frozen at -70°C to allow analysis of paired specimens under identical testing conditions. Also, a urine specimen was collected with the first blood collection, before stimulation, to screen for iodine deficiency. Factor assays were performed on a BCS coagulation analyser (Siemens) using standard one-stage assays and employing factor (F) deficient plasma from Siemens for F VII, F VIII, F IX, F X, F XI and F XII and from STAGO for F II and F V (STAGO, Sydney, Australia). Antithrombin (AT), protein C and protein S were performed using STA-R and commercial regents (STAGO, Sydney, Australia). The von Willebrand factor (VWF) assays for antigen (VWF:Ag) and collagen binding (VWF:CB) were performed using enzyme linked immunoabsorbent assays (ELISA) (108) employing polyclonal antibodies from Dako (Sydney, Australia) and collagen (type I/III mixture from bovine tendon; catalogue number 193492) from MP Biomedicals (Sydney, Australia). The sample size for this pilot study is twenty four samples. Normal reference intervals for the above tests are summarized as follows: F II 65-130 U/DL ; F V 55–140 U/dl; F VII 60-140 U/dL; F VIII 45–180 U/dl; F IX 60–150 U/ dl; F X 60–150 U/dl; F XI 50–140 U/dl; F XII 40–190 U/dl; VWF:Ag 45–200 U/dl; VWF:CB 50– 250 U/dl; AT 80-120 %; protein C 65-175 %; protein S 60-180 %. TSH and FT4 were analysed using Roche E170 (TSH 0.1-4.0 µU/mL and FT4 11.5-25.1 pmol/L). Testosterone, SHBG, E2 and progesterone were analysed using Immulite 2000 (Siemens, Sydney, Australia). Urine iodine was analysed using Sandell–Kolthoff reaction method; UI >100 µg/L [7].

RESULTS

After FSH stimulation, there were statistically significant difference in the levels of LH, FSH, testosterone, free androgen index (FAI), and F XI (Figures 1-A & 1-B). Also, despite a non-significant fall in F XII (Figure 2-A), there was a statistically significant negative correlation between E2 and F XII (figure 2-B) post treatment.

All other tested coagulation factors also showed a non-statistically significant fall after stimulation with FSH (Figure 3).

DISCUSSION

It is suggested that risk factors must be considered individually before each IVF attempt. In patients at high risk, clinical management of the post-transfer period is recommended [1].

According to our knowledge no systematic investigation has yet been conducted into haemostasis before and after treatment with FSH in women who undergo IVF. Although the mechanisms are not clear, it is reported that small doses of E2 enhance F XII concentrations in plasma [8].Another study showed that the level of F XII was about 20% higher in women using oral contraceptive than control group [9]. It is also reported that the F XII gene is modulated by E2 [9].Data reported in literature showed the transcriptional control of F XII by E2 and on the activation of F XII in the plasma. Multiple effects on haemostasis involving F XII have been reported in relation toE2 therapy [8].

Another study reported that the most consistent effects of E2, and partially to progesterone, on coagulation proteins are elevations of fibrinogen; F II, F VII, F IX, F X, and F XII; protein C; and plasminogen, while protein S and AT values are decreased [10].

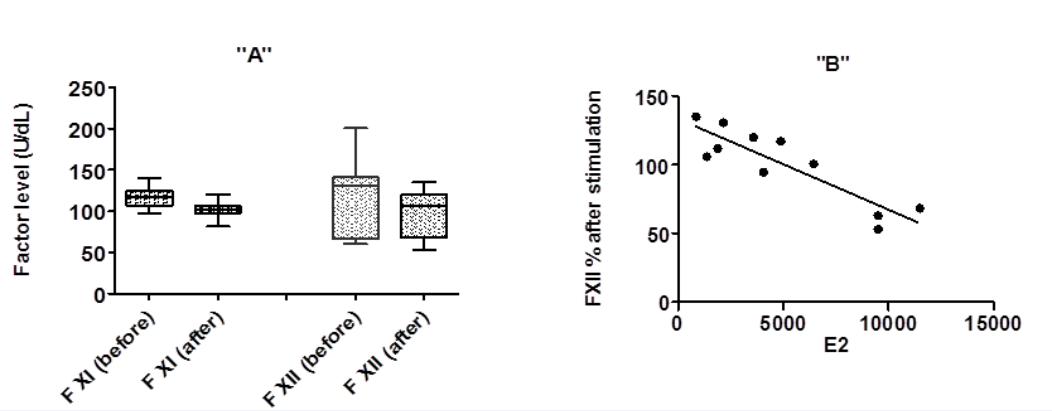

Figure 1 **A:** LH, Testosterone, FSH and FAI before and after stimulation with FSH. **B:** E2 and F XI before and after stimulation with FSH.

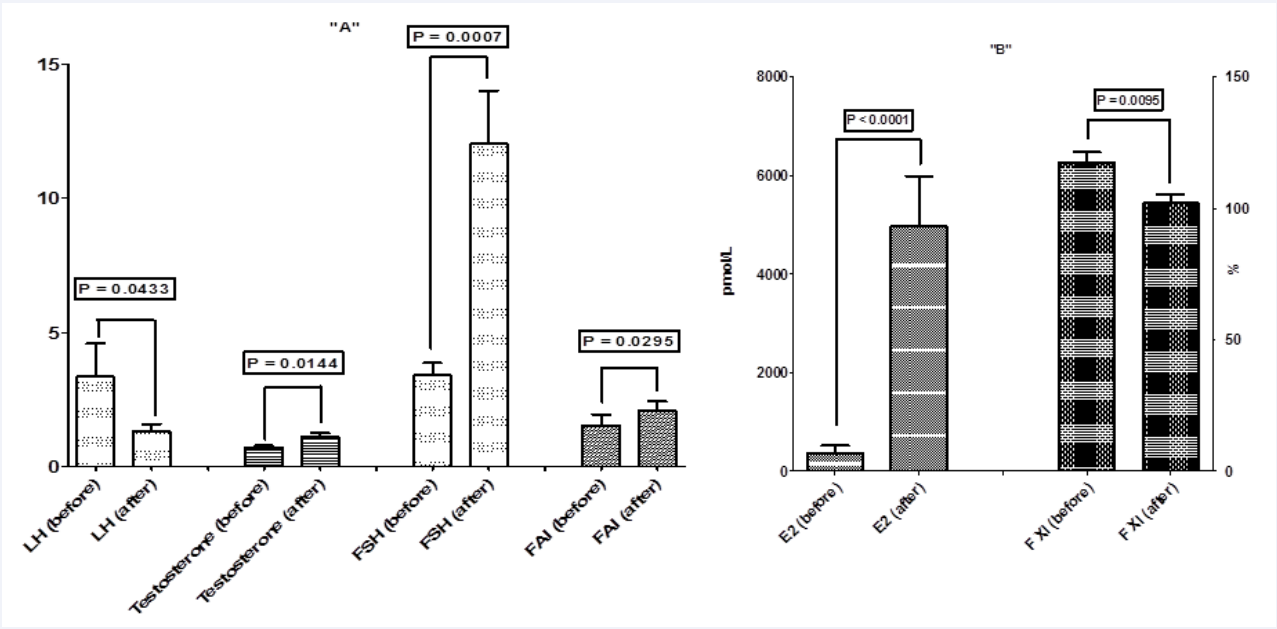

Figure 2 **A:** F XI and F XII before and after stimulation with FSH. **B:** The linear regression between F XII and E2 after stimulation with FSH) P = 0.003, R2 = 0.78).

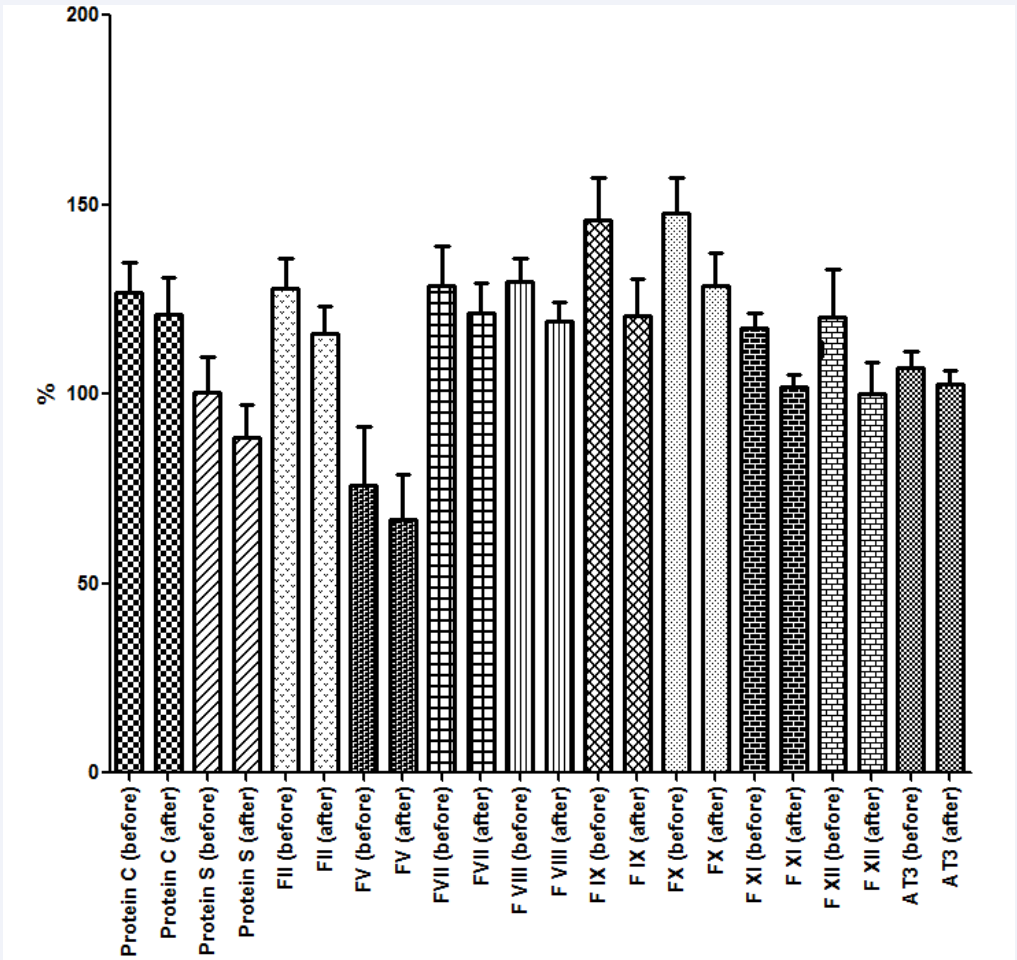

Figure 3 Blood factors and protein C and S before and after stimulation with FSH.

This pilot study showed that high concentrations of E2 may have a negative reverse effect on F XII levels as opposed to small E2 doses. F XI showed a similar trend in so far as yielding significant reduction post stimulation. Although this study also showed a statistically significant difference in testosterone and FAI, it is reported that physiological testosterone replacement does not adversely affect blood coagulation status [11].The pilot study also showed that under the study conditions, mean values for all tested factors, AT, protein C and S were lower after stimulation with FSH, while patients were on oral contraceptive to trigger their cycle in a timely manner.

Use of ART is increasing in many developed countries. Arterial and venous thromboembolic complications are reported during ART with an incidence of 0.1%. High E2 concentrations, and blood hyperviscosity play a major role in inducing a prothrombotic state. Therefore, before planning an ART, individual thromboembolic risk should be assessed and thromboprophylaxis offered to high risk patients. Prophylaxis should be initiated in women who develop moderate-to-severe OHSS [12,13]..

The pilot study data indicates that a more comprehensive investigation is warranted in this group of patients, to examine the long term effects of these changes and its relation to a possible OHSS.

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