Early Predictors of Gestational Diabetes Mellitus in IVF-conceived pregnancies

Abstract

Objective: Gestational diabetes mellitus (GDM) associates with adverse maternal and foetal outcomes. The aim of our study was to identify early and reliable GDM predictors, which will enable implementation of preventive and management measures.

Methodology: 28-week prospective cohort of IVF-conceived pregnant women (≤39years, BMI 18.5-38kg/m^2) without known history of diabetes mellitus. Fasting blood samples were analysed at baseline (pre-IVF) and 12-week gestation for reproductive hormones, glucose, serum insulin, lipids, thyroid function, adiponectin and LBP. At 28 weeks, 75-gram OGTT was used to screen for GDM.

Results: For the pregnant group overall at baseline, 22% had a BMI≥30kg/m^2, 45% with PCOS, 16% with HbA1c of 5.7–6.1% and 14% with a past history of GDM. At 28-week gestation (n=158): 34 women developed GDM and 124 did not. Significant baseline predictors of GDM onset included: greater BMI (29.0 vs. 25.8kg/m^2), older age (34 vs. 32years) and higher levels of FSH/LH ratio (1.2 vs. 1.0), HbA1C (5.5 vs. 5.2%), insulin (10.6 vs. 7.1μIU/mL), HOMA-IR (2.2 vs. 1.7), T-Chol (199 vs. 171mg/dL) and LDL-C (123 vs. 105mg/dL), and lower TG levels (74 vs. 76mg/dL). Significant 12-week GDM predictors included: greater maternal weight gain (delta: 3.4 vs. 1.5kg) and higher levels of insulin (11.3 vs. 7.6μIU/mL), TG (178 vs. 120mg/dL) and HOMA-IR (2.3 vs. 1.5). 12-week BMI is a predictor of GDM following adjustment for PCOS status and maternal age.

Conclusion: While preconception maternal BMI, age and FSH/LH ratio are predictors for subsequent development of GDM, early IVF-conceived gestational weight gain predicted best GDM onset.

Abbreviations:
IVF= in vitro fertilization, **LBP** = lipolysaccharide binding protein, **FSH** = follicle-stimulating hormone, **LH** = luteinizing hormone, **HOMAR-IR** = homeostatic model assessment of insulin resistance, **OGTT** = Oral Glucose Tolerance Test, **T-Chol** = total cholesterol, **LDL-C** = low density lipoprotein cholesterol, **TG** = triglycerides.

Clinical Trial Registration: NCT03426228

Introduction

Gestational diabetes mellitus (GDM) is a global health concern, associated with adverse outcomes for both mother and foetus, childbirth and possibly for offspring later in life(1).The worldwide increasing prevalence of GDM accounts for 5–20%, depending on specific population demographics and diagnostic criteria applied(2). The International Association of the Diabetes and Pregnancy Study Groups (IADPSG) defines GDM as "glucose intolerance resulting in hyperglycaemia, with onset or first recognition during pregnancy"(3). Established risk factors include maternal obesity (BMI>30 kg/m^2), advanced maternal age (>35 years), prior history of GDM, previous baby weight >4.5 kg, a first-degree relative with diabetes mellitus, abnormal weight gain during pregnancy and certain ethnicities(4,5). In addition, women with polycystic ovary syndrome (PCOS) have a significantly greater risk of developing GDM compared to women without PCOS(6). In usual clinical practice, pregnant women undergo screening for GDM at 24–28 weeks of gestation, using a 2-hour 75 gram oral glucose tolerance test (OGTT)(7).

Studies have explored possible GDM predictors during early pregnancy (<16 weeks), and these include higher levels of fasting plasma glucose (>126 mg/dL), HbA1c (5.7–6.4%) and serum insulin (≥ 30 mU/L)(8–10). Furthermore, changes in levels of thyroid hormones can also influence glucose metabolism through multiple mechanisms that include reduction of insulin half-life, promotion of hepatic glucose output and glycogenolysis(11,12). Serum triglycerides (TG) level is a further useful predictor of GDM, although association of GDM with other components of lipid profile is less clear(13). Adiponectin is suggested as another potential predictor of GDM onset, which is a type of adipokine with multiple regulatory impacts on glucose metabolism(14). Maternal secretion of adiponectin gradually declines during pregnancy, secondary to hormonal effects (including glucocorticoids). Low serum levels of adiponectin likely also contribute towards the development of pregnancy-related micro-inflammation (i.e. cytokines release), that in turn exacerbates insulin resistance(15). Changes in gut microflora during pregnancy may also trigger an inflammatory response, through mediation of a 'leaky gut'(16). A useful marker is lipopolysaccharide binding protein (LBP), an acute-phase protein that binds bacterial compounds such as lipopolysaccharides (LPS, endotoxin of outer membrane component of gram-negative bacteria of the gut). Increased permeability of LPS into the bloodstream contributes to the development of metabolic conditions (such as insulin resistance and type 2 diabetes mellitus)(17). Evidence is still controversial for using LBP as a surrogate marker of endotoxemia and resultant inflammation in place of LPS.

The aim of our study was to explore the utility of well-documented and novel metabolic, endocrine and inflammatory biomarkers as predictors of onset of GDM in early gestation in women undergoing IVF.

Methods

Recruitment

Participants were recruited from three separate fertility clinics in the United Arab Emirates (UAE). Convenient sample of multicultural women, scheduled to start i*n vitro* fertilization (IVF) therapy, were invited to enroll in the study, according to inclusion and exclusion criteria outlined below. Following informed consent, on their first (baseline) visit to the fertility clinic (preconception stage, first day of IVF), all recruited women completed medical history questionnaires and had anthropometric measurements taken. Fasting blood samples were also collected. Inclusion criteria included women who were scheduled to start IVF therapy, with an age range of 18-39 years and BMI 18-38 kg/m^2. Further inclusion criteria included confirmation of euglycaemia (HbA1c <6.4% and fasting glucose <126 mg/dL)(7) and normal thyroid function (0.4–4.0 μIU/mL)(5,18). Exclusion criteria included any current or past history of diabetes mellitus (but not of GDM), impairment of thyroid function and other chronic medical conditions (such as hepatic, haematological and cardiovascular). Further exclusion criteria included therapies such as anti-glycaemics, anti-hypertensives and anti-inflammatories. Patients taking thyroid drugs, growth hormone, oral steroids, bronchodilator or lipid-lowering drugs were also excluded.

Study Protocol

Ethical approval was obtained from local health authorities, and the study complied with the code of ethics of the Declaration of Helsinki. The study design was prospective cohort and non-experimental (observational cohort), whereby participants were followed for 28 weeks of gestation. As per protocol design, pregnancy was conceived by IVF. For ovulation stimulation and early support of follicle growth, all recruited women took follicle-stimulating hormone (FSH) either alone or combined with luteinizing hormone (LH) (300 IU/day) for about 8-10 days. Post embryo-transfer, progesterone (tablets: 10 mg three times/day; injection: 50 mg/day) and oestrogen (tablets: 2 mg three times/day) therapies were initiated and administered until around week 12 of pregnancy. Pregnancy status was confirmed with a serum beta-human

chorionic gonadotrophin (β-HCG) test performed at 4 weeks of IVF-therapy. At around 28 weeks of gestation, all pregnant women underwent a 2-hour 75-gram oral glucose tolerance test (OGTT) for identification of GDM. The international Association of Diabetes and Pregnancy Study Groups diagnostic GDM cut points during OGTT were used and include plasma glucose of: 95 mg/dL (5.3 mmol/L) fasting, 180 mg/dL (10 mmol/L) for 1 hour, and 153 mg/dL for 2 hours (8.5 mmol/L)(3,7).

Sample Size

Change in glucose homeostasis was the primary outcome of the study, and which was expected to occur earlier in IVF-conceived pregnancy as an effect of IVF hormones. In order to detect a moderate difference (standardized difference of 0.5) in glucose homeostasis at 12 weeks of IVF hormonal therapy, with 80% power and at 0.05 significance level, a sample size of 96 pregnant women was required. Assuming IVF pregnancy success rate is about 30%(19), we recruited 153 participants at baseline. In addition, prevalence of GDM in the UAE is about 30%(20); hence, we predicted that 30 participants will develop GDM at 28 weeks.

Outcome Measures

Fasting blood tests were taken at baseline and 12-week gestation. Women reproductive hormones (FSH, LH, oestrogen, progesterone and β-HCG), serum insulin and thyroid-stimulating hormone (TSH) levels were measured using Cobas E immunoassay analyzers, with electrochemiluminescence immunoassay ECLIA (Roche Diagnostics, Indianapolis, USA). Fasting plasma glucose was measured with hexokinase-glucose-6-phosphate dehydrogenase enzymatic method. Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated using the following formula(21): HOMA-IR= (fasting plasma insulin x fasting plasma glucose)/405. Fasting serum total cholesterol (T-Chol), TG and high-density lipoprotein cholesterol (HDL-C) were measured by homogenous enzymatic colorimetric method, from Roche/Hitachi Cobas C systems (Cobas C 311/501; Roche Diagnostics, Indianapolis, USA), and low density lipoprotein-cholesterol (LDL-C) level was deducted. Plasma LBP concentration was measured by human LBP ELISA with enzyme linked immunosorbent assay and adiponectin level by human ADP/Acrp 30 ELISA kit (Elab Science, Texas, USA).

Statistical Analysis

From the IVF-pregnant women, two main groups of participants were compared: pregnant women who developed GDM at 28 weeks, and those who did not. Statistical Package for

the Social Sciences (SPSS) software version 21.0 for Windows (SPSS, Chicago, IL) was used for data analysis. Normality of each parameter was tested with Shapiro-Wilk test, and most of them were not normally distributed. Accordingly, data are summarized using median and interquartile range (IQR). Non-parametric Mann-Whitney U test was used for two independent samples to compare data from the two groups (GDM and non-GDM pregnant women) at baseline and at 12 weeks of gestation. Non-parametric Wilcoxon test for two related samples assessed changes at baseline and 12 weeks within each group. In addition, linear regression analyses were used to explore association of delta change in fasting plasma glucose level from baseline to 12 week, with changes in other parameters for both GDM and non-GDM groups. Chi-square and fisher's exact tests were conducted to assess association of baseline maternal anthropometrics, pregnancy outcomes (categorical) and onset of GDM. Ethnicity of our participants (used in chi-square analysis) was divided into six groups: Middle East (from Lebanon, Syria, Jordan, Egypt, Iraq, Libya and Morocco), Gulf (from UAE, Saudi Arabia, Kuwait and Oman), Europe, South-Asia, East-Asia and Africa. Anthropometric and biochemical predictors of GDM were determined using binary logistic regression, and adjusted for age and history of PCOS. A *p*-value of ≤0.05 was deemed statistically significant. Confidence intervals (CI) were defined as 95%.

Results

Baseline Data

A total of 693 women were screened, 339 women either did not start IVF treatment or did not have an embryo transfer, and the remaining of 354 women were recruited into the study. Of the 354 women, 191 had a successful IVF-pregnancy outcome, 153 had a failed IVF and 10 presented with ectopic pregnancy. From the successful IVF-pregnancy group (n=191), 158 women completed the study tests at 12 weeks; and of these, two final subgroups were formed of 34 with confirmed GDM and 124 with no GDM at 28 weeks of gestation.

Data for anthropometric, metabolic and endocrine parameters of GDM and non-GDM women are presented in *Table* 1. For the pregnant group overall at baseline, 22% of women had a BMI ≥30 kg/m^2 (n=22), 45% had a confirmed diagnosis of PCOS (n=72), 16% had HbA1c in the pre-diabetic range between 5.7–6.1% (n=26), and 14% had a past history of GDM (n=22). Ethnicity of participants was multicultural: 53% Gulf nationals, 20% from Far East, 15% Middle Eastern, 8% Europeans and 4% with African origins. At baseline (pre-IVF) and compared to the non-GDM

subgroup, pregnant women who later developed GDM were 2 years older (p=0.03), 7kg heavier (p=0.01), with higher BMI (29.0 vs. 25.8 kg/m^2; p<0.001), and presented with significantly higher levels of the following parameters: ratio FSH/LH (1.20 vs. 1.0), insulin (10.60 vs. 7.14 μIU/mL) and HOMA-IR (2.20 vs. 1.70), all with p<0.05. Pregnant women who later developed GDM also had higher baseline T-Chol (199.0 vs. 171.0 mg/dL; p=0.002) and LDL-C (123.0 vs. 104.8 mg/dL; p=0.003), and lower TG levels (74.0 vs. 76.0 mg/dL; p=0.01) compared to the non-GDM subgroup. Ratio LH/FSH did not differ between the two groups.

Metabolic, Endocrine and Inflammatory Systems

At 12-week gestation, GDM subgroup had significant weight gain (delta change: 3.4kg; p=0.003), and reduction in fasting plasma glucose (88.38 to 80.0 mg/dL; p=0.007) and TSH levels (1.33 to 1.08 μIU/mL; p=0.05). There was also significant increase in 12-week levels of serum TG (74.0 to 177.9 mg/dL), T-Chol (199.0 to 211.4 mg/dL) and HDL-C (55.0 to 65.0 mg/dL), all with p<0.001. For non-GDM women, body weight also significantly increased at 12-week gestation (delta change: 1.5kg; p<0.001), and there was a reduction in fasting plasma glucose (85.3 to 81.0 mg/dL; p<0.001) and TSH levels (1.58 to 1.39 μIU/mL; p<0.001). In this subgroup, there were also significant changes in lipid profile, with increases in TG (76.0 to 120.0 mg/dL), T-Chol (171.0 to 198.4 mg/dL) and HDL-C (57.0 to 63.0 mg/dL) levels, all with p<0.001. Compared to baseline, there were no significant changes in fasting serum insulin, HOMA-IR, adiponectin and LBP levels at 12 weeks for both subgroups of women.

Regarding direct comparisons between the two subgroups, GDM women had significant higher levels of serum insulin (11.33 vs. 7.57 μIU/mL; p=0.02), HOMA-IR (2.30 vs. 1.50; p=0.01), TG (177.9 vs. 120.0 mg/dL; p=0.003), and delta change in body weight (3.4 vs. 1.5 kg; p=0.01) at 12-week gestation. Significant reductions in fasting plasma glucose and HbA1c levels occurred in both subgroups between baseline and 12-week gestation, although values remained within the normal range. Fasting levels of serum adiponectin and LBP were equivalent between GDM and non-GDM women at both baseline and 12 weeks of gestation.

Early Predictors of GDM

Association of change in glucose level compared to the change in other parameters (delta levels) from baseline to 12 weeks is presented in *Table 2*. There was an inverse association between delta change in glucose and insulin levels for both subgroups (GDM: B=-4.50; 95% CI:-5.84,-3.15; p<0.001; and non-GDM: B=-5.34; 95% CI:-6.05,-4.63; p<0.001). Delta change in glucose level was not associated with change in weight, lipids and TSH profile in both subgroups.

Within the whole pregnant group (regardless of future GDM status), change in serum insulin and HOMA-IR levels, were the best predictors of delta change in plasma glucose level during early gestation.

Associations of maternal and foetal outcomes (categorical variables) with onset of GDM are illustrated in *Table 3*. Baseline and 12-week BMI positively correlated with future development of GDM (p=0.001). Interestingly, LH/FSH ratio, ethnicity, history of PCOS and prior GDM showed no significant association with development of GDM. In regards to foetal outcomes, there was also no associations between number and sex of offspring and GDM risk. Maternal anthropometrics, metabolic and endocrine predictors of GDM are summarized in *Table 4*. Baseline maternal predictive factors for onset of GDM included the following: elevated FSH/LH ratio (OR=2.05; 95% CI:1.12,3.75; p=0.02), advanced age (OR=1.12; 95% CI:1.01,1.23; p=0.03) and greater BMI (OR=1.01; 95% CI: 0.73,1.39]; p=0.001) in an unadjusted comparison. At 12-week gestation, predictors of subsequent GDM included the following: greater levels of HOMA-IR (OR=1.59; 95% CI: 1.16,2.17; p=0.004), BMI (OR=1.16; 95% CI: 1.07,1.27; p<0.001) and insulin (OR=1.11; 95% CI: 1.03,1.18; p=0.004). Following adjustments for maternal age and known history of PCOS, 12-week maternal BMI remained as the only significant predictor of GDM (OR=1.11; 95% CI: 0.98,1.20; p=0.03).

Discussion

GDM rate of our participants is comparable with the latest local national statistics on GDM prevalence in spontaneous pregnancies, whereby one in every three pregnant women in the UAE develops GDM(20). In addition, during the first trimester (up to 12 weeks) of IVF-conceived pregnancies, plasma glucose of GDM women did not differ from the non-GDM subgroup and was also similar to data reported in spontaneously-conceived pregnancies(22).

Women who developed GDM were older (still below high-risk age group of >35 years(23)) and were more overweight (preconception) compared to the non-GDM subgroup. They also presented at baseline higher levels of glucose, insulin and lipids-related markers of GDM(24), and higher FSH/LH ratio. This hints the possibility of FSH/LH ratio being an early predictor of GDM; the mechanism remains unclear. During the first trimester (up to 12 weeks), GDM women exceeded the recommended weight gain of 0.5-2.0 kg during the first trimester(25). The decrease in glucose level (still within normal range) in both subgroups reflect the usual

physiological adaptation of metabolism regarding lipids and carbohydrates regulation in early pregnancy, favoring glucose availability to the foetus and maternal use of lipids (free fatty acids) as source of energy(26). Compared to the other aforementioned differences between the two subgroups, TG level was increased in both subgroups at 12 weeks, yet more in GDM women; this highlights its importance as a strong risk factor of GDM. Levels of adiponectin and LBP did not predict GDM risk.

Maternal and Foetal Outcomes and GDM

Estimates of GDM risks in our study tested a wide range of well-documented factors and predictors simultaneously rather than measuring them only in isolation. Numerous studies have emphasized the association between preconception BMI, gestational weight gain and GDM risk(27), and that being in the overweight (BMI: 25–29.5 kg/m^2) or obesity category (BMI >30 kg/m^2) is the strongest predisposing factor to GDM(28). Correspondingly, studies have reported that 5-10% preconception weight loss were sufficient to improve insulin sensitivity and risk of GDM in obese women with and without PCOS(29). Our study has evidenced similar findings with GDM risk being strongly associated with both preconception and prenatal obesity. However, when adjusting for age and PCOS history, only prenatal BMI (at 12 weeks) remained a significant predictor of GDM. Advanced age, essentially considered a powerful risk for obstetric adverse outcomes in pregnancy, was also an important predictor of GDM in our participants. Previous history of GDM and PCOS of participants did not predict onset of GDM, nor did their higher predisposed ethnicities (Gulf and Middle East)(4,6). In relation to pregnancy and foetus outcomes, multiple pregnancy has long been considered as a predisposing risk for complications and adverse medical outcomes such as GDM(30). However, we did not find any association between sex and number of offspring with risk of GDM.

Biomarkers and GDM Predictors

Baseline ratio of FSH/LH predicted best risk of GDM compared to the other biomarkers tested in the study. However, following adjustment for age and history of PCOS, FSH/LH ratio was no longer a significant early predictor of GDM risk. This may be caused by the higher prevalence of PCOS, advanced age and/or poor ovarian reserve in participants of this study; these conditions impair FSH and LH levels(31,32). Lower FSH level was also previously reported in prediabetes and diabetes post-menopausal women(33). Levels of FSH and LH were not measured during early pregnancy (at 12 weeks) to confirm the possibility of considering FSH/LH ratio as a novel predictor for GDM. Women with PCOS who are at higher risk of GDM, often

present higher preconception levels of LH/FSH ratio. Ratio level of LH/FSH in our participants was below the high-risk cut-off (ratio>2) in both group of participants(34). Surprisingly, even with a 45% prevalence of PCOS in our participants, baseline ratio of LH/FSH did not predict GDM risk. In addition, Hbac1 was not a good predictor of GDM in IVF-conceived pregnancy. The use of HbA1c for predicting and managing GDM remains inconclusive(24) and level of HbA1c can be influenced by different factors: anaemia, physiological hydraemia, hyperemesis gravidarum and dietary intake(9,35). Information in relation to participants' dietary intake was not collected. However, HbA1c may still assist in identifying women at high risk of adverse pregnancy outcomes, especially if presenting with HbA1c above 5.7% at <20 weeks of gestation(8).

Regarding insulin homeostasis, elevated levels of serum insulin in early gestation (<16 weeks) and HOMA-IR predispose to increased risk of GDM development(10,36). In our study, even with unchanged levels in insulin and HOMA-IR at 12 weeks, they predicted best early changes in glucose levels and onset of GDM. However, when adjusting for age and history of PCOS, HOMA-IR and insulin levels were no longer valid predictors of GDM. This may suggest that early gestation insulin and HOMA-IR levels of participants were related to their PCOS condition rather than a pregnancy effect. Nevertheless, rather than waiting for 24-28 weeks of gestation for doing the OGTT test, early gestation monitoring of insulin homeostasis will likely detect prompt changes related to GDM development and hence protect mother and foetus from adverse events.

In relation to lipid profile and GDM risk, previous studies highlighted a positive association between elevated TG and GDM risk. However, such association was not found in our study. In addition, the study by Li et al. reported increased serum TG, T-Chol, and LDL-C, and decreased HDL-C concentrations in GDM women, compared to their control groups(13). Participants in our study experienced instead an increase in HDL-C at 12 weeks, excluding its possibility in predicting GDM. Previous findings in relation to thyroid impairment and risk of GDM, reported that low TSH level decreases insulin sensitivity(12). The decreased glucose and TSH levels were not correlated in our study, and TSH level did not predict onset of GDM. It should be noted that levels of all these biomarkers remained within normal range(5), which might have affected power to show any association between parameters. Placental human chorionic gonadotropin (hCG) hormone influences serum TSH level, given their molecular and receptor homologies(37,38). Serum hCG level peaks around week 10 of pregnancy, and which explains the observed decrease in serum TSH level during the first trimester(18).

An adverse inflammatory profile has been shown to manifest during the first trimester of pregnant women who subsequently develop GDM later in their pregnancies(39). Given the association of adiponectin with anti-inflammatory effects, lower serum levels of adiponectin have been shown in pregnant women who also suffer from obesity and GDM(1,14). Furthermore, dysbiosis of the gut microflora associates with metabolic dysfunction (including insulin resistance) during pregnancy, and likely contributes towards the pathogenesis of GDM(40). In our study, there were no associations between reductions in fasting plasma glucose during the first trimester of IVF-conceived pregnancy and serum adiponectin and LBP levels. Furthermore, serum levels of adiponectin and LBP during the first trimester of IVF-conceived pregnancies were not predictive of subsequent onset of GDM. One explanation for these observations is that changes in inflammatory status (reflected by serum adiponectin) and gut microflora-related endotoxemia (reflected by serum LBP) only occur later in pregnancy, in the second and third trimesters; our study only focused on the first trimester. This hypothesis would be consistent with data from other studies(41,42). Collection of stool samples and direct culture of the faecal microflora would have enabled a more complete assessment of the gut microflora, and this should be a focus for future research.

Limitations of the study are acknowledged. Even though there are similarities between our IVF-conceived participants and those who conceive naturally (from the literature), there could be differences in the two groups, which makes the extrapolation of our findings to the general population questionable (such as no history of PCOS, other ethnicities and lifestyle factor). In addition, women undergoing IVF tend to be older than those who conceive naturally and they often have a poorer obstetric history, which predisposed them to increased obstetric risk and adverse outcomes(43). A well-matched spontaneously-conceived pregnant group would have hence allowed the determination of magnitude of change in all these parameters as an effect of pregnancy alone. In addition, using HOMA-IR to assess insulin sensitivity is not as precise as the “gold standard” euglycaemic clamp protocol, but it is still considered a practical and non-invasive surrogate measure for pregnant women(44). Another important biomarker of insulin resistance is sex hormone binding globulin (SHBG), which correlates with BMI and sex hormones (such as estrogen level). Low SHBG level serves as an early predictor of GDM(45). Further studies should consider measuring changes in SHBG levels in IVF-conceived pregnancies; this will help improving GDM risk assessment. Ethnicity and history of GDM were reported by participants, which may have been misreported. Adiponectin and LBP were tested in 42 participants overall,

making it difficult to assess change in these parameters and finding significant difference between the two groups. Finally, testing mid-pregnancy metabolic and endocrine parameters (including FSH/LH ratio) would have provided additional insight on GDM predictors.

To conclude, IVF hormonal therapy did not predispose to higher prevalence of GDM. Similarly to spontaneously-conceived pregnancies, preconception maternal BMI and age predicted best subsequent onset of GDM in IVF-conceived pregnancies. We also show, for the first time, that preconception FSH/LH ratio predicts subsequent onset of GDM; this finding should be further studied and including in non-PCOS populations. During the first trimester of IVF-conceived pregnancies, maternal weight gain was the best predictor of subsequent GDM onset. Achieving a healthy preconception weight and gestational weight gain are important preventive measures against GDM. We recommend using our data when updating clinical guidelines in regards to optimal management of IVF-conceived pregnancies, and require validation from other independent studies. Any woman undergoing IVF therapy should hence have clinical preconception phenotyping and careful clinical monitoring during pregnancy. This approach would enable focused allocation of resources, especially to those women most at risk of GDM development.

Declaration of interest

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

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Data availability

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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***Table 1*. Comparison of Anthropometrics and Metabolic Parameters at Baseline and 12 Weeks of gestation for Pregnant Women with and without Gestational Diabetes Mellitus (GDM)**

Variables	Baseline		*p* value	12 Weeks		*p* value
	GDM (n=34)	Non-GDM (n=124)		GDM (n=34)	Non-GDM (124)	
Age (years)	34.0 (3.00)	32.0 (5.00)	0.03			
Weight (kg)	72.5 (20.50)	64.5 (11.77)	0.01	75.9 (15.0)	66.0 (11.90)	**0.01**
Body mass index (kg/m^2)	29.0 (7.20)	25.8 (6.30)	0.001	29.2 (7.28)	25.0 (4.90)	**0.001**
Female Hormones						
FSH (IU/L)	7.00 (1.58)	6.53 (2.54)	0.23			
LH (IU/L)	5.60 (3.07)	6.25 (3.67)	0.12			
Ratio FSH/LH	1.20 (1.40)	1.00 (0.50)	0.02			
Ratio LH/FSH	0.75 (0.54)	0.91 (1.04)	0.06			
Oestrogen (pg/mL)	49.21 (25.10)	41.10 (35.69)	0.36	*848.1 (800.4)	410.1 (884.8)	0.06
Progesterone (ng/mL)	0.21 (0.21)	0.23 (0.25)	0.63	*40.15 (38.25)	42.13 (37.78)	0.49
Metabolic and Endocrine						
Fasting glucose (mg/dL)	88.38 (14.00)	85.30 (8.00)	0.14	80.00 (8.4)	81.0 (8.44)	0.16
Fasting insulin (µIU/mL)	10.60 (10.53)	7.14 (6.86)	0.01	11.33 (14.45)	7.57 (8.01)	0.02
HbA1c (%)	5.50 (0.79)	5.20 (0.60)	0.06	5.27 (0.86)	4.90 (0.47)	0.25
HOMA-IR	2.20 (2.00)	1.70 (1.80)	0.01	2.30 (2.97)	1.50 (1.70)	0.01
T-Chol (mg/dL)	199.0 (28.80)	171.0 (39.0)	0.002	211.4 (71.38)	198.4 (47.0)	0.14
TG (mg/dL)	74.0 (67.0)	76.0 (38.10)	0.01	177.9 (84.23)	120.0 (46.10)	0.003
LDL-C (mg/dL)	123.0 (35.0)	104.8 (32.50)	0.003	104.5 (50.75)	108.0 (29.20)	0.20
HDL-C (mg/dL)	55.0 (16.20)	57.0 (15.50)	0.33	65.0 (12.95)	63.0 (22.0)	0.93
TSH (µIU/mL)	1.33 (0.93)	1.58 (1.30)	0.86	1.08 (0.73)	1.39 (1.39)	0.36
ŦAdiponectin (µg/mL)	9.21 (1.08)	8.68 (2.26)	0.47	8.62 (2.88)	8.70 (2.37)	0.83
ŦLBP (µg/mL)	58.51 (136.8)	63.76 (77.02)	0.97	73.32 (73.22)	43.55 (58.42)	0.94

Data presented in median and interquartile range (IQR); Ŧn= 42 pregnant; *Levels at 4 weeks; $p<0.05$ vs. GDM by independent test; FSH: follicle-stimulating hormone; LH: luteinizing hormone; HbA1c: glycated haemoglobin A1c; HOMA-IR: homeostatic model assessment of insulin resistance; T-Chol: total cholesterol; TG: triglycerides; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TSH: thyroid-stimulating hormone; LBP: lipopolysaccharide binding protein

***Table 2*: Change in Glucose Level (Baseline to 12 Weeks) Compared to Changes in Other Parameters in GDM and non-GDM Women using Linear Regression Analysis**

Variables	GDM (n=34)			Non-GDM (n=124)		
	B	95% CI	*p* value	B	95% CI	*p* value
BMI	2.37	-0.59, 5.33	0.11	0.03	-0.67, 0.73	0.93
HbA1c	6.28	0.85, 11.70	0.03	-0.36	-2.46, 1.76	0.74
Insulin	-4.50	-5.84, -3.15	<0.001	-5.34	-6.05, -4.63	<0.001
HOMA-IR	22.52	16.96, 28.07	<0.001	25.91	22.80, 29.01	<0.001
T-Chol	-0.03	-0.26, 0.21	0.83	0.003	-0.03, 0.03	0.82
TG	0.02	-0.36, 0.09	0.42	-0.004	-0.02, 0.01	0.57
LDL-C	-0.02	-0.27, 0.24	0.90	-0.01	-0.05, 0.02	0.52
HDL-C	0.13	-0.11, 0.36	0.27	0.02	-0.04, 0.08	0.51
TSH	1.76	-9.36, 12.89	0.72	3.54	-0.40, 7.47	0.08
ŦAdiponectin	0.003	-0.02, 0.03	0.76	<0.001	-0.01, 0.01	0.97
ŦLBP	-0.004	-0.06, 0.05	0.88	0.02	-0.01, 0.04	0.24

Ŧn=73(42 pregnant, 31 non-pregnant); HbA1c: glycated haemoglobin A1c; HOMA-IR: homeostatic model assessment of insulin resistance; T-Chol: total cholesterol; TG: triglycerides; LDL-C: low density lipoprotein-cholesterol; HDL-C: high-density lipoprotein-cholesterol; TSH: thyroid-stimulating hormone; LBP: lipopolysaccharide binding protein

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***Table 3*: Association of Women Characteristics and Pregnancy Outcomes with Development of Gestational Diabetes Mellitus (GDM)**

Variables		**GDM** (n=34) % (n)	**Non-GDM** (n=124) % (n)	***p* value**
BMI_Baseline (kg/m^2)	< 18.5	0 (0)	4.0 (5)	0.001*
	18.5-24.9	32.4 (11)	50.8 (63)	
	25-30	23.5 (8)	29.0 (36)	
	30-35	38.2 (13)	13.7 (17)	
	>35	5.9 (2)	2.4 (3)	
BMI_12 weeks (kg/m^2)	< 18.5	0 (0)	1.6 (2)	0.001*
	18.5-24.9	23.6 (8)	47.6 (59)	
	25-30	29.4 (10)	33.9 (42)	
	30-35	29.4 (10)	14.5 (18)	
	>35	17.6 (6)	2.4 (3)	
Ethnicity	Middle East	14.7 (5)	15.3 (19)	~~0.12*~~
	Gulf	70.5 (24)	49.2 (61)	
	Europe	0 (0)	9.7 (12)	
	~~North America~~	~~0 (0)~~	~~0 (0)~~	
	South Asia	14.5 (5)	16.9 (21)	
	East Asia	0 (0)	4.0 (5)	
	Africa	0 (0)	4.8 (6)	
History of PCOS	With	38.2 (13)	47.6 (59)	0.33**
	Without	61.8 (21)	52.4 (65)	
History of GDM	With	14.7 (5)	13.7 (17)	0.88**
	Without	85.3 (29)	86.3 (107)	
Number of offspring	Single	67.6 (23)	65.3 (81)	0.80**
	Twin	32.4 (11)	34.7 (43)	
Sex of offspring	Male	41.2 (14)	45.2 (56)	0.82**
	Female	38.2 (13)	38.7 (48)	
	Mix	20.6 (7)	16.1 (20)	

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p<0.05 vs. GDM, by *Fisher's exact test, **Chi-squared test; PCOS: polycystic ovary syndrome

***Table 4*: Anthropometrics, Metabolic and Endocrine Predictors of Gestational Diabetes Mellitus (as dependent variable) in Pregnant Women (n=158), adjusted for Age and PCOS**

Variables	Unajusted Analysis			Adjusted Analysis		
	OR	**95% CI**	***p value***	***OR***	**95% CI**	***p value***
Age	1.12	1.01-1.23	0.03	1.14	0.99-1.26	0.04
PCOS History	0.68	0.31-1.48	0.33			
Ratio FSH/LH_Baseline	2.05	1.12-3.75	0.02	1.61	0.94-2.78	0.08
Ratio LH/FS_Baseline	0.51	0.21-1.21	0.13			
BMI_Baseline	1.01	0.73-1.39	0.001			
BMI_F	1.16	1.07-1.26	<0.001	1.11	0.98-1.20	0.03
HbA1c_F	1.77	0.64-4.48	0.27			
Glucose_F	1.05	0.99-1.11	0.11			
Insulin_F	1.11	1.03-1.18	0.004	1.13	0.78-1.70	0.53
HOMA-IR_F	1.59	1.16-2.17	0.004	0.85	0.14-4.88	0.85
TG_F	1.01	0.99-1.01	0.75			
T-Chol_F	1.01	0.99-1.02	0.13			
ŦAdiponectin_F	1.00	0.99-1.00	0.71			
ŦLBP_F	0.99	0.99-1.01	0.52			

Ŧn=75; PCOS: polycystic ovary syndrome; F: final (12 weeks); FSH: follicle-stimulating hormone; LH: luteinizing hormone; HbA1c: glycated haemoglobin A1c; HOMA-IR: homeostatic model assessment of insulin resistance; TG: triglycerides; T-Chol: total cholesterol; LBP: lipopolysaccharide binding protein; OR: odds ratio; C.I.: confidence interval

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