Simvastatin effects on androgens, inflammatory mediators, and endogenous pituitary gonadotropins among patients with PCOS undergoing IVF: results from a prospective randomized placebo-controlled clinical trial

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Abstract

Objective: To evaluate effects of simvastatin on selected biochemical parameters and reproductive outcome among patients with polycystic ovary syndrome (PCOS) who undergo in vitro fertilization (IVF). Methods: PCOS patients were randomized to receive either oral simvastatin 20mg/d (n=32) or placebo (n=32) in a prospective, double-blind randomized clinical trial (NCT 005-75601) in parallel with controlled ovarian hyperstimulation for IVF. All patients were determined to be at average risk for cardiovascular disease, based on high sensitivity C-reactive protein (hsCRP) measurement at entry. Following an eight-week treatment interval concluding at periovulatory hCG administration, selected clinical and laboratory parameters were measured. Results: Mean serum total testosterone level decreased by 25% in the simvastatin group, compared to a 10% reduction in the placebo group (p<0.001). A trend of lower serum LH levels was noted in experimental and control groups (29 vs. 22%, respectively) although this difference was not significant (p>0.05). Neither fasting insulin nor QUICKI were significantly impacted by simvastatin (p>0.05). As expected, total cholesterol was not modified among placebo patients but was significantly reduced following simvastatin (p=0.001). Additionally, hsCRP and VCAM-1 were both significantly lower after simvastatin therapy compared to controls (p≤0.005, for both). At study completion, no important change in BMI was observed in either group (p≥ 0.60). While oocyte maturation rate, fertilization rate and clinical pregnancy rate were all higher following simvastatin, none of these improvements were statistically significant. Conclusions: This report presents data from the first prospective, randomised, placebo-controlled clinical investigation of simvastatin in the setting of PCOS and IVF. Simvastatin appears to be compatible with gonadotropin therapy for IVF and can offer beneficial endocrine and cardiovascular effects for PCOS patients who undergo embryo transfer. While the observed improvements in reproductive function were mild, the reductions in hsCRP and VCAM-1 following simvastatin treatment were significant, suggesting the need for further clinical trials to clarify simvastatin’s impact on reproductive physiology.
Introduction

Polycystic ovary syndrome (PCOS) is a common multisystem disorder associated with abnormal (or absent) ovulation, affecting up to 10% of reproductive age females [1-4]. While the specific etiology of PCOS remains poorly understood, a broad range of endocrine, inflammatory and metabolic derangements have been recognized. Specifically, PCOS is frequently associated with insulin resistance, systemic inflammation and oxidative stress which result in endothelial dysfunction. For many women with PCOS, these consequences are multiplied by chronically elevated serum androgens, leading to dyslipidemia and cardiovascular sequelae [5]. For IVF patients with PCOS, it is not unusual to observe a high cycle cancellation rate due to ovarian hyper-response, as well as large numbers of oocytes retrieved with relatively poor fertilization [6].

Dyslipidemia is one of the most common metabolic abnormalities among PCOS women, with a prevalence approaching 70% [7-9]. Statins are a pharmacologic class of agents that selectively inhibit 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in the cholesterol biosynthetic pathway [5]. Because reduced serum testosterone and improvement of hirsutism have been reported following simvastatin treatment of PCOS patients [10-12], this has evoked some speculation as to how this medication might impact ovarian function. To date, statins have not been systematically studied in an assisted reproductive context, however. By directly inhibiting cholesterol production, simvastatin alters the downstream availability of testosterone, resulting in markedly reduced ovarian androgens. Additionally, simvastatin may attenuate intracellular insulin and IGF-I signaling in the ovary [13] as well as reverse theca hyperplasia and diminished steroidogenic enzyme production. From these earlier investigations, we hypothesized that incorporation of simvastatin into an IVF regime for PCOS patients would yield desirable metabolic effects, translating to improved oocyte quality and potentially improve reproductive outcome following embryo transfer [14,15].

Against this background, the dyslipidemia observed in PCOS patients suggested a worthwhile role for statin therapy [16]. Unfortunately, the use of this class of medication in females of reproductive age—and particularly the inclusion of statins into an assisted reproduction sequence—presents a special challenge, due to its pregnancy
contraindication. Unlike oral contraceptive pills which render the risk of unplanned pregnancy very low, PCOS patients treated with statins might still establish a pregnancy and any possible teratogenic consequences secondary to statin use would be unacceptable. Accordingly, a novel study design was explored where statin administration was incorporated with IVF but limited to an eight-week interval terminating with periovulatory hCG administration. This effort represents the first known randomized, double-blind, placebo-controlled clinical trial to evaluate simvastatin during IVF.
Methods

Patient selection and study design

Following approval by the institutional review board at the Tehran University Faculty of Medicine, subjects \((n=64)\) were enrolled in a prospective, double-blind, randomized placebo-controlled clinical trial [NCT 005-75601]. Written informed consent was obtained from all study patients. Criteria to enter the study included female patient age between 18-40yrs, PCOS diagnosis confirmed by meeting 2004 Rotterdam criteria [17], and currently undergoing IVF+ICSI for a male factor indication. Exclusion criteria were cycle day three serum FSH >10mIU/l, stage III or IV endometriosis, congenital adrenal hyperplasia, thyroid disease, Cushing syndrome or hyperprolactinemia. Commercially available immunoassay kits were used to measure serum LH and FSH. Serum DHEAS, testosterone and prolactin levels were determined via chemiluminescence assay (DiaSorin; Turin, Italy). Sex hormone binding globulin was measured by ELISA (DRG; Marburg, Germany). Additionally, fasting plasma glucose (FPG) and fasting insulin were recorded, and a standard 75g oral glucose load was used to perform a glucose tolerance test with calculation of area under the curve (AUC) for insulin and glucose. Insulin sensitivity was estimated via quantitative insulin-sensitivity check index [18]. A photometry assay (Pars Azmon; Tehran, Iran) was used to measure serum HDL and LDL cholesterol, and a high-sensitivity C-reactive protein ELISA kit (Diazyme Laboratories; Poway, California USA) was used to record hsCRP. Serum vascular cell adhesion protein-1 (VCAM-1) was measured via ELISA kit (Abcam; Cambridge Massachusetts USA). Intra- and inter-assay coefficients of variation were <10% for all evaluations performed. Study patients did not receive oral contraceptives, steroids or other medications affecting ovarian function, insulin sensitivity or lipid metabolism for a minimum of three months before the investigation. Blood samples were uniformly obtained between 7:00-8:00am after a 12h fast. Specimens were taken in the follicular phase of spontaneous menses, or following oral medroxyprogesterone acetate. Randomization was by sequentially-numbered opaque, sealed envelope (SNOSE) methodology; envelopes were opened sequentially after participant (assignment) details were written on the envelope. Aluminum foil was used inside envelopes to shield contents from intense light. In this non-crossover study, allocation was blinded both for patients and clinic staff. Group A received 20mg oral simvastatin \((n=32)\), while Group B received placebo \((n=29)\). Both groups received study medication for eight weeks before initiating their IVF treatment sequence.
Controlled ovarian hyperstimulation sequence

Both patient groups underwent pituitary down-regulation followed by ovulation induction via daily injection of 150IU recombinant FSH (Gonal-F®, Serono; Bari, Italy), which was initiated on cycle day three. Periovulatory hCG was administered when serum estradiol levels exceeded 500 pg/mL and if there were at least two follicles with mean diameter ≥18mm. Both simvastatin and placebo were discontinued on day of hCG injection. Transvaginal ultrasound-guided oocyte retrieval was performed 36h after hCG administration, and transfer of 1–2 embryos was carried out two days after oocyte retrieval. 400mg Cyclogest (Actavis Pharmaceuticals; Barnstaple, UK) twice daily was used for post-embryo transfer luteal support, beginning the day after oocyte retrieval and continuing until day of clinical pregnancy assessment. Clinical pregnancy was defined as at least one intrauterine gestational sac with fetal cardiac activity documented by transvaginal ultrasound approximately five weeks after embryo transfer. Supplementary progesterone was discontinued when a negative hCG test was registered. Repeat endocrine and metabolic evaluations were obtained eight weeks after embryo transfer.

Statistical analysis

Assuming simvastatin results in a 10% improvement in the number of MII (metaphase II, “mature”) oocytes, a sample of 30 in each arm was calculated to yield a significance of 0.05 with power of 0.8 (Sigmastat/Jandel Scientific; San Rafael, CA, USA). The primary outcome was improvement in metaphase II oocytes and secondary outcomes was decrease in testosterone and inflammatory markers. Comparisons were carried out according to intention to treat by Mann–Whitney, $\chi^2$ or Fisher’s exact test, as appropriate. Statistical analysis was performed using SPSS Version 12.0 (IBM; Chicago, USA). A $p$-value of <0.05 was considered statistically significant.
Results

Sixty-four IVF patients were enrolled and randomized in this investigation, while three subjects did not complete IVF for personal reasons. Of the remaining 61 women, 32 were assigned to Group A (simvastatin) and 29 women entered Group B (placebo) as shown in Figure 1. Patients in both IVF groups received the same cumulative gonadotropin dose (1500IU). Pre- and post-treatment comparisons for both groups are summarized in Table 1. The median number of retrieved oocytes during IVF was similar for both groups (12.3±6.96 in Group A vs. 8.8±4.5 in Group B; p=0.066). As depicted in Table 2, the median number (and proportion) of MII oocytes observed in the two groups was not significantly different (8.42±5.1 (76.13%) in Group A vs. 6.71±3.7 (68.2%) in Group B; p=0.296). The 2pn fertilization rate for patients in Group A and Group B was also similar (74.1±17.25% for patients receiving statin vs. 64.9%±24.1% for patients receiving placebo; p=0.128). The clinical pregnancy rate was somewhat higher for patients in the statin group (28% vs. 21% for patients receiving placebo), although this difference was not statistically significant (p=0.25). The observed incidence of mild or moderate ovarian hyperstimulation syndrome was similar in both groups (0.66% in statin group vs. 0.77% in placebo group; p=0.65). No appreciable change in body mass index was noted in either group.

During this study, significant decreases in serum (total) testosterone were observed in both groups with an average reduction of 25% among statin patients and an average reduction of 10% in the placebo group (p=0.001, for both). Of note, the difference in serum testosterone reduction between the two groups was highly significant (p=0.0001). Likewise post-treatment serum DHEAS was lower in both groups, but a mean DHEAS reduction of 30% was noted in statin patients vs. 44% in placebo patients; p=0.03. Assessment of pituitary hormones revealed a significant decline of serum LH, but a limited effect on serum FSH. Specifically, patients in the statin group demonstrated an average serum LH decline of 29% and a 30% reduction of the LH:FSH ratio. As summarized in Table 2, these effects were significantly greater than those observed in the placebo group. Total serum cholesterol was reduced by an average of 24% in Group A (p=0.001), but a mean cholesterol reduction of only 1% was observed among Group B (control) patients (p>0.05). In Group A, mean serum low-density lipoprotein levels
decreased by 15% ($p=0.001$) but among patients in the placebo group (Group B) LDL-cholesterol increased by an average of 8% ($p=0.91$). In this study, serum high-density lipoprotein levels increased by an average of 15% and 14% in statin and placebo group, respectively ($p=0.98$, for difference between groups). Simvastatin did not significantly impact fasting insulin or QUICKI marker compared to placebo control during this investigation. In contrast, simvastatin was observed to have a significant beneficial effect on systemic inflammatory markers, as statin therapy was noted to result in a 58% decline in hsCRP compared to a mean 28% reduction in hsCRP among patients in the placebo group ($p=0.0001$). Similarly, we measured mean decreases in sVCAM-1 of 18% and 7% in statin and placebo groups, respectively ($p=0.004$). All patients tolerated study medications well; there were no discontinuations due to adverse effects.
Characterized by multisystem endocrine derangements, polycystic ovary syndrome (PCOS) is frequently encountered in routine clinical practice [19]. The protean manifestation of the condition sometimes leads PCOS patients to seek treatments for specific symptoms, rather than correct fundamental, causative pathophysiology. However, because modern research has yet to bring exactly why or how this syndrome emerges within reach, the laudable aim to address the basic elements of PCOS remains therapeutically unsatisfying. In the meantime, strategies to reduce androgens, suppress follicular development and/or ovulation, and attenuate insulin insensitivity are among common treatments deployed in the management of PCOS.

Although PCOS patients undergoing IVF typically produce increased numbers of oocytes for retrieval, these often are of poor quality and associated with impaired fertilization and implantation. Several authors have speculated on potential metabolic mechanisms leading to increased miscarriages among IVF patients with PCOS [20-22]. It has been postulated that LH hypersecretion, elevated androgens, and/or relative insulin excess may negatively affect the granulosa cell-oocyte interaction, oocyte maturation, and potential embryonic developmental competence, thus contributing to poor outcomes for PCOS patients undergoing assisted reproduction [23]. Prior research in a non-IVF setting has demonstrated that simvastatin can decrease serum testosterone, serum LH, as well as the LH:FSH ratio [24-26]. These corrections would be beneficial for PCOS women who require IVF, since high testosterone and the “inverted” LH:FSH ratio are considered hallmarks of hypothalamic-pituitary-ovary dysfunction often seen in PCOS. The capacity of simvastatin to attenuate serum testosterone derives from its mevalonate pathway inhibition, which reduces testosterone from decreased upstream availability of cholesterol (a necessary substrate for androgen production), as well as suppression of the theca compartment in the ovary [27,28]. Statins may also modulate serum LH by hypothalamic and/or pituitary effects. For example, studies of rat pituitary tumour have shown that statins can influence plasma membrane Gs and Gi proteins, as well as adenyl cyclase activity [29]. With respect to overall reproductive outcome, an improved clinical pregnancy rate among patients who received simvastatin was noted in this study, but this increase was too limited to reach statistical significance. We also observed a small but insignificant improvement in MII oocyte yield following simvastatin
treatment compared to placebo (76.1% vs. 68.2%; \( p > 0.05 \)). Additional research is needed to validate the impact of statin-mediated serum lipid improvements on the reproductive outcome of PCOS patients in IVF.

Not surprisingly, simvastatin in tandem with gonadotropin treatment for IVF produced more pronounced effects on serum lipids in our study, as significant reductions in total serum cholesterol and LDL cholesterol were noted among PCOS patients receiving simvastatin. In particular, we recorded a significant increase in HDL cholesterol after statin therapy, although no important change was noted in serum triglyceride levels. It should be noted that improvements in HDL (and DHEAS) were also seen among control patients, and this finding requires further clarification. Improvement in serum HDL is especially beneficial for PCOS patients, in whom dyslipidemia and other cardiovascular risk factors have special relevance. HDL is believed to play a key role in human oocyte health, influencing embryo fragmentation and embryo cell number [30]. It has been hypothesized that oocytes from PCOS women could have lower numbers of meiotic spindles compared in to controls because of impairments in antioxidant defense in follicular fluid [23]. This would seem to agree with earlier observations that have placed HDL as the sole lipoprotein present in follicular fluid, where the membrane is permeable to serum proteins up to 300 kDa but excludes low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and larger HDL2s [31,32]. Serum HDL correlates well with HDL present in follicular fluid, and HDL particle concentration has been shown to be negatively associated with embryo fragmentation score on post-fertilization day three [30].

Evidence is also accumulating that endothelial dysfunction and low-grade chronic inflammation are important components of PCOS [33]. Recent work has suggested that hsCRP is a useful marker for adverse cardiovascular events in women [29,34], and in our investigation all study patients had baseline hsCRP levels consistent with “average” cardiovascular disease risk (where: <1mg/l = low risk, 1-3mg = average risk, >3mg/l = high risk) [35]. The endothelial dysfunction seen in PCOS coexists with (and appears to be influenced by) VCAM-1 [36]. VCAM-1 is expressed by vascular endothelium under pro-inflammatory conditions, and plays a key role in the pathogenesis of atherosclerosis by mediating adhesion of activated leukocytes to the vessel wall [37]. Our data confirmed a significant decrease in mean hsCRP levels among PCOS patients who received simvastatin during IVF, while also producing pronounced decreases in soluble VCAM-1 levels. These observed lower VCAM-1 levels may be more
germane to circulatory and/or perfusion parameters rather than blastocyst-endometrial interactions, since VCAM-1 is not absolutely required for embryo implantation [38]. Instead, the VCAM-1 attenuation noted in our study patients would lead to reduced migration and adhesion of lymphocytes, monocytes, eosinophils and basophils to vascular endothelium.

The physiologic impact of PCOS on reproductive outcome following IVF remains the target of active investigation. One meta-analysis of clinical IVF studies reported that, although a significantly higher number of oocytes were recovered from PCOS patients, the number of good quality embryos available for transfer was not significantly different between controls and PCOS subjects [6]. Other investigators have found a significantly lower number of mature oocytes and reduced fertilization rate in PCOS patients compared to controls [39]. Yet, others have reported comparable numbers of MII oocytes harvested at retrieval, similar fertilization rates, and comparable embryo development when PCOS women were compared against controls undergoing ICSI [40].

Because studies of statins and reproductive physiology are rare, data from the current investigation are particularly useful. Although the current classification of statins as contraindicated in pregnancy [41] can raise substantial barriers to research in this arena, there are precedents in routine clinical IVF practice for similar short-term use of other “Category X” medications, with no reported adverse maternal/fetal health consequences. For example, pituitary desensitization with a GnRH-agonist is common before initiating ovulation induction with gonadotropins, yet both groups of pharmaceuticals are classed as contraindicated in pregnancy. Indeed, the terminal elimination half-life of simvastatin is comparable to leuprolide [42], and the latter agent is routinely administered alongside gonadotropins during the follicular recruitment phase in IVF. Considering the fact that we confined statin dosing only to the period up to periovulatory hCG administration (i.e., no gestational exposure), the actual bioactivity of simvastatin at or near the time of blastocyst nidation was likely negligible. These data provide helpful evidence regarding a beneficial role of simvastatin on cardiovascular risk factors in this young but at-risk population of PCOS patients undergoing IVF. How simvastatin might modify lipid parameters and/or markers of vascular inflammation among heavier, older PCOS patients at even higher baseline risk for cardiovascular disease awaits future study. Our findings align with previous research on how simvastatin affects serum testosterone in
women with PCOS, and show how simvastatin can be used with IVF to attenuate inflammatory markers that predict cardiovascular sequelae. Further prospective, randomised double-blind placebo controlled clinical trials of simvastatin in IVF are needed to confirm these initial findings.
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