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# Effects of in vitro fertilization (IVF) therapies on metabolic, endocrine and inflammatory status in IVF-conceived pregnancy

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## Abstract

**Rationale:** In vitro fertilization (IVF) is a common treatment for infertility. In mice, IVF is associated with development of glucose intolerance. However, human data are limited regarding the metabolic, endocrine and inflammatory effects of IVF therapy in IVF-conceived pregnancies.

**Objective:** To explore effects of IVF therapies on metabolic, endocrine and inflammatory parameters in IVF-conceived pregnancy.

**Methodology:** Twelve-week prospective observational study of adult normoglycaemic women, BMI 18.5-38 kg/m$^2$ and ≤ 39 years awaiting IVF therapy. Fasting blood samples were collected at baseline and 12 weeks, and serum analysed for reproductive hormones, glucose, lipids, insulin sensitivity, thyroid status, adiponectin inflammatory marker and lipopolysaccharide-binding protein (LBP).

**Results:** Two hundred and seventy-five women were analysed: 158 IVF-conceived pregnant women and 117 with failed IVF. Compared with baseline, nonpregnant women had significant ($P < .001$) increases in 12-week glucose (86.04-87.62 mg/dL), insulin (8.72-9.37 μIU/mL), HOMA-IR (1.9-2.1), T-Chol (169.5-174.9 mg/dL), TG (71.0-83.7 mg/dL) and HDL-C (52.0-54.11 mg/dL) levels. At 12 weeks, pregnant women also had ($P < .001$) increases in T-Chol (177.5-199.5 mg/dL), TG (73.5-126.78 mg/dL) and HDL-C (55.3-65.1 mg/dL), while a significant reduction in glucose (86.15-82.19 mg/dL), HbA1c (5.3-5.08%) and TSH (1.71-1.36 μIU/mL) levels from baseline. Adiponectin and LBP levels remained the same in either group.

**Conclusion:** In vitro fertilization hormonal therapy impairs glucose and insulin levels; these effects are masked in early pregnancy. Changes in lipid profile occur following IVF therapies regardless of pregnancy outcome. Neither adiponectin nor LBP is affected by IVF therapies and during early IVF-conceived pregnancy.

KEYWORDS

endocrinology, in vitro fertilization, in vitro fertilization pregnancy, inflammation, metabolism, pregnancy

Clinical Trial Registration: NCT03426228

## 1 | INTRODUCTION

Infertility is a growing health concern and affects 20% of couples of reproductive age.[1] Accordingly, assisted reproductive technologies have emerged as important therapeutic options for the management of infertility, primarily in vitro fertilization (IVF).[2] Similar in composition to IVF, long-term usage of oral contraceptive hormones (based on oestrogen and progesterone) associates with adverse effects on metabolic, endocrine and inflammatory status, changes in gut microflora and gastrointestinal side effects.[3] However, IVF therapies constitute much higher doses but shorter exposure duration to these hormones and their safety on both maternal and foetal outcomes remains incompletely understood.[4,5]

Pregnancy is characterized by hormonal-driven changes with consequences on metabolic, endocrine and inflammatory status and possibly on gut microflora. Insulin sensitivity deteriorates starting from the second trimester and subsequent hyperinsulinemia.[6] Metabolic response to oral contraceptive therapies bear similarities to those that occur during the second trimester of pregnancy.[7,8] Furthermore, in mice IVF therapy is associated with development of glucose intolerance.[9] Reproductive hormonal therapies also alter thyroid function with increased serum thyroxine-binding globulin and total serum thyroxine levels; a comparable effect is seen during pregnancy.[10,11] Additionally, increase in lipid profile occurs normally mid-pregnancy[12] and with contraceptives use.[7]

Physiological changes of pregnancy may also induce micro-inflammation and synthesis of inflammatory markers[13]; a similar effect was observed in obese women on oral contraceptive therapies.[14] Inflammation (as a stressor) increases permeability of the gut lining[15]; a surrogate marker of gut endotoxemia is lipopolysaccharide-binding protein (LBP). LBP binds bacterial compounds, including lipopolysaccharides (LPS; outer membrane component of gram-negative bacteria of the gut). Similarly, long-term use of oral contraceptives impairs gut permeability, with enhanced levels of LBP and LPS signalling and associated cytokine-mediated inflammatory diseases.[16] Adiponectin is another anti-inflammatory and insulin sensitizer marker, which gradually declines in pregnancy.[17]

Given the high doses of IVF hormones combined with gestational hormones, physiological changes may hence be manifested earlier in IVF-conceived pregnancies. The aim of this study was to assess metabolic, endocrine and inflammatory effects of IVF hormonal therapies within 12 weeks following its administration in the two groups: women with successful IVF-conceived pregnancy and women with failed IVF.

## 2 | METHODS

### 2.1 | Subjects

Women from multicultural population were recruited from three IVF clinic branches in the United Arab Emirates (Dubai, Abu Dhabi and Al Ain). Convenient sampling method was used to recruit participants who were to start IVF therapy and meeting the inclusion criteria. A list of women who were to commence IVF treatment was reviewed daily. Following exclusion of diabetes mellitus and thyroid dysfunction, those subjects who consented for recruitment into the study were invited to attend for a baseline fasting blood test (for 10 hours) on their screening visit (first day of their IVF treatment program). Anthropometric data were also obtained (weight, height, BMI), and medical history questionnaires completed by the principle investigator. Ethical approvals were obtained from local health authorities for each of the study centres, and the study complied with the code of ethics of the Declaration of Helsinki.

### 2.2 | Inclusion and exclusion criteria

Women aged 18-39 years of age, presenting with any infertility concern and BMI 18.5-38 kg/m$^2$, were eligible for inclusion in the study. In addition, women were eligible to participate if it was their first ever IVF cycle. Exclusion criteria included current or past history of diabetes mellitus, thyroid dysfunction and any other chronic medical condition such as hepatic, respiratory, haematological and cardiovascular disease. Other exclusion criteria included use of any therapy that may affect glucose homeostasis, thyroid and/or lipid profile, such as growth hormones, oral steroids, anti-inflammatory and bronchodilator drugs.

### 2.3 | IVF intervention

In this prospective cohort (observational), participants were followed for 12 weeks of their first IVF cycle. Each subject underwent IVF therapy as per clinical need, using the 'antagonist protocol', which relies on administration of agents to prevent premature ovulation (ie, gonadotropin-releasing hormone antagonist) and to ensure adequate oocyte growth.[18] The study consisted of four stages (shown in Figure 1):

#### 2.3.1 | Stage 1: ovarian stimulation and follicle growth (1-12 days)

Depending on baseline levels, follicle-stimulating hormone (FSH) was administered alone or combined with luteinizing (LH) (300 IU/day). While FSH is needed for ovarian follicular growth and endometrial development, LH ensures proper oocyte maturation. Follicle growth (size and numbers) was monitored with frequent ultrasound and blood tests for assessment of serum levels of reproductive hormones, and appropriate adjustment of IVF therapies. On day 6 of stimulation, gonadotropin-releasing hormone antagonist injection was administered (0.25 mg/day) for a better control of endogenous FSH and LH concentrations. One dose of human chorionic gonadotropin hormone 'trigger' (0.5 mg)

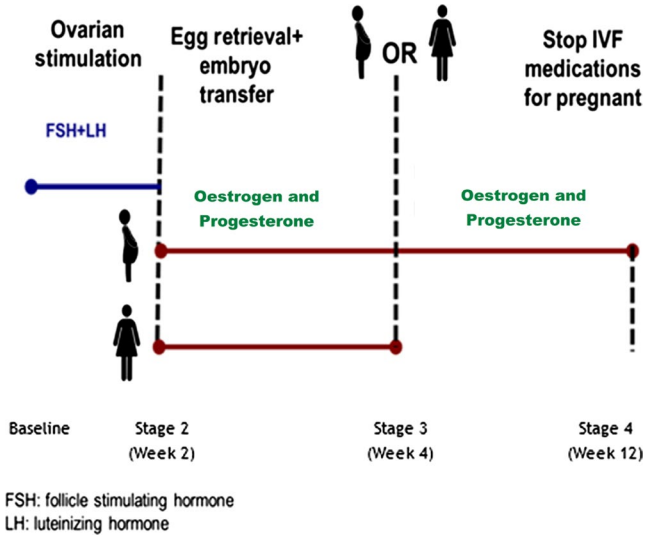


**FIGURE 1** Study stages and IVF hormonal intervention. IVF, in vitro fertilization

was given 36 to 40 hours before schedule of egg retrieval to induce final egg maturation. All other medications were discontinued at that point.

### 2.3.2 | Stage 2: egg retrieval to embryo transfer (week 2)

This includes the period from egg retrieval ('oocyte pick-up', OPU) to embryo transfer (ET) five days post-OPU. Egg retrieval is done by transvaginal ultrasound aspiration. During this stage, each retrieved egg undergoes fertilization with collected semen, under a microscope and using intracytoplasmic sperm injection technique (sperm is directly injected into cytoplasm of mature egg). Transvaginal ultrasound guidance is used during ET, which is associated with a higher percentage of pregnancy per transfer compared with transabdominal ultrasound guidance transfers.[19] Post-OPU, progesterone (tablet: 10 mg three times/day and injection: 50 mg/day) and oestrogen therapies (tablet: 2 mg three times/day) were initiated.

### 2.3.3 | Stage 3: first pregnancy test (week 4)

This includes measurement of serum beta-human chorionic gonadotropin (β-HCG). Successful IVF therapy is defined as a clinically confirmed pregnancy with a positive serum β-HCG test and a gestational sac is observed on ultrasound, while unsuccessful refers to a negative β-HCG test at 4 weeks. Biochemical pregnancy represents a pregnancy confirmed by a positive β-HCG but no sac is visible on ultrasound, and ectopic pregnancy is the case where the embryo abnormally implants outside the uterus.[20] With all cases of negative β-HCG, ectopic or biochemical pregnancies, all reproductive therapies were discontinued at this stage. Biochemical and ectopic pregnancies were not included in the unsuccessful group data. For all successful pregnancies, subjects were required to continue taking their reproductive therapies (oestrogen and progesterone) for the first trimester (until around week 12 of pregnancy).

### 2.3.4 | Stage 4: final blood tests (week 12)

This included assessment of the two groups: women with successful IVF-conceived pregnancy and women with failed IVF.

## 2.4 | Sample size

The primary outcome of the study was to assess changes in glucose homeostasis in response to IVF therapy and during IVF-conceived pregnancy. Significant changes in glucose and insulin levels are expected to occur earlier in IVF-conceived pregnancy as an effect of IVF hormones. In order to detect a moderate difference (standardised difference = 0.5), with 80% power, at significance level of 0.05 and a ratio of 2:1 for pregnant to nonpregnant women, the sample size consisted of 96 pregnant and 48 nonpregnant women. According to the latest statistics, pregnancy success rate post–egg retrieval is about 30% and this declines with age.[21] Therefore, 275 participants were recruited initially to end up with 96 clinically confirmed pregnant.

## 2.5 | Outcome measures

Blood tests were conducted at baseline and 12-weeks and included the following: female reproductive hormones (FSH, LH, oestrogen (oestradiol E2 form) and progesterone), fasting plasma glucose, serum insulin, glycated haemoglobin A1c (HbA1c), lipid profile, thyroid-stimulating hormone (TSH), adiponectin and LBP. At 4-weeks of IVF hormonal therapy, fasting glucose and insulin levels were also measured, as well as oestrogen, progesterone and β-HCG pregnancy test.

Female reproductive hormones, insulin and TSH were measured with the electrochemiluminescence immunoassay ECLIA, using Cobas E immunoassay analyzers from Roche Diagnostics. Fasting plasma glucose was measured by enzymatic reference method with hexokinase-glucose-6-phosphate dehydrogenase. Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as follows: (fasting plasma insulin x fasting plasma glucose)/ 405.[22,23] Total cholesterol (T-Chol), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were measured by homogenous enzymatic colorimetric method, with Roche/Hitachi Cobas C systems (Cobas C 311/501; Roche Diagnostics). Enzyme-linked immunosorbent assay was used to determine plasma LBP concentration with human LBP ELISA kit. Adiponectin was measured using human ADP/Acrp 30 ELISA kits, from Elabscience.

### 2.6 | Statistical analysis

Data analysis was performed with Statistical Package for the Social Sciences (SPSS) software version 21.0 for Windows (SPSS). Non-normal distribution of parameters was identified using Shapiro-Wilk test, and results are hence presented as median and interquartile range (IQR). Nonparametric Mann-Whitney *U* test for two independent samples was used to compare the two groups (pregnant vs non-pregnant), at baseline and at 12 weeks. Nonparametric Wilcoxon's test for two related samples was used to assess changes at baseline vs at 12 weeks within each group (pregnant or nonpregnant). A *P* value of ≤0.05 was used for significance level, with 95% confidence interval (CI).

## 3 | RESULTS

### 3.1 | Recruitment

A total of 702 women were prescreened by telephone, from whom 673 were eligible for enrolment in the study. Of these, 354 women were recruited into the study as they had ET. Post–embryo transfer, biochemical and ectopic pregnancies (n = 10) were excluded and participants were divided into two groups: clinical successful pregnancy based on positive β-HCG (pregnant, n = 191) and unsuccessful pregnancy outcome based on negative serum β-HCG (nonpregnant, n = 153). In the pregnant group (n = 191), 16 participants did not complete the study, 17 experienced a miscarriage (8%) before 12 weeks and they were hence excluded from the study. In total, 275 participants completed the study, of which the two groups included successful IVF-conceived pregnancy (n = 158) and unsuccessful IVF pregnancy (n = 117; 36 participants did not complete the study). Successful pregnancy accounted for 105 single (66%) and 53 multiple pregnancies (34%). An overview of the recruitment flow is shown in Figure 2. The reasons for undergoing IVF therapy were as follows: 30% female infertility, 30% male infertility, 15% for gender selection and 25% related to other causes (genetic disorders, combination of both male and female infertility and unknown infertility). Overall, 45% of women undergoing IVF had a confirmed diagnosis of polycystic ovary syndrome (PCOS) (n = 122; 50 nonpregnant and 72 pregnant), but this was not necessarily the main reason for undergoing IVF in these cases. For the pregnant group overall at baseline, 22% of women had a BMI ≥ 30 kg/m$^2$ (n = 22), 16% had HbA1c in the prediabetic range between 5.7-6.1% (n = 26), and 14% had a past history of GDM (n = 22).

At baseline, there was no difference in anthropometrics, endocrine and metabolic parameters between pregnant and nonpregnant women (data shown in Table 1). Participants overall had a median age of 32 (6) years, BMI of 25.4 (6.9) kg/m$^2$, HbA1c of 5.2 (0.52) % and TSH of 1.82 (1.4) μIU/mL. At baseline, 21% had a BMI ≥ 30 kg/m$^2$. Ethnicity of participants was multicultural: 53% Gulf nationals, 20% from Far East (South and East Asia), 15% Middle Eastern, 8%

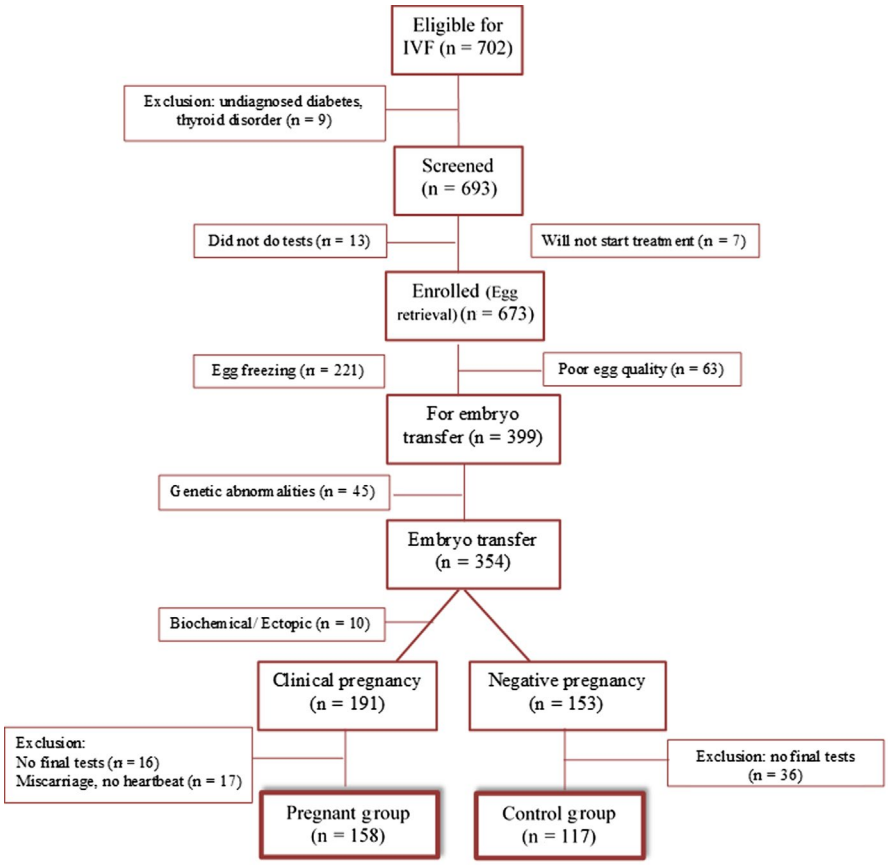


**FIGURE 2** Flow chart of participants' recruitment and enrolment in the study

**TABLE 1** Comparison of anthropometrics, metabolic and endocrine parameters at baseline and 12 wk of IVF treatment for pregnant and nonpregnant women

| | Baseline | | | 4 wk | | | 12 wk | | |
|---|---|---|---|---|---|---|---|---|---|
| Variables | Pregnant (n = 158) | Nonpregnant (n = 117) | *P* value | Pregnant (n = 158) | Nonpregnant (n = 117) | *P* value | Pregnant (n = 158) | Nonpregnant (n = 117) | *P* value |
| Age (y) | 32.0 (7.0) | 32.5 (7.00) | .32 | | | | | | |
| Weight (kg) | 65.5 (18.95) | 64.0 (13.97) | .58 | | | | 66.9 (15.9) | 64.7 (15.05) | .21 |
| Body mass index ($kg/m^2$) | 24.8 (7.30) | 25.55 (6.15) | .62 | | | | 25.7 (6.90) | 25.75 (5.73) | .86 |
| Female hormones | | | | | | | | | |
| FSH (IU/L) | 6.46 (2.51) | 6.65 (2.47) | .25 | | | | | | |
| LH (IU/L) | 5.99 (3.16) | 5.75 (2.70) | .39 | | | | | | |
| Ratio FSH/LH | 1.10 (0.60) | 1.10 (0.50) | .14 | | | | | | |
| Oestrogen (pg/mL) | 41.9 (24.2) | 41.04 (19.15) | .41 | 412.15 (857.10) | 220.5 (197.90) | <.001 | | | |
| Progesterone (ng/mL) | 0.23 (0.23) | 0.24 (0.20) | .84 | 41.07 (37.61) | 20.96 (23.95) | <.001 | | | |
| Metabolic and endocrine | | | | | | | | | |
| Fasting glucose (mg/dL) | 86.15 (8.0) | 86.04 (10.0) | .73 | 84.47 (7.61) | 86.09 (6.55) | .01 | 82.19 (7.19) | 87.62 (8.34) | <.001 |
| Fasting insulin (μIU/mL) | 8.84 (6.81) | 8.72 (6.41) | .93 | 9.95 (9.28) | 11.82 (6.29) | .01 | 9.45 (6.95) | 9.37 (5.4) | .86 |
| HbA1c (%) | 5.3 (0.58) | 5.20 (0.50) | .77 | | | | 5.08 (0.53) | 5.19 (0.47) | .003 |
| HOMA-IR | 1.95 (1.52) | 1.90 (1.50) | .99 | 2.00 (2.00) | 2.60 (1.45) | .01 | 2.00 (1.60) | 2.10 (1.5) | .17 |
| T-Chol (mg/dL) | 177.5 (44.95) | 169.5 (39.33) | .15 | | | | 199.5 (44.35) | 174.9 (48.03) | <.001 |
| TG (mg/dL) | 73.5 (44.0) | 71.0 (41.98) | .94 | | | | 126.78 (60.3) | 83.7 (35.15) | <.001 |
| LDL-C (mg/dL) | 103.0 (38.95) | 101.3 (44.0) | .61 | | | | 103.2 (32.43) | 102.57 (38.83) | .47 |
| HDL-C (mg/dL) | 55.3 (15.94) | 52.0 (18.82) | .12 | | | | 65.1 (18.3) | 54.11 (14.30) | <.001 |
| TSH (μIU/mL) | 1.71 (1.29) | 1.95 (1.46) | .34 | | | | 1.36 (1.10) | 1.80 (1.05) | <.001 |
| ŦAdiponectin (μg/mL) | 8.87 (1.86) | 8.47 (2.17) | .17 | | | | 8.66 (2.41) | 8.46 (1.94) | .82 |
| ŦLBP (μg/mL) | 62.96 (78.83) | 55.60 (70.70) | .97 | | | | 45.18 (71.82) | 41.29 (88.16) | .65 |

*Note:* Data presented in median and interquartile range (IQR; IQR = Q3-Q1); Ŧn = 73 (42 pregnant, 31 nonpregnant); $P < .05$ vs pregnancy by independent test.

Abbreviations: FSH, follicle-stimulating hormone; HbA1c, glycated haemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; LBP, lipopolysaccharide-binding protein; LDL-C, low-density lipoprotein cholesterol; LH, luteinizing hormone; T-Chol, total cholesterol; TG, triglycerides; TSH, thyroid-stimulating hormone.

Europeans and 4% with African origins. There was a significant increment in weight at 12 weeks in both groups: pregnant women BMI: 24.8-25.7 $kg/m^2$, $P < .001$; nonpregnant women BMI: 25.6-25.8 $kg/m^2$, $P = .002$.

## 3.2 | Glucose and insulin homeostasis

Compared with baseline, glucose level was significantly lower at 4 weeks (Δ: −1.68 mg/dL, $P = .007$) and more so at 12 weeks (Δ: −3.96 mg/dL, $P = .001$) in the pregnant group. At week 12, pregnant women had also significant reduction in HbA1c (5.3 to 5.08%, $P < .001$). There were no changes in fasting insulin and HOMA-IR measures at 4 and 12 weeks of pregnancy compared with baseline levels. In contrast, nonpregnant 4-week glucose level did not change compared with baseline, while insulin (Δ: 3.1 μIU/mL, $P < .001$) and HOMA-IR measures (Δ: 0.7, $P = .01$) were increased. At 12 weeks, nonpregnant women showed statistically significant increase in glucose (Δ: 1.56 mg/dL), while insulin (Δ: −1.17 μIU/mL) and HOMA-IR (Δ: −0.2) were slightly reduced at week 4 but remained higher compared to baseline levels, with $P < .001$ (Table 1 and Figure 3). Regardless of pregnancy status, significant changes in glucose and insulin homeostasis occur at 4 weeks of IVF therapy and dependent on IVF outcome. In relation

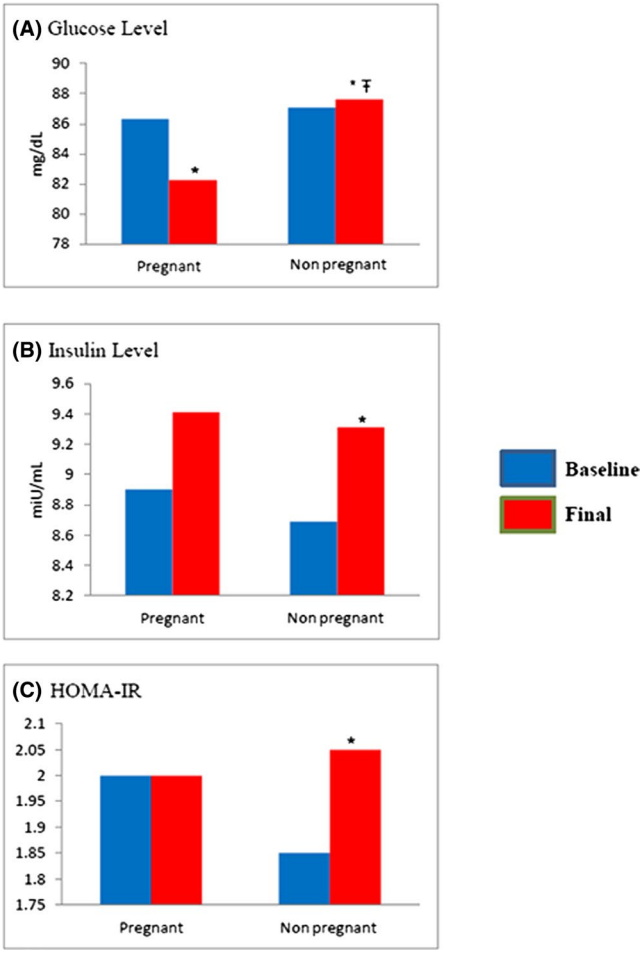


**FIGURE 3** Comparison of glucose homeostasis at baseline and 12 wk of IVF therapy between pregnant and nonpregnant women (Graph A. Fasting glucose level; B. Fasting insulin level; C. HOMA-IR). *$P < .05$ vs at 12 wk of IVF therapy; ŦP < .05 vs pregnancy; HOMA-IR, homeostatic model assessment of insulin resistance; IVF, in vitro fertilization

to history of PCOS, in the pregnant group, 72 women had a history of PCOS and 86 were non-PCOS prior to pregnancy. At baseline, women with PCOS presented higher insulin (11.3 vs 9.5 μIU/mL) and HOMA-IR (2.5 vs 2.05) levels compared to the non-PCOS group, with $P < .001$. At 12 weeks of pregnancy, there were no differences in insulin homeostasis parameters between the two groups of pregnant women. In the nonpregnant group, baseline and 12-week insulin homeostasis did not differ between PCOS and non-PCOS women.

### 3.3 | Lipids

At 12 weeks of pregnancy, lipid profile increased significantly, including T-Chol (177.5-199.5 mg/dL), TG (73.5-126.8 mg/dL) and HDL-C (55.3-65.1 mg/dL), with $P < .001$. Similarly, in nonpregnant women, T-Chol (169.5-174.9 mg/dL), TG (71.0-83.7 mg/dL) and HDL-C (52.0-54.1 mg/dL) increased ($P < .001$).

### 3.4 | Endocrine and inflammatory outcomes

Pregnant women had significant reductions in TSH (1.7-1.4 μIU/mL; $P < .001$) at 12 weeks, while for nonpregnant group, there was no change in TSH level. For all subjects overall, and for each pregnant and nonpregnant groups, there were no changes in serum adiponectin or LBP between baseline and 12-weeks.

## 4 | DISCUSSION

This study showed that IVF hormonal therapy raises levels of glucose, insulin and lipids parameters and reduces insulin sensitivity in women with failed IVF (ie, nonpregnant). A similar but more intense effect on impaired insulin sensitivity and glucose intolerance was reported with long-term use of oral contraceptives,[24,25] suggesting that the duration of the treatment might have more impact on glucose and insulin homeostasis than a high dose for a short period of time. The effect of IVF hormones on glucose and insulin homeostasis was down-regulated by pregnancy, with no change observed in insulin level and resistance (HOMA-IR), while glucose level was reduced (still within normal range). Glucose level may drop early in pregnancy, secondary to a physiological adaptation for increased foetoplacental needs, with a focus on carbohydrates as a source of energy.[6,26] During early gestation, glucose homeostasis remained similar to nonpregnancy level, confirming also other studies' findings conducted in spontaneous pregnancy in maintaining euglycaemic levels.[27,28] The diabetogenic state from hyperinsulinaemia and increased insulin resistance is usually manifested during mid-preganncy[27]; such changes were not observed yet at 12 weeks of IVF-conceived pregnancy. In relation to history of PCOS, despite higher baseline levels of insulin resistance markers (insulin and HOMA-IR; levels still within normal range) in PCOS women, 12-week levels were down-regulated to non-PCOS pregnant levels.

Unlike the expected effect of oestrogen therapy on raising TSH, level remained the same at week 12 for nonpregnant women. Two possible explanations may be proposed: (a) IVF medications were stopped at 4 weeks and oestrogen therapy has already been cleared out from the body; (b) duration of IVF hormone administration was too short to induce changes in TSH level. Drop in TSH level in the pregnant group is consistent with previous studies reporting 20-50% suppression due to the sharp increase in hCG concentrations.[29,30] Given the potential adverse effect of IVF hormones on impairing glucose and insulin homeostasis and thyroid function, more attention should be paid with repeated IVF cycles or if IVF hormones were to be provided for a longer period during pregnancy.

Female reproductive hormones stimulate synthesis of inflammatory markers, which may be associated with change in gut permeability[13]; a similar response was expected with IVF hormonal therapy. Interestingly, our data do not support an effect of IVF therapies on serum LBP levels of participants, and by inference change

in gut permeability yet up until 12 weeks. More research is needed to elucidate whether LBP can act as a surrogate marker of LPS and its related impact on inflammation and gut microflora. Additionally, dietary intake is one of the important modulators of intestinal microflora diversity and richness[31]; such information was not collected in the study. While it is nowadays common to link gut microflora impairment with the pathogenesis of certain metabolic disorders (such as obesity and T2D), which are also associated with low-grade inflammation, it is still however not yet confirmed whether inflammation is the cause or a consequence of the condition.[32]

Adiponectin is another useful marker of inflammation and has glucose-lowering properties.[17] Serum adiponectin inversely associates with BMI, fasting glucose and insulin, and TG levels, and positively associates with HDL-C levels.[17] These effects cannot be ascertained in our study, since interpretations are only based on unchanged levels of adiponectin within and between groups. In addition, the type of association between adiponectin and inflammation remains controversial in the literature. In contrast to the negative typical correlation between adiponectin, obesity and metabolic disease, a positive association was presented with inflammatory and immune-mediated diseases in one study.[33]

The interplay between inflammation and lipid metabolism is well documented, and the two play important role in the pathophysiology of metabolic conditions, such as insulin resistance.[34] Similarly to oral contraceptives, IVF hormones augmented lipid parameters, but values remained within normal range possibly due to the short duration of the treatment.[7,25] Metabolic and hormonal changes of pregnancy impose changes on lipid homeostasis, which play an important role in the provision of energy for the foetus. At 12 weeks of pregnancy, lipid profile complied with normal gestational changes with increased TG, T-Chol and HDL-C levels.[12] The attributable effect of IVF hormones on lipids in pregnancy cannot be however determined for the following reasons: (a) disparities in duration of IVF hormonal administration between the two groups (4 weeks for nonpregnant vs until 12 weeks of pregnancy); (b) a cumulative effect would have been identified if lipids parameters exceeded the reference ranges in pregnancy[35]; and (c) including a well-matched spontaneous-pregnant group would have allowed determination of the magnitude of change in lipid profile as an effect of pregnancy alone.

Baseline metabolic and endocrine parameters may differ between normal vs obese classified BMI, and this may have affected our data at 12 weeks. A limitation of this study hence relates to the large BMI range of participants. We did not perform subgrouping of BMI categories due to lack of power. Dietary intake, urine and stool sample collection of participants would have enabled more accurate assessment of early changes in gut microflora. Moreover, although the euglycaemic clamp protocol is considered as the 'gold standard' test for assessing changes in insulin sensitivity,[36] HOMA-IR is a surrogate measure, yet not as precise as the clamp but more practical and noninvasive for pregnant women. It is also worth mentioning that comparing the effect of IVF hormones to oral contraceptives is questionable, since the two therapies may differ in their type of bioactive oestrogen and progesterone and the duration of the treatment. In addition, although IVF therapies constitute much higher doses but they present a transient term of exposure to these hormones. In addition, pregnancy is a complex condition with significant inherent confounding effects on metabolic and inflammatory systems, which makes the direct comparison between pregnant and nonpregnant groups delicate. Finally, we acknowledge that other potential confounding factors may have influenced our data; such factors include psychological status and diet of participants. A well-matched spontaneous-pregnant group would have allowed the determination of magnitude of change in all these parameters as an effect of pregnancy alone. Future studies may want to compare more thoroughly pregnancies conceived spontaneously vs by IVF.

## 5 | CONCLUSION

In vitro fertilization therapy induces weight gain and impairment in glucose, insulin and lipid homeostasis in failed IVF. Improvement of glucose homeostasis, decrease in thyroid profile and increase in lipid profile in clinical pregnancy are likely a pregnancy-related effect. Neither adiponectin nor LBP is affected by IVF therapies and during early IVF-conceived pregnancy. Hence, monitoring of metabolic and endocrine parameters in 3 months following IVF should be implemented in clinical practice, particularly with repeated and failed IVF attempts.

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### CONFLICT OF INTEREST

There is no conflict of interest or financial disclosure to declare.

### AUTHOR CONTRIBUTION

All authors contributed substantively to the preparation of this manuscript.

### DATA AVAILABILITY STATEMENT

Data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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