Effect of in vitro fertilisation (IVF) hormonal therapy on metabolic, endocrine and inflammatory status in IVF-conceived pregnancy

by

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Statement of Contribution of Work

All the work outlined in this thesis is mine, except for the following:

- Adiponectin and Lipopolysaccharide binding protein (LBP) assays were performed by Mrs. Hema Unnikannan (University of Sharjah).
- All biochemical assays were performed at the in-house lab at Fakih IVF clinic.

Declaration

This thesis is submitted to the University of Warwick in support of my application for the degree of Doctor of Philosophy in Medicine. It has been composed by myself and has not been submitted in any previous application for any degree.

Abstract

Rationale: Infertility is the inability to conceive following 12 months of unprotected intercourse and affects 20% of couples worldwide. *In vitro* fertilisation (IVF) therapy has become more common in recent times. In mice, IVF associates with glucose intolerance. In humans, there is controversy regarding possible deleterious effects of IVF hormones in pregnancy, including worsening of metabolic, diabetogenic and inflammatory status.

Objectives: To explore effects of IVF therapy in pregnancy on glucose and lipid homeostasis, and other metabolic and inflammatory parameters. To explore early predictors of onset of gestational diabetes mellitus (GDM), including whether use of IVF therapy may hasten the onset of GDM.

Methodology: Adult non-diabetic women (n=275), BMI: 18.5-38kg/m^2, age ≤39years were recruited (n=158 pregnant, 117 non-pregnant). Collection of blood samples occurred throughout IVF cycle: baseline, 2, 4 and 12 weeks. Outcome variables included reproductive hormones, glucose, lipid profiles, insulin sensitivity, thyroid, gut microflora and inflammatory status.

Results: At 12 weeks, non-pregnant women experienced increased levels of glucose (86.04 to 87.62mg/dL), insulin (8.72 to 9.37μIU/mL), HOMA-IR (2.1 to 1.9), $p<0.01$; and lipid profile: T-Chol (169.5 to 174.9mg/dL), TG (71.0 to 83.7mg/dL), HDL-C (52.0 to 54.11mg/dL), with $p<0.001$. For pregnants, glucose (86.15 to 82.19mg/dL), HbA1c (5.3 to 5.08%) and TSH (1.71 to 1.36μIU/mL) were significantly lower and lipids were higher: TG (73.5 to 126.78mg/dL), T-Chol (177.5 to 199.5mg/dL), HDL-C (55.3 to 65.1mg/dL); all $p<0.001$. Prenatal BMI (OR=1.11; $p<0.05$) was the main predictor of GDM risk.

Conclusion: IVF therapy worsens lipid profile regardless of IVF outcome. Divergent effect of IVF therapy on glucose homeostasis depends on pregnancy status, with improved glycaemia in early IVF-conceived pregnancy, and worsened in the short term following unsuccessful IVF. The possible longer-term metabolic effects of IVF should be a focus for future research. Prenatal BMI appears to be the best predictor for future onset of GDM.

Glossary

AMH	Anti-müllerian hormone
ART	Assisted reproductive technology
ASRM	American Society for Reproductive Medicine
B	Difference calculated from baseline
BID	Twice a day
BMI	Body mass index
β-HCG	Beta-human chorionic gonadotrophin
CD2	Cycle day 2
CI	Confidence interval
CRH	Corticotrophin-releasing hormone
CRP	C-reactive protein
DHA	Dubai Health Authority
ET	Embryo transfer
F	Final
F3	Triiodothyronine
F4	Thyroxine
FPG	Fasting plasma glucose
FPI	Fasting plasma insulin
FSH	Follicle-stimulating hormone
GDM	Gestational diabetes mellitus
GnRH	Gonadotrophin-releasing hormone
GT	Genetic testing
HAAD	Health Authority Abu Dhabi
HbA1c	Glycated haemoglobin A1c
hCG	Human chorionic gonadotrophin
HDL-C	High-density lipoprotein cholesterol
HOMA-IR	Homeostatic model assessment of insulin resistance
HPL	Human placenta lactogen
ICSI	Intracytoplasmic sperm injection

IL-6	Interleukin-6
IQR	Interquartile range
IVF	*In vitro* fertilisation
LBP	Lipopolysaccharide binding protein
Lbs	Pounds
LDL-C	Low-density lipoprotein cholesterol
LH	Luteinizing hormone
LPS	Lipopolysaccharides
MOH	Ministry of Health
NICE	National Institute for Health and Clinical Excellence
OR	Odds ratio
OHSS	Ovarian hyperstimulation syndrome
OPU	Oocyte pick-up
PCOS	Polycystic ovary syndrome
QUICKI	Quantitative insulin sensitivity check index
SART	Society of Assisted Reproductive Technology
SD	Standardised difference
SCFA	Short chain fatty acids
sCD14	Soluble CD14
T-Chol	Total cholesterol
T2DM	Type 2 diabetes mellitus
TG	Triglycerides
TID	Three times a day
TNF-α	Tumour necrosis factor
TSH	Thyroid-stimulating hormone
UAE	United Arab Emirates
WHO	World Health Organization

Section 1: Introduction, Literature Review and Aims

Chapter 1: Introduction

Infertility is a multifaceted global health concern, and affects 20% of couples of reproductive age. In recent decades, there has been a tendency to delay childbearing age with contraceptives. Furthermore, the obesity epidemic and prolonged exposure to environmental and lifestyle-related stressors are major contributors to the increasing incidence of infertility, and the need for its effective treatment. The use of assisted reproductive technologies (ART), including primarily *in vitro* fertilisation (IVF), is becoming more prevalent. In the United Arab Emirates (UAE), with infertility affecting 50% of women of reproductive age, IVF is also very popular because of the accentuated importance of parturition, for gender selection and preventing genetically predisposed diseases of first degree relative marriages (e.g. thalassemia).

Oral contraceptive hormones commonly associate with gastrointestinal side effects from changes in gut microflora, in addition to possible adverse effects on glucose and lipid metabolism, which in turn may promote insulin resistance and inflammation. The concentration of reproductive hormones is much higher when used for IVF therapy than for oral contraception. However, confirmation of the safety of IVF (both maternal and foetal) remains tenuous. Previous studies have focused mainly on risk of obstetric complications in IVF-conceived pregnancies, with relatively few studies investigating possible effects of IVF hormones on maternal metabolic, endocrine and inflammatory status.

Pregnancy is characterised by profound hormonal-driven changes with consequences for metabolic, endocrine and inflammatory status and possible implications in gut microflora. Dyslipidemia, hyperglycaemia, insulin resistance and/or oxidative by-products result in adverse maternal and foetal outcomes in the short-term and may predispose to chronic conditions later in life. The “diabetogenic state” of pregnancy results from exaggerated adiposity accretion, impairment in glucose and insulin homeostasis, and lipolysis. Growing evidence supports the notion of intestinal microbiota as an endocrine-metabolic organ, given its significant contribution in different physiological regulations. Functional and composition distortion in intestinal microbiota contribute to immunity impairment, inflammation and metabolic disorders including insulin resistance and possibly

gestational diabetes mellitus (GDM).

Given the elevated concentrations of IVF hormones combined with gestational hormones, metabolic, endocrine and inflammatory changes may manifest earlier in IVF-conceived pregnancies. The relationship of IVF hormones to the known diabetogenic and atherogenic effects of pregnancy requires further investigation. Reliable predictive factors for possible metabolic, endocrine and inflammatory sequelae of IVF therapies are required. Such factors could help prevent or at least identify at an early stage women who are at “high risk” for maternal and/or foetal complications, such as GDM. The first objective of this study was to assess the impact of IVF-related hormones on maternal glucose and insulin homeostasis, metabolic profile and inflammatory status. The second objective was to explore early predictors for GDM and assess the applicability of existing well-documented ones.

Section 1: Introduction, Literature Review and Aims

Chapter 2: Literature Review

In this section, the main causes of infertility will be outlined followed by an overview of the common treatment modalities including known maternal and foetal risks and complications, with a focus on IVF therapy. Also, a review of the existing literature on maternal metabolic, endocrine and inflammatory effects of oral contraceptives and IVF therapies in pregnancy will be conducted. Finally, the well-documented and potential novel predictors of GDM will be elaborated.

2.1. Infertility

Infertility is a global health concern, and affects 20% (1 in 5) of couples of reproductive age. Infertility is defined as the "inability to conceive after 12 months of unprotected intercourse, and 6 months for women 35 years of age or older"[1,2]. Subfertility refers to couples who can still conceive but with more difficulty, an example of which is a 40-year-old woman who can get pregnant but her chances are lower compared with a younger woman[3]. In the UAE, women infertility affects 30 to 60% depending on the age group of women considered[4].

2.1.1. Female Infertility

Many factors are associated with infertility in women. Mechanical impairment of the reproductive system accounts for about 35% of female infertility, and includes damaged or blocked fallopian tubes, fibroids and endometriosis[1,5]. Age has a significant impact on female fertility, affecting both quality and quantity of eggs: reproductive age peaks in the 20s and early 30s and starts to decline after the age of 35 years. Consequently, the chances of pregnancy are about 25–30% in the early 30s and 10% or lower after 40 years of age[6–8]. Problems with ovulation and hormonal-related disturbances are common causes of infertility in women. Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age and is associated with obesity, hyperinsulinemia and insulin resistance[9–12]. Although frequently co-existent with PCOS, there is evidence to support the notion that overweight and obesity are independent contributors to female infertility, mediated through adverse effects on reproductive hormones,

manifesting with anovulation[13–15]. Furthermore, a lower serum levels of anti-müllerian hormone (AMH, measurement of ovarian reserve) were reported in obese women with PCOS[16]. Due to the adverse effects of obesity on female fertility outlined here, obese women undergoing IVF may require higher doses of stimulating hormones. Obesity reduces the success rate of IVF, with a diminished endogenous hormonal response and increased risk of adverse pregnancy outcome[17–19].

Extensive studies have asserted the importance of preconception weight-loss in obese women with or without PCOS to improve metabolic parameters, reproductive performance and pregnancy outcomes[20,21]. Multiple esteemed societies (including American College of Obstetricians and Gynaecologists, the Maternal Fetal Medicine Association and the European Society of Human Reproduction and Embryology[21–23]) uniformly recommend delaying fertility treatments, with a greater focus on weight-loss preconception in obese women. The British Fertility Society[24] recommends women to have a BMI <35 kg/m^2 before commencing any fertility treatment. The British National Institute for Health and Clinical Excellence (NICE)[25] recommends a preconception BMI of 19–30 kg/m^2.

In the UAE, the incidence of PCOS and infertility is a rising concern especially with increased obesity prevalence amongst women. In fact, the UAE is listed amongst the top 10 countries worldwide for obesity prevalence[26,27] and has one of the highest rates of PCOS, accounting for 60% of Gulf women and 30% of women of Indian origin[28]. Regarding recommendations for fertility treatments, the health authority of Abu Dhabi (HAAD) in UAE sets the BMI upper limit to 38 kg/m^2. The BMI cut-off is less strict in advanced maternal age, since delaying fertility treatment for such cases may result in inevitable infertility. Underweight women (BMI<18.5) may also experience irregular menstrual cycles and infertility, often secondary to insufficient body fat stores with adverse implications for normal sex axis functioning[29–32]. Thyroid dysfunction can also impair menstrual cyclicity and fertility[33,34].

Studies have compared different dietary compositions and their effect on fertility (e.g. vegetarian, low fat and low carbohydrate diets)[35–38]. Understandably,

there is much controversy in this literature, and the optimal "fertility diet" has yet to be identified. A healthy and balanced lifestyle improves egg quality[7]. Heavy caffeine consumption (500 mg/day or more than 5 cups/day) and high mercury levels from seafood impair fertility[39]. Smoking hastens depletion of follicle reserves[8]. Validation of the effects of these dietary and environmental factors on female fertility requires further research focus.

2.1.2. Male Infertility Factors

Male factors represent 30% of infertility cases[40,41]. Assessment of male fertility involves evaluation of semen (including volume and concentration), sperm (including number, shape [morphology] and motility) and the reproductive tract. Oligospermia (low sperm count), teratospermia (abnormal sperm morphology) and asthenozoospermia (impaired sperm motility) are the most commonly reported causes of male infertility. Azoospermia (complete absence of sperm production) is a less common cause of male infertility[42,43]. In men, fertility is adversely affected by advanced age, smoking and obesity, similar to women[41,44,45], although age-related effects are less pronounced in men, with fertility only declining after the age of 50 years[8]. High alcohol and caffeine intake and the use of recreational drugs may also contribute to male infertility[46,47]. Additionally, poor control of medical conditions (such as diabetes and cystic fibrosis) can adversely affect sperm quality and ejaculation[48].

2.1.3. Other Infertility Factors

A combination of male and female factors account for 20% of infertility cases, and the remaining 15% of infertility cases are idiopathic or unexplained [40,41]. Having outlined the underlying causes of infertility, there follows an outline of treatment options including common associated risks and complications.

2.2. Assisted Reproductive Technology

Assisted reproductive technology (ART) describes various procedures that help couples to conceive. ART options include natural cycle, time intercourse, intrauterine insemination (IUI), gamete intra-fallopian transfer (GIFT), zygote intrafallopian transfer (ZIFT), egg donor, and *in vitro* fertilisation (IVF)[41,49] (Table 1).

Table 1: Summary of Fertility Therapy at IVF Clinic

Techniques	Ovarian Stimulating Agents	Egg retrieval	Uterine Sperm Insertion	Embryo Transfer	Recommended
Ovulation monitoring (natural cycle)	-	-	-	-	First treatment of choice
Time intercourse	✓	-	-	-	Second choice of treatment
IUI	*S*mall dose	-	✓		Male factor
Regular IVF	✓	✓	-	✓	-Gender selection -Genetic abnormalities -Failure of other fertility treatments -Severe male factor -Immunological factors[1]
Mini IVF	Small dose	✓	-	✓	-At risk of OHSS -Minor infertility factors
Natural IVF	-	✓	-	✓	- Poor responders to other fertility treatment -Poor ovarian reserve -At risk of OHSS[41]

IUI: Intrauterine insemination; IVF*: in vitro* fertilisation; OHSS: ovarian hyper-stimulation syndrome

2.2.1. *In Vitro* Fertilisation (IVF)

In vitro fertilisation (IVF) is the most commonly performed ART-based technique, given its high success rate. IVF is the treatment of choice for unexplained infertility[20,49]. In addition to the listed factors in Table.1, IVF is very popular in the Arab World because of the accentuated importance of parturition regarding gender selection, and prevention of genetically predisposed diseases of first relative marriages (e.g. thalassemia). The success rate of IVF depends on multiple factors. Women's age is one of the main predictors of IVF outcome. Success of IVF declines with age, especially after the age of 35 years[1]. Obesity also negatively affects IVF outcome, with severity of obesity strongly associates with poorer outcome. According to Khairy (2017)[20], obesity induces molecular changes in oocytes, which impairs embryo quality and endometrial receptivity. Furthermore, number of transferred embryos may predict IVF success: with more embryos transferred there is a higher chance of pregnancy. The number of transferred embryos depends on the women's age[41].

2.2.2. Obstetric Risks and Complications of IVF Therapy

2.2.2.1. Risks of IVF Therapy on Maternal Outcomes

Despite a steady increase in the medical treatment of infertility with ART, there is still a lack of published evidence on its safety. Compared with other ART treatments, IVF associates with increased maternal risks and complications, since there is alteration of the normal physiological development of pregnancy during IVF. Furthermore, use of stimulating agents can also adversely affect pregnancy outcome, including association with ovarian cysts, ovarian enlargement and Ovarian Hyper Stimulation Syndrome (OHSS)[41]. In extreme cases (3–5%), a serious complication of ovarian stimulation, OHSS may ensue. OHSS is an exaggerated ovarian response, with a significant increase in oestrogen level and enlarged ovaries, resulting in shift in fluid to extravascular spaces (mainly abdominal), and in severe cases around the lungs[50].

Ectopic pregnancy is considered as a complication of pregnancy and is twice more common in IVF compared to a spontaneously-conceived pregnancy. It

is related to increased volume of fluid injected with the embryo, or the location of the embryo transfer being close to the fallopian tubes. Pregnancy loss after 12 weeks is also more common in IVF-conceived pregnancies[51,52]. Furthermore, multiple pregnancies account for 25% of IVF-conceived pregnancies, given that more than one embryo can be transferred to increase chances of pregnancy[49]. However, although twin and triplet pregnancies have a higher complication rate overall compared with singleton ones (including preeclampsia, GDM, thromboembolism and pre-term delivery)[41,53,54], complication rates overall are similar between IVF-conceived and spontaneously-conceived pregnancies[55,56].

Future studies should focus on obstetric outcomes with IVF-conceived pregnancies in larger cohorts, well matched with spontaneous pregnancies, including those that result in multiple pregnancies. One reason for the frequent categorization of women undergoing IVF therapy as 'high risk' may relate to higher rates of maternal adverse outcomes with IVF-conceived pregnancies, as they usually present with advanced age, high BMI (>30 kg/m^2) or a pre-existing medical condition such as PCOS or impaired thyroid function[57,58]. A higher prevalence of spontaneous abortion occurs in IVF-conceived pregnancies in women who are obese and/or have a history of PCOS[10].

IVF-conceived pregnancy is a 'high-risk' intervention with increased risk for maternal and obstetric complications. These include miscarriage, vaginal bleeding, frequent hospitalisation, gestational hypertension, GDM and preterm labour[6,52,59–62]. However, there is some controversy in the literature regarding the actual risk of adverse obstetric and maternal outcomes in IVF-conceived pregnancies: in a large retrospective study by Kozinszky et al.[55], data did not show increased rates of obstetric complications with IVF pregnancies. Caesarean section is also more common with IVF pregnancy[63]. Women may consider IVF-conceived pregnancy as "precious" after many years of infertility, and choose caesarean section to prevent perceived complications from a natural vaginal delivery, and not necessarily because of medical necessity[64].

IVF hormonal therapy may also associate with increased risk of breast and ovarian cancer post-IVF, although, this association remains poorly described and

more studies are needed[65–67].

2.2.2.2. Risks of IVF Therapy on Foetal Outcomes

Risk of congenital malformation was shown to associate with IVF, especially with multiple pregnancies[62,66,68,69]. However, when controlling for age and parity, this association is not always significant[70]. Mixed reports exist regarding foetal outcomes of IVF-conceived pregnancies. Whilst some studies suggest that IVF may predispose to intrauterine growth retardation, foetal anomalies, birth defect, and perinatal mortality[56,71,72], others show no difference in foetal outcomes between spontaneous and IVF-conceived pregnancies[59,69,73]. In one study, it was shown that IVF-conceived children are predisposed to obesity, insulin resistance, type 2 diabetes mellitus (T2DM) and cardiovascular disease in adulthood[74]. Further prospective studies are required to clarify the nature and extent of adverse effects of IVF on offspring, regarding both foetal development and longer-term effects that may manifest in adulthood.

2.3. IVF Therapy, Metabo-Endocrine, Inflammatory and Gut Profiles

2.3.1. Endocrine Function in IVF-conceived Pregnancy

Key hormones control the female reproductive system: luteinizing hormone (LH), follicle-stimulating hormones (FSH), oestrogen, progesterone and beta-human chorionic gonadotrophin (β-HCG). A rise in serum LH triggers ovulation. The rise in LH also stimulates the ovaries to secrete progesterone. Whilst oestrogen and progesterone have multiple metabolic and endocrine effects throughout the body, there is confinement of the effects of FSH and LH to the ovary. Under normal physiological control, oestrogen and progesterone (also called ‘gestational’ or ‘maternal’ hormones) rise linearly during pregnancy, and play crucial roles in supporting pregnancy and normal foetal development[75–77]. Progesterone, produced primarily from the ovaries, acts on the uterus to prepare its lining for embryo implantation. During pregnancy, progesterone is also produced by the placenta[1]. Secretion of oestrogen also originates from the ovaries and placenta. Oestrogen and

progesterone dominate the first and second halves of gestation respectively. β-HCG hormone is one of the early indicators of pregnancy. Addressing the well-known physiological effects of reproductive hormones from use of oral contraceptives and pregnancy should enable prediction of the potential adverse maternal impact of IVF therapy.

2.3.2. Metabolic Profile in IVF-conceived Pregnancy

2.3.2.1. Glucose and Insulin Homeostasis in Pregnancy

During early pregnancy, fasting plasma glucose level is similar to that of non-pregnant women, and usually remains constant throughout pregnancy. Glucose tolerance is also commonly within normal range or slightly enhanced in early pregnancy[78]. Insulin, the principal modular of glucose homeostasis, often only rises significantly in the serum after the second trimester. Insulin sensitivity is unchanged or even increases in early gestation, to ensure sufficient glucose supply to the foetus[79–81]. During the second and third trimesters, insulin sensitivity frequently diminishes to 50–70% of first trimester levels (measured by hyperinsulinemic-euglycaemic glucose clamp technique). In addition, insulin requirement increases after 26th week of gestation, to possibly 50% more compared to pre-pregnancy levels[82–86]. Insulin resistance, reflected by increased homeostatic model assessment of insulin resistance (HOMA-IR), drives increases in serum insulin level. The presence of normoglycaemia in pregnancy despite prevailing insulin resistance may reflect a physiological adaptation of metabolism regarding lipid and carbohydrate regulation[87–89]. The state of insulin resistance favours glucose availability to the foetus, maternal fat accretion and use of lipids (free fatty acids) as source of energy by the mother[88]. Reduced insulin sensitivity in pregnancy can sometimes reach comparable levels to that in T2DM[88,90].

Gestational hormones play important roles in insulin homeostasis. Whilst oestrogen enhances insulin release and binding to its receptor, progesterone actually reduces insulin binding to its receptor and hence impairs glucose transport. Therefore, a possible explanation for the diabetogenic effect of pregnancy relates to the rise in serum levels of progesterone (secreted by the placenta.) during the

second and third trimesters, that manifests in reduced insulin sensitivity, hyperinsulinemia, and impairment of 'pre-implantation environmental state'[10]. Other placental hormones contribute to the diabetogenic effect of pregnancy, which include: human placental lactogen (HPL), human chorionic gonadotrophin (hCG), corticotrophin-releasing hormone (placental CRH), relaxin, kisspeptin and growth hormones. Concentrations of these hormones augment exponentially from the second trimester. Placental CRH triggers production of maternal adrenocorticotrophin hormone, and in turn provokes cortisol secretion typically from mid-gestation[91]. Placental hormones, referred as diabetogenic hormones, induce anti-insulin action and trigger lipolysis[92]. Consequently, there is a reduction in peripheral and hepatic insulin sensitivity, sparing of carbohydrates (glucose) for the foetus and decreased maternal use of glucose as energy. As a result, insulin-stimulated glucose uptake is reduced at the muscle and liver levels and hence promote glycogenolysis (breakdown of stored glycogen into glucose)[78,88,92].

The diabetogenic effect of pregnancy stems hence from impairment of insulin sensitivity, and increased beta-cell activity in response to a greater requirement for insulin[86,93–95]. Although insulin resistance plays an important role in the aetiology of numerous adverse outcomes during pregnancy (such as GDM, preeclampsia and miscarriage), the mechanisms implicated remain incompletely understood[81,96,97].

2.3.2.2. Glucose and Insulin Homeostasis and IVF Therapy

Interestingly in mice models, IVF associates with glucose intolerance[98]. In humans, studies on oral contraceptive therapies (oestrogen and progesterone combination) report comparable findings in relation to their impact on glucose metabolism and insulin homeostasis. Impairment of insulin sensitivity and glucose tolerance were commonly shown with the use of oral contraceptives, as evidenced by higher glucose and insulin levels[99–101]. Whilst some research findings suggest that insulin resistance is induced by progesterone, others suggest that this is likely oestrogen-related, and that progesterone only affects insulin half-life[99,102,103]. Oestrogen therapy at a dose of 0.625 mg/day may decrease fasting serum levels of

glucose and insulin, whereas 1.25 mg/day was associated with a 25% decrease in insulin sensitivity. Given that the dose of gestational hormones administered during IVF therapy is higher than that used for combined oral hormonal contraception, it is not possible to extrapolate glycaemic and metabolic effects of combined oral hormonal therapies to those used for IVF.

Compared to spontaneously-conceived pregnancies, women with IVF-pregnancies are more likely to develop GDM. This association remains following adjustment for maternal and gestational age, and parity[104,105]. Higher prevalence of GDM in IVF-conceived pregnancy likely results from the large dose of exogenous hormones (oestrogen and progesterone) administered during the treatment and which in turn alter the normal physiological development of pregnancy (levels of reproductive hormones are adjusted with medications for optimal control of IVF cycle). It is also possible that the increased risk for GDM in IVF-conceived pregnancies may stem from association with prenatal obesity or maternal PCOS (conditions that are not always specified)[106,107]. Alternatively, association of IVF with GDM may develop indirectly from the effects of IVF therapy on body fat accumulation, or directly from the procedure itself, through incompletely understood mechanisms. Multiple pregnancies, more common in IVF-pregnancy compared to spontaneously-conceived pregnancy, also constitute a powerful risk factor for GDM[108]. However, human data are severely limited in relation to the effects of IVF on insulin and glucose homeostasis during early pregnancy. Further studies are required to explore the potential for IVF-related hormonal therapy to hasten (physiological changes to occur in early pregnancy instead) or augment the diabetogenic effect of pregnancy. Such data will likely provide further insight into early predictors of GDM.

2.3.3. Cardiovascular and Inflammatory Risks in IVF-conceived Pregnancy

2.3.3.1. Inflammatory Markers with IVF Therapy

Pregnancy-related inflammatory response is induced by physiological and hormonal changes, and detectable as early as embryo implantation[109]. Gestational hormones play an important role in the synthesis of inflammatory parameters (such

as C-reactive protein, CRP)[110]. A list of inflammatory and gut-related parameters to assess pathophysiological changes of pregnancy is presented in Table 2.

C-reactive protein (CRP), one of the commonly measured inflammatory markers, appears to increase with the use of oral contraceptives, mainly in women <35 years[111,112]. Based on this observation, IVF-related therapy may also stimulate an inflammatory response. Serum levels of high-sensitivity CRP (hs-CRP) also correlate with age and BMI, factors that both associate with infertility and often occur in those seeking IVF therapy. Furthermore, obesity associates with inflammation and is itself often an independent cardiovascular risk factor in obese women who use oral contraceptive therapies[113,114]. There is controversy regarding the predictive utility of CRP for conception failure post-IVF[109].

Adiponectin, a useful marker of inflammation and insulin sensitivity, has not been comprehensively studied in women who undergo IVF[115]. Furthermore, serum adiponectin levels gradually decline during pregnancy, secondary to hormonal fluctuation[113,116].

Table 2: Inflammatory and Gut Microflora Parameters

Biomarkers	Description	Specification/Use
Interleukin (IL-6), tumour necrosis factor (TNF-α), TNFsR1	Pro-inflammatory markers as a result of increased levels of endotoxins or stress[117,118]. Levels increased in second trimester of pregnancy and may predict insulin resistance[119].	- IL-6 is over-expressed in obesity and inflammation[119]. - More studies are needed to assess TNF-α potential role as GDM predictor independently of BMI[119]. - Indirect effect on insulin resistance and gut-related changes.
Adiponectin	Type of adipokines, adipocyte-derived hormone with anti-inflammatory, anti-glycaemic and insulin sensitizing properties[120]. Low levels in early pregnancy predicts increased risk of GDM[113].	- Involved directly in glucose and lipids metabolisms with receptors in the liver, muscle and adipose tissue (AdipoR1 and AdipoR2)[121]. - Low level in PCOS[113], and should be more elaborated.
Leptin	Type of adipokines, adipocyte or placental-	Involved indirectly in glucose metabolism, and

	derived hormone with pro-inflammatory properties[120]. Contribute to regulation of gonadotrophin releasing hormones secretion[116]. Hyperleptinemia in early pregnancy predicts a higher risk of GDM[122].	acts instead on the brain (hypothalamic receptors)[123].
Visfatin	Type of adipokines, adipocyte-derived hormone, which triggers secretion inflammatory cytokines and hence contributes to insulin resistance[120].	Insulin-like action, but more prospective studies should be conducted in regard to its role in glucose homeostasis[116].
Pentraxin, such as pentraxin 3 and CRP	In early pregnancy, low level is associated with subsequent development of GDM[124].	Pentraxin 3 has anti-microbial and anti-inflammatory properties but is produced at site of inflammation[124].
Lipopolysaccharide binding protein (LBP)	Soluble acute-phase protein, produced by hepatocytes and intestinal epithelial cells. Binds to LPS and provokes immune response by triggering cluster of differentiation 14 (CD14) inflammatory signal cascade[125,126].	- Surrogate marker of endotoxemia[127,128] and a better marker of microbial translocation (abnormal passage of bacteria of the intestinal lumen through the epithelial mucosa barrier and possibly reaching external tissues) compared to LPS[125]. - Stable in blood and half-life is about 24 hours[127].
Lipolysaccharides (LPS)	Bacterial from gram-negative cell walls in gut lumen.	- High variability throughout the day[125]. - Half-life is about 2 hours, and hence evaluation of endotoxins by LPS may not be reflective enough[126]. - Analysis technique is more complex than LBP and more costly. - Assay is more sensitive to effect of detergents, urea, and pH[125].
rRNA	Placental or faecal test for microbiome test with 16s RNA ribosomal sequencing[129,130].	Cumbersome at a clinic setting and costly.

Short chain fatty acids (SCFA)	Intestinal bacteria fermentation of non-digestible carbohydrates, protein and bile acids into SCFA (butyrate, acetate and propionate)[131]. Beneficial for host and used as source of energy for epithelial cells and maintaining gut homeostasis[118]. Abnormal level of SCFA triggers inflammatory response[132].	- Measured in blood (serum or plasma) or stool. - Hepatic and/or other tissues absorption and rapid uptake, measurement of SCFA in stool and blood engenders little information[131]. - Low concentration in blood[133].
Branched chain amino acids	Microbial synthesis: isoleucine, valine, leucine, tyrosine and phenylalanine. Contribute to glucose homeostasis[131]; and levels associate with increased diabetes risk[115].	- Tracer needed for analysis. - Invasive, time consuming and not practical for pregnant women[131].
Soluble CD14 (sCD14)	Secreted by the liver and intestinal monocytes in response to LPS and other bacterial substances[125]	Useful biomarker for clinical endotoxemia[118].

2.3.3.2. Lipid Metabolism in Pregnancy

In addition to an inflammatory response, pregnancy also induces changes in lipid and lipoprotein metabolism, usually evident following the first trimester. Early pregnancy dyslipidemia (hypercholesterolemia and principally hypertriglyceridemia) associates with increased risk of adverse outcomes both for the mother (including preterm delivery, preeclampsia and GDM) and for the foetus (including macrosomia and large-for-gestational-age)[134–136]. Consequently, screening for lipid disorders prior to pregnancy and its appropriate management is important.

Lipid-lowering agents (e.g. statins) are possible teratogens and hence not recommended during pregnancy. However, available evidence to support such recommendations is limited[137]. Whilst some studies report an increase in all lipid

parameters during pregnancy[135,138], others show only significant increases in triglycerides (TG) and very-low-density lipoprotein (VLDL), and a decrease in low-density lipoprotein cholesterol (LDL-C)[134,135,138,139]. Hypertriglyceridemia results from pregnancy-related increased body fat and lipolytic activity, required to support pregnancy and in preparation for breastfeeding[134,139]. Impairment in insulin sensitivity during pregnancy may also impair lipid metabolism, through reduced ability of insulin to suppress lipolysis[116]. Insulin resistance and hyperinsulinemia also occur commonly in obesity and PCOS, and associate with preconception hyperlipidemia[140]. Given the high prevalence of obesity and/or PCOS in women undergoing IVF, screening and management of dyslipidemia preconception is important to reduce the likelihood of development of pregnancy-related complications.

2.3.3.3. Lipid Metabolism and IVF Therapy

Studies on the effects of oral contraceptive therapies on lipid profile report conflicting data in relation to changes in LDL-C and high-density lipoprotein cholesterol (HDL-C) levels, but strong evidence regarding increases in TG level[101,141–143]. Elevated serum levels of oestrogen triggers hepatic synthesis of lipids, with increased serum levels of TG, total cholesterol (T-Chol) and HDL-C[138,144,145]. In line with this, hypertriglyceridemia is thought to be oestrogen-dose-related[86,142]. Hypertriglyceridemia subsequently impairs insulin sensitivity[101]. According to Godsland et al.[143], oral contraceptives predispose to a higher risk for coronary heart disease, mediated through increased TG, LDL-C and insulin levels, and decreased HDL-C. The literature is deficient regarding data on the effects of IVF hormonal therapies on lipid profile, and potential effects of the latter on augmentation of the atherogenic nature of pregnancy.

2.3.4. Gut Microflora in IVF-conceived Pregnancy

2.3.4.1. Gut Microflora in Pregnancy

The gut microbiota can trigger an inflammatory response through mediation of ‘leaky gut’[131,146]. A useful serum marker for this process is lipopolysaccharide-

binding protein (LBP), an acute-phase protein. LBP binds bacterial compounds, including lipopolysaccharides (LPS). LPS is an outer membrane component of gram-negative bacteria that normally reside within the gut and form the microbiota. Under normal condition, LPS (also called endotoxins) remains in the gut, but when it crosses over a leaky gut wall into the circulation it becomes problematic. Gut wall permeability (with leakage of LPS into the bloodstream) is likely influenced by stress, a high fat/energy-dense diet, or the use of certain hormonal therapies. In the presence of endotoxins in the circulation, LBP is rapidly synthesized by the liver, and released into the circulation to bind LPS. The binding of LBP with LPS provokes an immune response and triggers cluster of differentiation 14 (CD14) inflammatory signal cascade[125,126]. This results in a condition called 'metabolic endotoxemia'[117,147–149]. LBP is thought to be a surrogate marker of endotoxemia, given that it is considered as a more stable biomarker than LPS[126]. More research is needed to elucidate whether LBP can act as a strong surrogate marker of LPS and its related impact on inflammation and gut flora. LBP have also been associated with metabolic diseases, such as obesity, diabetes, and non-alcoholic fatty liver disease[127]; further research is required to assess its possibility in predicting metabolic impairments of pregnancy, including insulin resistance and early GDM[150].

Maternal gut microbiota undergoes microbial adaptation with restriction of species count and floral diversity from early to late pregnancy, which may predispose to gestational micro-inflammation and metabolic impairments[151] (Refer to table 2 for a list of markers). Pregnancy-related changes in the gut microbiota may mediate metabolic dysfunction including reduced insulin sensitivity[152]. Accordingly, reduced maternal microfloral diversity was reported in the first trimester in those who later developed GDM[132]. In fact, maternal gut floral dysbiosis (imbalance of gut microbiota) associates with various pregnancy-related complications, such as insulin resistance, preeclampsia, miscarriage, intrauterine growth retardation and preterm delivery. Furthermore, maternal dysbiosis of the gut likely contributes to the pathogenesis of GDM[151,153,154].

2.3.4.2. Gut Microflora and IVF Therapy

Female sex hormones influence profoundly microbiota composition within the mouth, vagina and gut. There is a likely role for oral contraceptives in the development of inflammatory bowel disease (Crohn's disease and ulcerative colitis)[155]. Furthermore, gastrointestinal side effects commonly occur with oral contraceptive therapies. Oral oestrogen and progesterone treatment has been shown to affect gut permeability, LPS signalling and cytokine-mediated inflammatory diseases[156,157]. Dysbiosis is possibly related to insufficient or overloaded oestrogen and engenders an inflammatory response, resulting in metabolic and immunological disorders (e.g. T2DM)[149]. It is speculated that changes in gut microflora, serum LBP and LPS levels may also occur with IVF-related therapies, given the higher dose of reproductive hormones used compared with oral contraceptives and the stress of the procedure. Mediation of the inflammatory effects of IVF therapies may occur through changes in the gut microbiota and serum levels of LBP and LPS. Such inflammatory effects may extend throughout the IVF-conceived pregnancy. There are currently no reported studies on the effects of IVF treatments and IVF-related pregnancies on lipopolysaccharides markers (such as LBP and LPS) and gut microbiota. Assessment of gut permeability during pregnancy (through IVF- and spontaneous conception) would form a novel focus for future research.

2.3.5. Endocrine Function in IVF-conceived Pregnancy

2.3.5.1. Thyroid Function in Pregnancy

Thyroid dysfunction impairs menstrual cyclicity, female fertility and pregnancy outcome, and is classified as the second most common endocrine disorder in women of reproductive age[33,158]. Hypo- and hyperthyroidism are the two main types of thyroid disorder that adversely affect sex-hormone-binding and accordingly their own serum levels. Thyroid dysfunction affects both the duration and flow of menstrual cycle. Disorders in length of cycle include: oligomenorrhea (light or infrequent menstruation, ≥35 days), polymenorrhea (cycles with intervals of ≤21 days) and amenorrhea (absence of menstruation for more than three months),

and disorders of menstrual flow are: hypomenorrhea (decreased flow), hypermenorrhea (increased flow) and menorrhagia (heavy and prolonged menstrual period)[159]. In women of reproductive age, oligomenorrhea and amenorrhea are the most common abnormalities associated with hyperthyroidism, followed by hypomenorrhea, and anovulation[160,161]. Plasma oestrogen and LH levels were found to be higher in women with hyperthyroidism compared to their controls during all phases of the menstrual cycle[162]. Hypothyroidism is associated with polymenorrhea, menorrhagia and hypermenorrhea[161–163].

In early stages of pregnancy, the foetal thyroid gland is not yet fully developed and hence maternal thyroxine plays a vital role for the foetus. After 10 weeks, both maternal and foetal thyroid hormones are necessary to satisfy the increased needs of gestation and adequate foetal neurological development[158,164]. Oestrogen has a significant impact on thyroid-stimulating hormone (TSH) secretion and thyroid gland activity[165]. TSH is in charge of triggering thyroid hormones secretion (T3: triiodothyronine and T4: thyroxine). During pregnancy, "oestrogen dominance" interferes with thyroid metabolism by stimulating hepatic thyroxine-binding globulin secretion, thereby reducing levels of free thyroid hormones (T3 and T4)[166–168]. In addition, serum TSH level is suppressed throughout pregnancy, with typically a low-normal level in the first trimester[169,170]. There is also an increase in serum β-HCG (pregnancy indicator hormone) during pregnancy, the effect being particularly pronounced in twin pregnancies[171]. β-HCG has stimulatory effects on the TSH receptor and may drive over-production of thyroid hormones during pregnancy, and also contributes to suppression of TSH production[33,172].

Recurrent pregnancy loss, preterm birth and placenta abruption associate with high TSH level[40,96,172]. Consequently, it is not uncommon for pregnant women to be prescribed with thyroid medications on their first trimester, as soon as thyroid impairment is detected[58,164]. In addition, previous studies reported that pregnant women with hypothyroidism (TSH level elevated, T4 and T3 levels normal or low[164]) often require increased doses of thyroxine by about 30–45%[169,173,174]. Both hypo- and hyperthyroidism impair insulin sensitivity, and this may ultimately affect pregnancy outcome. Hyperinsulinemia may also block the conversion of T4 to its

active form T3[175,176].

2.3.5.2. Thyroid Function and IVF Therapy

Oral contraceptives and pregnancy alter thyroid function in similar ways, likely an oestrogenic effect. However, although the mechanisms are similar, more pronounced changes in thyroid hormones likely occur during gestation. The difference in magnitude of thyroid effects between pregnancy and oral contraceptive therapies likely relates to exogenous oestrogen therapy having a dose-dependent effect on increasing serum thyroxine-binding globulin and total serum thyroxine levels in those with normal thyroid function[93,145,173]. In contrast, few studies have determined changes in thyroid hormone level to be progesterone-related[177].

Regarding IVF therapies, in addition to the exogenous oestrogen, GnRH is also administered, the latter having been reported to affect levels of thyroid hormones (likely through indirect stimulation of gonadotrophin release and increased production of oestrogen)[178]. GnRH hormone (from the hypothalamus) stimulates FSH and LH synthesis that in turn influence serum levels of oestrogen.

Impaired thyroid function predicts poor IVF fertilisation outcome, hinting a role for thyroxine in oocyte physiology, and more importantly emphasising the importance of treating abnormal thyroid levels preconception[179]. The acceptance and validity of TSH range of 0.4–4.0 μIU/mL is still debatable with IVF therapy[180] and the American Thyroid Association recommends a cut-off of <2.5 μIU/mL[170,181,182]. Preconception TSH level exceeding 2.5 μIU/mL was correlated with a lower gestational age (38.5 vs 38.0 weeks for singletons and 36.0 vs 34.6 weeks for twin pregnancy) and lower birth weight (7.33 vs 6.78 lbs for singletons and 5.36 vs 4.83 lbs for twin pregnancy) according to Baker et al.[183]. In contrast, Kilic et al.[184] stated that neither the embryo grades nor the number of fertilised eggs differ among women undergoing IVF, but the pregnancy ratio was lower if presenting with impaired thyroid level and/or positive anti-thyroid antibodies. There is a lack of data in the current literature on thyroid status in IVF-conceived versus spontaneously conceived pregnancies. Given the potential cumulative

effects of oestrogen-related thyroid dysfunction during IVF-conceived pregnancy, this should be a focus for future research.

2.4. Early Predictors of Gestational Diabetes Mellitus

2.4.1. Anthropometrics and Medical History

Gestational diabetes mellitus (GDM) is a growing concern worldwide and a threat for maternal and foetal health during pregnancy, childbirth and possibly later in life for offspring[119,132]. Given the high dose of diabetogenic-related hormones of IVF therapy, it may be expected that GDM has a higher prevalence in IVF-conceived pregnancies. It is therefore important to identify early predictors and biomarkers for future onset of GDM in IVF-conceived pregnancies.

GDM is defined as "glucose intolerance resulting in hyperglycaemia, with onset or first recognition during pregnancy". GDM has a prevalence of 5–30% depending on population characteristics and diagnostic criteria[132,185]. In the UAE, one in every three pregnant women develops GDM[186–188]. Obesity (body mass index, BMI>30 kg/m^2), advanced maternal (>35 years) and gestational age, and previous GDM are important contributors to the rising incidence of GDM[180]. Other risk factors of GDM suggested by NICE and the American Diabetes Association (ADA) include previous baby weight >4.5 kg (or the equivalent to 9.9 lbs), a first-degree relative with diabetes mellitus, abnormal weight gain during pregnancy and certain ethnicities (Hispanic, African-Americans, Native American, South or South-East Asian, Pacific Islander or Indigenous Australian South, East Asia and Middle East)[55,88,189–192]. In addition, evidence-based studies report that women with PCOS have a significantly higher risk of developing GDM compared to non-PCOS controls, independently of obesity. The risk of GDM is particularly high when both PCOS and obesity coexist[106,193]. Current guidelines recommend selective screening during pregnancy depending on the presence of high-risk factors, but early general screening remains controversial[194]. The International Association of Diabetes and Pregnancy Study Groups recommends all pregnant women to be screened for GDM around 24–28 weeks of gestation with the 75-gram oral glucose tolerance test

(OGTT). The OGTT is also accepted by the American Diabetes Association for this purpose[195,196]. The 2-hour OGTT glucose cut offs for GDM suggested by the International Association of Diabetes and Pregnancy Study Groups are ≤92 mg/dL (5.1 mmol/L) for fasting, ≤180 mg/dL (10 mmol/L) for 1-hour and ≤153 mg/dL (8.5 mmol/L) for 2-hours[197]. The World Health Organization (WHO) and the American Diabetes Association guidelines 2-hour OGTT criteria for diagnosing GDM are a fasting glucose ≥126 mg/dL (≥7.0 mmol/L), 2h ≥ 140 mg/dL (≥7.8 mmol/L) and/or HbA1c ≥6.5% (≥48 mmol/mol). Pre-diabetes is diagnosed by a fasting glucose of 100–125 mg/dL (5.6–6.9 mmol/L) and/or HbA1c of 5.7–6.4% (39–47 mmol/mol)[195,198,199].

GDM has an adverse impact on both mother and foetus. Maternal complications include preeclampsia and caesarean delivery. Foetal outcomes include macrosomia, large-for-gestational-age, hyperinsulinemia, hypoglycaemia, and future risk of obesity and T2DM[119,195,198]. The literature provides evidence to support early detection and management of GDM to prevent maternal and foetal complications and improve pregnancy outcome. It is important therefore to identify those women at high risk of developing GDM early in pregnancy. Studies have explored possible biomarkers for GDM for use as early predictors. These include elevated level of glucose, insulin, HbA1c, adiponectin, CRP, TG, HDL-C, vitamin D and B 12, LBP and short chain fatty acid[196,200,201].

2.4.2. Glucose Homeostasis Markers

Numerous glucose-related markers were tested and being used as screening tools and early markers of potential GDM. Plasma glucose is the first biomarkers to monitor during gestation, and which level usually remains within a normal range throughout pregnancy. During early pregnancy, dysglycaemia (abnormal glucose level) and HbA1c (5.7–6.4% or the equivalent to 39–47 mmol/mol) associate with increased risk of future development of GDM later in pregnancy[202–204]. HbA1c has a high sensitivity but low specificity, and therefore unlikely to be a useful screening tool in a multi-ethnic population, as in the UAE population[205,206]. To maintain euglycaemia during later stages of pregnancy, insulin secretion is normally

increased to counteract the associated reduction in insulin sensitivity[207]. Consequently, elevated levels of serum insulin during early pregnancy (measured <16 weeks of gestation) may also predict increased risk of future GDM development[208,209]. A list of well-documented methods and formulas to measure glucose and insulin homeostasis, and evaluating glucose tolerance and insulin resistance, is presented in Table 3.

Table 3: Methods to Assess Glucose Homeostasis and Insulin resistance

Methods	Description	Specification/Use
Hyperinsulinemic euglycaemic clamp	Known as "gold standard" protocol[210,211]; consists of a continuous intravenous insulin infusion and variable infusion rates of glucose[212].	- Costly, time consuming, not practical and invasive. - Mainly used in research. - Clinical application to pregnancy is limited[213].
Frequently sampled intravenous glucose tolerance test	Computer-assisted model also referred a 'minimal model' which generates an insulin sensitivity index and a measure of the acute endogenous response of insulin to glucose[212,214].	- Particularly efficient in non-diabetic. - Costly, invasive, time consuming and not practical. - Mainly used in research. - Clinical application to pregnancy is limited[213].
Insulin sensitivity index	Calculated using fasting and 120 min post OGTT insulin and glucose levels[215].	- Sensitive but pregnant women may refuse doing an OGTT early in pregnancy if not clinically indicated.
Insulin suppression test	Intravenous infusion of glucose and insulin with somatostatin or epinephrine (to suppress endogenous secretion of insulin and glucagon)[212,214]	- Not practical, invasive and complex. - Mainly used in research. - Clinical application to pregnancy is limited[213].
Insulin tolerance test	Four bolus of insulin are provided and blood test is collected throughout, and plasma glucose decrease is measured[214].	- Risk of hypoglycaemia, invasive and not practical.
75g Oral glucose tolerance test (OGTT)	Plasma fasting, 1h and 2h glucose levels are measured following 75 g standard oral glucose solution[195,196].	- Conducted around 24-28 weeks for GDM or earlier if risk factors present. - Provide information about glucose tolerance but not of insulin resistance[214].

Insulinogenic index	Ratio of fasting insulin to fasting glucose	Practical, safe, not costly and easy.
Homeostatic model assessment of insulin resistance (HOMA-IR)	Quantify insulin resistance and beta-cell function. Described glucose-insulin homeostasis, and is calculated as follow: fasting glucose (mg/dL) x fasting insulin (μIU/mL) / 405[216,217]	- High sensitivity and specificity for assessing insulin resistance[218]. - Estimates of insulin resistance and deficient beta-cell function by HOMA-IR correlates with estimates from the euglycaemic hyperglycaemic clamp[216]. - Similar results to insulin sensitivity index[219]. - More reliable than QUICKI method[218]. - Practical, safe, not costly and easy to use. - Non-invasive and a clinically convenient to assess pancreatic beta-cell function and insulin resistance[217]. .
Quantitative insulin sensitivity check index (QUICKI)	Quantify insulin resistance and β-cell function; and calculated as follows: 1/ [log insulin + log glucose])[214,218] or 1/Log HOMA	Practical, safe, not costly and easy to use.
Matsuda index	From the OGTT, estimates of hepatic and muscle insulin sensitivity as follows: (10,000/square root of [fasting glucose x fasting insulin] x [mean glucose x mean insulin during OGTT])[214,220]	Time consuming and pregnant women may refuse doing an OGTT early in pregnancy if not clinically indicated.

2.4.3. Other Endocrine and Metabolic Markers

Thyroid hormones influence glucose metabolism through multiple mechanisms that include reduction of insulin half-life, promotion of hepatic glucose output and glycogenolysis[221]. In early pregnancy, serum level of thyroid hormones are lower in women who later develop GDM compared to non-GDM

women. Given that both hypothyroidism and subclinical hypothyroidism (mild form of hypothyroidism with only high TSH level but normal T4 and T3[158,170]) correlate with insulin resistance, early gestation hypothyroidism may be a potential risk factor for the development of GDM[221]. The association between subclinical hypothyroidism and increased risk of developing GDM remains controversial and warrants further investigation[222].

In relation to pregnancy-associated dyslipidemia and risk of GDM, abnormal TG level predisposes to increased risk of GDM compared to those with a normal TG level. Elevated levels of TG (>137 mg/dL or the equivalent to 1.55 mmol/L) was associated with higher GDM risk even after adjusting for pre-pregnancy adiposity[223]. Possible association of GDM with other lipid markers (T-Chol, LDL-C and HDL-C) is less clear[223–225]. A study by Abell et al. reported that HDL-C ≥85.5 mg/dL during the first trimester of pregnancy reduced GDM risk[119]. The logarithm of TG/HDL-C ratio is commonly used as an atherogenic marker, and elevated ratio level (Log TG/HDL-C >0.099) may identify pregnant women with higher risk of GDM before 24 weeks of gestation[226].

2.4.4. Adiponectin

Recent studies explore adipokines (such as adiponectin and leptin) as an early predictor of GDM. Adipokines are produced by adipose tissues and serve as a network that communicates different organs and physiological processes, such as glucose and lipid metabolisms, energy balance, insulin sensitivity, immunity and inflammation[116].

Adiponectin plays a key role in glucose homeostasis regulation through anti-glycaemic and insulin sensitizing effects. Adiponectin decreases hepatic glucose production and increases insulin action and peripheral glucose uptake. Low serum level of adiponectin associates with T2DM, insulin resistance, obesity, PCOS and preeclampsia. Serum adiponectin inversely associates with BMI, fasting glucose and insulin, and TG levels, and positively associates with HDL-C levels[116]. Maternal adiponectin secretion gradually declines during pregnancy, secondary to hormonal effects, mainly mediated by oestrogen and prolactin. These hormonal

changes are more pronounced with advancing gestational age[113]. Furthermore, adiponectin has anti-inflammatory effects[116]. Low levels of serum adiponectin in the later stages of pregnancy may contribute towards the development of pregnancy-related inflammation, with release of pro-inflammatory cytokines (TNFα ad IL-6). This in turn may further exacerbate insulin resistance[116].

A lower serum level of adiponectin occurred early in pregnancy in overweight and obese women who later developed GDM compared to their non-GDM controls. This association of serum adiponectin with GDM was still present after adjusting for differences in pre-pregnancy BMI and insulin sensitivity between the two groups[116,119,120]. Level of adiponectin ≤6.4 μg/mL was associated with higher risk of developing GDM later in gestation[113,227]. GDM is characterised by inflammation and insulin resistance, interconnected with adiposity. Inflammation aggravates insulin resistance, which results in a vicious circle[116].

In relation to lipid metabolism, adiponectin stimulates fatty acid oxidation and reduces TG level[113,228]. Maternal adiposity is an important risk factor for the development of GDM, and more insight on the link between adiposity and glucose intolerance is required[120,227]. Low levels of serum adiponectin associate with reduced lipid oxidation, impairment of insulin signalling with insulin resistance and stimulation of hepatic gluconeogenesis[113]. Therefore, measurement of adiponectin along with other biomarkers may serve as early predictors of GDM (early in pregnancy). IVF therapy may influence adiponectin release in pregnancy, although this remains speculative based on the current literature.

2.4.5. Gut Microbiota Markers

The relationship between intestinal microbiota and metabolic health is very topical and of much interest. Changes in the gut microbiota may influence development of much 21st century chronic illness, including diabetes mellitus, cardiovascular disease and dyslipidemia[229]. A list of inflammatory and gut-related markers to assess glucose homeostasis, insulin resistance and early markers of subsequent GDM development is summarised in Table 2 (refer to section 2.3.3). LBP strongly correlates with both obesity and insulin resistance [117,230]. High levels

of serum LBP strongly correlate with LPS, and associate with insulin resistance, obesity and T2DM[131,229,231,232]. Assessment of gut permeability during pregnancy (through both IVF- and spontaneously-conceived pregnancies) would form a novel focus for future research, and provide insight into possible links between IVF and future risk of GDM.

Table 4: Summary of Effects of Oral Contraceptives, Spontaneous Pregnancy and Expected Changes in IVF-conceived Pregnancy

Parameters	**Oral Contraceptives**	**Spontaneous Pregnancy**	**Expectation of Combined Effect of IVF therapy with pregnancy**
Fasting Glucose	↑	No change during first trimester	↑
Fasting Insulin	↑	No change during first trimester	↑ starting from first trimester
HOMA_IR	↑	↓ or no change during first trimester	↑ starting from first trimester
Lipid Profile	↑	↑	↑↑
TSH	↑	No change or ↓ during first trimester	No change
Adiponectin	No data	No change during first trimester	↓
LBP	No data	No change during first trimester	↑

HOMA-IR: homeostatic model assessment of insulin resistance; TSH: thyroid-stimulating hormone; LBP: lipopolysaccharide binding protein

Section 1: Introduction, Literature Review and Aims

Chapter 3: Aim of the study and Objectives

3.1. Rationale

Previous studies have focused on risk of obstetric complications and foetal outcomes in IVF-conceived pregnancies, and the effect of oral contraceptives on some metabolic parameters. However, limited knowledge is available in relation to the safety of IVF hormonal therapies on maternal metabolic, endocrine and inflammatory status, and the relationship to the known diabetogenic and atherogenic effects of pregnancy. As stated earlier, glucose and insulin levels do not normally change in early pregnancy. Increased insulin resistance and requirement occur during mid-gestation and more toward late gestation[88]. Given that IVF hormones are thought to play an important role in glucose homeostasis (tested on mice), physiological changes of pregnancy are expected to occur earlier in IVF-conceived pregnancy as a result of exogenous IVF hormones combined with those of pregnancy. Change in glucose homeostasis at 12 weeks was the primary outcome of this study and with the speculations that:

1. Baseline levels of glucose and insulin levels do not differ between the two groups (pregnant and non-pregnant)
2. Glucose and insulin levels do not differ at 4 weeks (as both groups receive the same IVF hormonal therapy)
3. Glucose and insulin levels differ at 12 weeks given that non-pregnant women will have stopped hormonal treatment and are not pregnant (levels go back to baseline).

The secondary outcome of this study was related to the change in insulin sensitivity at 12 weeks compared to baseline levels and between groups. Other secondary outcomes included changes in lipid and thyroid profiles, as well as, in inflammatory and microflora-related markers at 12 weeks.

This research will enhance the understanding of the short-term effect of IVF hormonal therapy on the metabolic, cardiovascular, endocrine and inflammatory systems, which include glucose and insulin homeostasis, lipid profile, gut microflora and thyroid function. The study will also provide insight into early risk of pregnancy-related complications and metabolic disturbances, and identification

of potential predictors of GDM.

3.2. Study Objectives

1. To explore the short-term effects of IVF hormonal therapy on maternal metabolic and inflammatory status, including:

   i. Risk of glucose intolerance and insulin resistance
   ii. Risk of other metabolic, endocrine and inflammatory disturbances (including lipid profile, gut microflora and thyroid function)

2. To identify possible early predictors of GDM and other metabolic-related adverse outcomes in IVF-conceived pregnancies.

3.3. Hypothesis

Null hypothesis (H0): at 12 weeks, changes in glucose and insulin levels (from baseline), lipid profile, thyroids and inflammatory markers in pregnant women are equal to changes in non-pregnant women.
Alternate hypothesis (H1): at 12 weeks, change in glucose and insulin levels (from baseline), lipid profile, thyroids and inflammatory markers in pregnant women is not equal to changes in non-pregnant women.

3.4. Research Questions

1. Do IVF hormonal therapies impair glucose homeostasis and insulin sensitivity starting from the first trimester of pregnancy?
2. Do IVF hormonal therapies impair other endocrine, cardio-metabolic and inflammatory parameters (such as lipid and thyroid profiles)?
3. Given the preconception measures and early gestational screening in IVF-conceived women, can early predictors of GDM be identified?

Section 2: Methods and Analysis

Chapter 4: General Methods

4.1. Ethics and Consents

The study was initially reviewed by Warwick Medical School upon enrolment in the PhD program. Approval to conduct the study was first granted by the University of Warwick's Biomedical and Scientific Research Ethics Sub-Committee (BSREC) (REGO-2018-2232) (Appendix 1). The study protocol was also reviewed by the two main health authorities in the UAE, as recruitment of participants occurred from three centres located in Dubai, Abu Dhabi and Al Ain. Dubai Health Authority (DHA) "University Students Application to Conduct Research" recognised centres and "Dubai Scientific Research Ethics Committee" (DSREC) (DSREC-11/2017_09) approved the study for the Fakih IVF Dubai branch (Appendix 2) and the Medical Research Department at the Ministry of Health (MOH) (HCQ-190-18) for Abu Dhabi and Al Ain Fakih IVF branches (Appendix 3). The study was registered under the name of Warwick University at ClinicalTrials.gov (NCT03426228). Ethical approvals were renewed and revised yearly by both DHA and MOH.

Informed consent was obtained from each participant who agreed to participate in the study before undergoing IVF therapy (Appendix 5). Prior to informed consent, there was provision of an explanation about the nature and purpose of the study to all potential participants. Confidentiality and anonymity of all participants was maintained throughout the study, and this was made clear to them. All participants could withdraw from the study at any time. In case a patient decided not to participate in the study, this decision did not affect ongoing clinical care in any way. There was also clear explanation of this information to all potential participants.

4.2. Study Design

The presented research is a longitudinal quantitative cohort study, whereby blood samples were collected at different stages during IVF therapy. The reference point is prospective and the nature of the investigation is correlational (observational) and non-experimental.

4.3. Study Setting

The study took place at Fakih IVF Clinics in Dubai, Abu Dhabi and Al Ain branches in the UAE. Fakih IVF is the leading and most advanced infertility clinic in the UAE, where approximately 4000 IVF cycles are conducted annually. At Fakih IVF, pre- and post-fertility treatments and in-house obstetric facilities are provided, including blood tests, scans, IVF procedures (e.g. laparoscopy, egg retrieval, embryo transfer, microdissection) and in-house genetic testing. Most patients are followed up to the point of delivery.

4.3.1. Participant Recruitment and Screening

On the first visit with the IVF specialist, patients discuss the fertility treatment that is appropriate for them. Female reproductive hormone tests are conducted to help identify the type of fertility protocol to apply. Hormonal assays included AMH, FSH, LH, oestrogen, progesterone and prolactin. Thyroid-stimulating hormone (TSH) and HbA1c are also tested to screen for thyroid dysfunction and dysglycaemia respectively. Any fertility treatment would usually start on the second day of the menstrual cycle (labelled as CD2). Hence, patient comes on their second day of their following cycle if she agreed with her doctor to start the IVF therapy. On a daily basis, all CD2 files for the next day were reviewed to check if they will be undergoing an IVF therapy and if so, their eligibility for the study. In case the patient was eligible (first screening steps, non-diabetic and normal thyroid function on file), the main investigator would call her to introduce the study and the purpose of the tests. If the patient was willing to participate, she was asked to undergo an overnight fast of 8 hours on the day of her appointment.

Consent forms were signed on their first day of the IVF treatment; anthropometrics and medical history questionnaire were completed as well (Appendix 4). The nurse in the phlebotomy room was informed to add the following hormonal tests: lipid profile, fasting glucose and insulin, TSH and HbA1c. The list of tests and the consent form were attached in patients' chart; a copy was provided to patients and another to the main investigator (Appendices

5 and 6).

4.4. Study Population

4.4.1. Inclusion and Exclusion Criteria

Adult obese (BMI: 30–38 kg/m^2) and non-obese (BMI: 18.5–29.9 kg/m^2) women (≤39 years of age), presenting with any infertility concern (such as PCOS, fallopian tube obstruction, endometriosis, fibroids, male factor), with or without insulin resistance or a combination of these factors were eligible participants for the study. Patients were excluded if they presented with diabetes (confirmed by impaired or abnormal fasting glucose and/or HbA1c), and/or abnormal thyroid function. Patients were also excluded if they had any pre-existing serious medical concerns, such as cancer, hepatic, haematological, renal (e.g. impaired kidney function), pulmonary, cardiovascular (e.g., stroke, myocardial infarction) dysfunctions. An additional exclusion factor included medications that may affect glucose homeostasis, thyroid and/or lipid profile. This included glucose-lowering, thyroid medications, growth hormones, oral steroids, anti-inflammatory, immunosuppressant, bronchodilators and antiarrhythmic drugs[233,234]. Advanced pre-gestational age and obesity are one of the main risk factors for GDM. These factors also confound egg quality and quantity. It was important therefore to minimise the confounding effect of age on metabolic outcomes, through adopting an upper age limit of 39 years, given that most patients who are seeking IVF treatment at the clinic are usually under the age of 39 years, and still have good ovarian reserve and response.

4.4.2. Study Groups

- ***Pregnant group***: received IVF therapy and tested positive for pregnancy at 4 weeks, and continued taking IVF hormonal therapy until 12 weeks of pregnancy.

- ***Non-pregnant***: received IVF therapy and tested negative for pregnancy at 4 weeks, and stopped taking IVF hormonal therapy at that point.

4.5. Sampling Technique

The non-probability convenience sampling method was used to recruit participants for the study. Patients who were to start a fresh IVF treatment at any of the IVF Clinic branches (Dubai, Abu Dhabi or Al Ain) and meeting the inclusion criteria were eligible to participate.

4.6. Sample Size Calculation

During the first trimester of a spontaneously-conceived pregnancy, glucose and insulin levels are thought not to differ from non-pregnant levels. Fasting insulin level starts increasing in the second and third trimesters, and follows a reciprocal relationship with steadily diminishing insulin sensitivity[81,88]. In this study, the diabetogenic effect of pregnancy on glucose and insulin homeostasis is expected to occur earlier (starting from the first trimester) as an effect of IVF hormonal therapy.

If the proportion of attribute (≈45% infertility couples) in this study is set to be p and the standard deviation of changes at 12 weeks from baseline is σ, to detect a mean difference of size δ with $(1-\beta)\%$ power at α significance level, the sample size for the pregnant women group:

$$n = \left(\frac{1}{1-p}\right)\frac{\left(z_{\alpha/2} + z_{\beta}\right)^2}{\delta/\sigma^2}$$

It follows that the sample size for non-pregnant women is $\left(\frac{1-p}{p}\right)n$. The hypothesis was tested at 5% significance level, corresponding to $z_{\alpha/2} = 1.96$ and 80% power to $z_{\beta} = 0.84$.

Significant change in glucose and insulin levels can be expected at the first trimester with IVF-conceived pregnancy (as an effect of exogenous hormones). Such information is however not available in the literature, since insulin measurements are only tested at around the second trimester when it usually starts changing, and these studies were conducted in spontaneous pregnancy and not IVF-conceived pregnancy. Given that the ratio δ/σ cannot be determined, a standardised difference can be specified with:

- 0.3 corresponding to a small difference
- 0.5 corresponding to a moderate difference
- 0.7 corresponding to a big difference

It will be very difficult to convince a non-pregnant participant, who spent 10,000$ on the treatment which failed to come back after 12 weeks for a blood test. Consequently, the number of non-pregnant participants was narrowed to a ratio of 2:1 pregnant to non-pregnant, and which corresponded to $p=0.67$.

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 non-pregnant women, the sample size consisted of 96 pregnant and 48 non-pregnant women. According to the latest statistics, pregnancy success rate post-egg retrieval is about 30% and this declines with age[235,236]. This success rate is comparable to the rate reported by Fakih IVF Clinics. Therefore, 275 participants were recruited initially to end up with 96 clinically confirmed pregnant.

4.7. Data Collection

4.7.1. IVF Therapy Protocol

The IVF protocol consists of three phases: egg maturation preparation, egg and sperm collection and embryo transfer. The IVF intervention type that was used with the study participants was the "antagonist protocol", which relies on administering agents to prevent premature ovulation (i.e. gonadotrophin-releasing hormone antagonist)[237] (Figure 1 and 2).

On day 2 or 3 of the menstrual cycle, ovarian stimulation began with daily administration of FSH alone or combined with LH injection, depending on baseline hormonal levels, to stimulate follicle growth and development into eggs. Follicle growth (size and numbers) were monitored throughout the stimulating phase with frequent ultrasound and hormonal blood tests (FSH, LH, oestrogen and progesterone hormones). IVF therapy was adjusted accordingly. Ovarian stimulation lasted from 8 to 12 days. On day 6 of stimulation, gonadotrophin-releasing hormone (GnRH) antagonist injection was administered daily, as a means

to block endogenous GnRH activity, which normally stimulates FSH and LH release from the pituitary gland. Under normal physiological conditions, FSH influences egg growth and maturation, and stimulates oestrogen production. LH triggers the late stage of egg maturation and ovulation, and stimulates progesterone production from the ovaries. The ovaries normally secrete oestrogen and progesterone post-ovulation. GnRH administration enables better control of the reproductive environment (through suppression of endogenous hormones), and prevents premature ovulation during IVF therapy[41]. Similar in function to LH, a human chorionic gonadotrophin (hCG) hormone "trigger" injection is administered 36 to 40 hours before the schedule of egg retrieval to induce final egg maturation and to trigger the rupture of eggs from ovarian follicles[41]. At this stage, there is discontinuation of all other IVF-related hormones. On the day of egg retrieval, also called 'oocyte pick-up' (OPU), patients are required to come having fasted for 8 hours. OPU is performed under sedation and using an ultrasound guided needle. The number of eggs collected depends on the number of follicles which responded to the stimulation; on average, 8 to 15 eggs are retrieved[238]. After OPU, patients are prescribed progesterone and oestrogen therapies ('luteal therapy') to help prepare the uterine lining for embryo implantation and support early pregnancy. The IVF hormones were administered in different forms: intramuscularly, orally and vaginally. The dose and type of oestrogen and progesterone depended on the patient history, stage of pregnancy and purpose (for instance if bleeding or spotting is present, vaginal administration may be appropriate).

The collected semen sample is injected into retrieved eggs under microscope, and following the intracytoplasmic sperm injection (ICSI) method. The ICSI method increases the chances of successful fertilisation and consists of injecting a single sperm directly into the cytoplasm of an egg[41]. Embryos are assessed based on morphology and rate of cells division to advance from zygote to blastocyst. Genetic testing (GT) of embryos is conducted on day 3 or 4 post-OPU. This includes pre-implantation chromosomal screening and/or pre-implantation genetic diagnosis. The latter helps screening for inherited diseases. Embryo transfer (ET) is conducted five days post-OPU and without sedation. Occasionally, transfer

would be cancelled when ovarian hyperstimulation syndrome (OHSS) occurred. OHSS is one of the complications of ovarian stimulation when undergoing ART, and may necessitate embryo freezing and re-scheduling of ET for the next menstrual cycle in severe OHSS cases.

Pregnancy success and the risk of multiple pregnancies depend on the number of eggs transferred[238]. On day 8 post-embryo transfer, the first β-HCG test (also known as pregnancy test) is conducted and repeated on day 10 and if needed on day 12. In a successful pregnancy, serum β-HCG usually doubles every 48 hours. An ultrasound scan is scheduled two weeks post-embryo transfer to assess for pregnancy viability and the presence of a sac. At 8 weeks of gestation, foetal heartbeat is usually detected with ultrasound. Serum oestrogen and progesterone levels are measured regularly (until the first ultrasound) to adjust the dose of IVF-hormonal therapy. Hormonal therapies were discontinued (at 4 weeks) in all cases of negative, ectopic or biochemical pregnancy. Otherwise, participants were instructed to continue taking hormonal therapies until around week 12 of pregnancy. Biochemical pregnancy (also called early miscarriage) implies a pregnancy confirmed by a positive pregnancy test (i.e. β-HCG), but no sac is visible on ultrasound. Ectopic pregnancy also implies a positive β-HCG but the level increases at slower rate. The latter is considered as a complication of pregnancy, whereby the embryo implants outside the uterus (such as in the fallopian tube), resulting from the amount of fluid injected with the embryo not being minimal, or when the location of the embryo transfer is closer to the fallopian tubes[239]. A clinical pregnancy is confirmed by both high serum β-HCG level and ultrasound confirmation of a gestational sac[41].

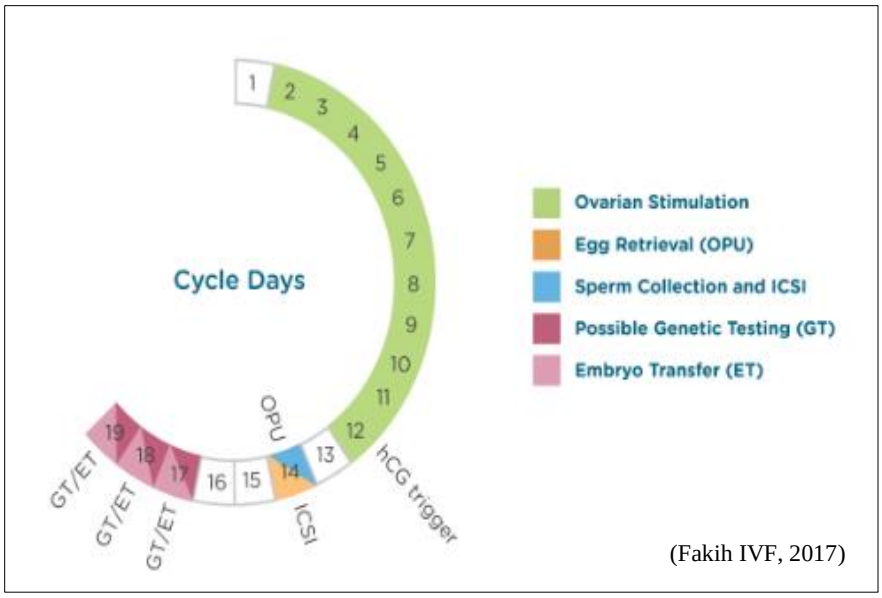

Figure 1. *In Vitro* Fertilisation Protocol Steps[240]

4.7.2. UAE Regulations

In the UAE, it is strictly prohibited to have egg or sperm donors, whilst gender selection is allowed (Article 10, p.7)[241]. The optimal number of embryos to transfer is determined based on the patient characteristic, age and medical history[41]. According to Health Authority Abu Dhabi (HAAD) regulations, patients with a favourable prognosis should be transferred no more than two embryos if under 35 years of age, but no more than three if 37 years and older (Article 13, p.8)[241,242]. These guidelines are also compatible with the American Society for Reproductive Medicine (ASRM) regulations[243].

4.8. Study Protocol

4.8.1. Study Stages

After an overnight fast of 8 hours, there was collection of 10 ml of blood at four pre-defined time points during the IVF protocol:

1. ***Phase 1***– At baseline, egg maturation (starting IVF therapy)

2. ***Phase 2*** – Week 2 – Egg retrieval (OPU procedure)

3. ***Phase 3*** – Week 4 – At pregnancy test (β-HCG test)

4. ***Phase 4*** – Week 12 (one week after completing IVF hormonal therapy for pregnant group).

4.8.2. IVF Therapies

Ovarian reserve and age of the women are important factors to consider when defining the type of IVF treatment and dosage of IVF hormones to take (Table 5). Anti-müllerian hormone (AMH) and FSH levels and antral follicle count (through transvaginal ultrasound) determine ovarian reserve. AMH is a hormone secreted by the cells of growing follicles of the ovaries[244]. High FSH level, low AMH or low follicle count are all indicators of low ovarian reserve and may predict low chances of pregnancy[245]. In that case, higher dose of IVF hormones may be required. Older women may require higher doses of stimulating hormones, as they tend to have a lower response and a higher risk of miscarriage compared to younger women[1,41].

For pregnant group, the final blood sample (phase 4) was collected a week post-discontinuation of hormonal therapies at around week 12 of pregnancy (half-life of exogenous hormones is about 15–50 hours), to ensure sufficient time for body clearance of IVF therapies[246,247].

Table 5: Description of IVF Hormonal Therapy

IVF Stage	Categories	Function	Doses
Ovarian Stimulation.1 (also called gonadotrophins)	Recombinant FSH Recombinant FSH and LH	Follicles recruitment and development.	Tablets: 300 IU/day
Ovarian Stimulation.2	GnRH antagonist	Prevents spontaneous rupture of follicles and helps in controlling the stimulation cycle by preventing premature ovulation.	Tablets: 0.25 mg/day
Triggering	GnRH agonist or recombinant hCG	Similar effect to LH surge for final maturation of eggs, 36 hours before egg retrieval.	One injection (0.5 mg)
Post-embryo transfer	Progesterone	Synthetic progesterone to support early pregnancy and optimise uterine lining for embryo implantation.	Injection: 50 mg/d; Tablets: 10 mg/ TID; Vaginal: 100 mg/BID; Gel: 1.125 mg/TID
	Oestrogen	Synthetic oestrogen to prepare uterine lining	2 mg/TID

FSH: follicle-stimulating hormones; LH: luteinizing hormone; GnRH: gonadotrophin-releasing hormone hCG: human chorionic gonadotrophin; TID: three times a day; BID: twice a day

4.8.3. Intervention Plan

During the stimulation period, patients are required to visit the clinic every two days for hormonal blood tests to monitor their response to the treatment and to take their injections, and then at 8 and 10 days post-embryo transfer for the pregnancy test (β-HCG) (Figure 2 and Table 6). Once pregnancy is confirmed (at

8 weeks with heartbeat detected), they were scheduled for a prenatal follow-up every three weeks. Hormonal profile is measured at each of their visits to adjust the dose of IVF stimulating agents (i.e. FSH, LH, progesterone and oestrogen).

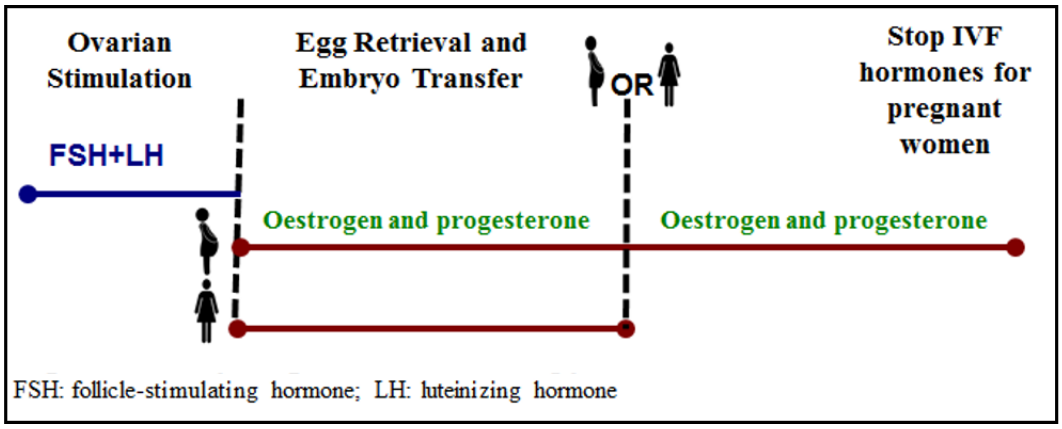

Figure 2: Study Stages and IVF Therapy Intervention

Table 6: Lists of IVF Hormonal Therapy at Each Stage of the Study

IVF hormones	Phase 1-Baseline (Start IVF treatment)	Phase 2-Week 2 (Egg retrieval and embryo transfer)	Phase 3-Week 4 (β-HCG test)	Phase 4-Week 12 (Final)
Control Group (Non-pregnant)	FSH and LH GnRH or recombinant HCG	Oestrogen and Progesterone	Oestrogen and Progesterone	
Pregnant group	FSH and LH GnRH or recombinant hCG	Oestrogen and Progesterone	Oestrogen and Progesterone	**Oestrogen and Progesterone**

*Green highlight represents stage where non-pregnant vs. pregnant women groups were distinguished; hCG: human chorionic gonadotrophin; FSH: follicle-stimulating hormones; LH: luteinizing hormones; GnRH: gonadotrophin-releasing hormone; β-HCG: beta-human chorionic gonadotrophin

4.9. Choice of Biomarkers and Measuring Techniques

The standard blood test panel was conducted and included fasting glucose and insulin, HbA1c, lipid profile and thyroid level. In addition, after comparing the different well-recognized methods to measure glucose homeostasis and insulin resistance, HOMA-IR was selected for early gestation assessment (up to 12 weeks). This method (formula-based) provides a highly reliable estimates of insulin resistance and which also correlates with estimates by the "gold standard" euglycaemic clamp technique[216] (refer to Table 3). Additionally, HOMA-IR method is validated in pregnancy and even with the presence of gestational obesity[248]. It is also simple, practical and safe in pregnancy. The OGTT test was conducted around 28 weeks of gestation to diagnose GDM. In the present study, the 2-hour OGTT glucose cut offs suggested by the International Association of Diabetes and Pregnancy Study Groups were used for GDM diagnosis, with two abnormal levels from the following: glucose fasting ≥92 mg/dL (5.1 mmol/L), ≥180 mg/dL (10 mmol/L) for 1-hour and ≥153 mg/dL (8.5 mmol/L) for 2-hours[197].

In regards to inflammation, different markers were evaluated and the following criteria were taken into account: cost, test acceptability in pregnancy, earliest possible indicator of any change, and availability and accessibility of measuring kits in the UAE (refer to Table 2). Given the well-demonstrated direct association of adiponectin with inflammation and insulin resistance, this parameter was selected for the present study as a potential early marker of changes in glucose homeostasis and inflammation. Additionally, most studies focused on the association between adiponectin and risk of T2D[113]. Further insight into the association between adiponectin and onset of GDM is hence needed.

In relation to gut endotoxemia and related inflammatory biomarkers (refer to Table 2), LBP was thought to be appropriate in reflecting changes in both mechanisms. Strong evidence have suggested using LBP as a surrogate marker of endotoxemia and resultant inflammation in place of LPS. Consequently, given its high stability, LBP biomarker was selected in this study as a potential early indicator of change in inflammation and gut microflora during IVF therapy and in the first trimester of pregnancy (up to 12 weeks). The literature is still limited in

regards to the effect of hormonal therapy on LBP and further studies on its predisposition to onset of GDM are required.

In addition, well-documented and appropriate anthropometric and metabolic cut-offs were also tested at 12 weeks to assess participants at high risk of GDM. These parameters included age ≥35 years[1,7], BMI ≥30 kg/m^2 from NICE guidelines[25], BMI ≥35 kg/m^2 by British Fertility Society[24], HbA1c ≥5.7% (39 mmol/mol)[200,203], TSH ≥2.5 μIU/mL[183], TG ≥137 mg/dL (≥1.55 mmol/L)[223], HDL-C ≤85.5 mg/dL (≤2.21 mmol/L)[119], log TG/HDL-C ≥0.099[226] and adiponectin ≤6.4 μg/mL[113,227]. A narrowed ethnicity classification into two main groups (Arabs vs. non-Arabs) was also tested given the higher prevalence of GDM in the Arab community[192,249].

4.10. Blood Tests

A trained nurse was in charge of measuring anthropometrics and clinical parameters (e.g. weight and blood pressure), in addition to taking fasting blood samples (Table 7). The socio-demographics, medical and pregnancy histories of each patient were recorded in their file. Blood tests were conducted at Fakih IVF (in the phlebotomy room) and transferred to the lab (in-house) for analysis. Tests results were available on Fakih IVF internal server at the end of each day.

Table 7: List of Blood Tests at Each Stage of the Study

Type of Tests	Phase 1- Baseline (Start IVF treatment)	Phase 2- Week 2 (Egg retrieval)	Phase 3- Week 4 (β-HCG test)	Phase 4- Week 12 (Final)
Female reproductive hormones: FSH, LH, oestrogen, progesterone	√	√	√	
HbA1c	√			√
Fasting glucose	√	√	√	√
Fasting insulin	√	√	√	√
TSH	√			√
Fasting lipid profile	√			√
Adiponectin	√			√
LBP	√			√
β-HCG pregnancy	√		√	
Body weight	√			√

*Green highlight represents stage where non-pregnant vs. pregnant women groups were distinguished; FSH: follicle-stimulating hormones; LH: luteinizing hormone; HbA1c: glycated haemoglobin A1c; TSH: thyroid-stimulating hormone; LBP: lipopolysaccharide binding protein; β-HCG: beta-human chorionic gonadotrophin

4.11. Infertility

Within 24 hours of blood collection, participants received a call from the clinic to provide them with an overview of their results. If there was any abnormal result with clinical significance (such as new diagnosis of diabetes), participants and the physician in charge were promptly informed by the principal investigator (Ayla Coussa). Appropriate clinical management (including any additional investigations) would be arranged within the clinical setting at Fakih IVF Centres and independently of the research setting.

4.12. Risks and Benefits of Participation

This study did not present any risk to participants, since no additional injections were required and tests were analysed from the same serum sample used for standard IVF therapy. The study did not affect in any sense the flow of fertility treatment. Responsibility for any risks of the tests throughout the treatment period was covered by the IVF Clinic. Findings from this study have the potential to reduce the prevalence of complications and metabolic disturbances in relation to IVF-pregnancy outcomes through identification of early predictors of onset of metabolic dysfunction. A copy of all blood test results was provided to participants at the end of the study.

4.13. Data Analysis

4.13.1. Assays

4.13.1.1. Hormones

Female hormones (AMH, FSH, LH, oestrogen, progesterone, and β-HCG) and TSH levels were measured with the electrochemiluminescence immunoassay (ECLIA) using Cobas E analysers (Elecsys 2010, E170), which was supplied by Roche Diagnostics (Indianapolis, USA).

4.13.1.2. Glucose Homeostasis and Insulin Resistance

Fasting plasma insulin (FPI) was also measured by ECLIA kit using Cobas E immunoassay analysers (Elecsys 2010, E170) from Roche Diagnostics (Indianapolis, USA). Fasting plasma glucose (FPG) was measured by enzymatic reference method with hexokinase-glucose-6-phosphate dehydrogenase using Roche/Hitachi Cobas C systems (Roche Diagnostics, Indianapolis, USA). Both fasting FPI concentration (μIU/mL) and FPG (mg/dL) were used in the homeostatic model assessment (HOMA) to quantify insulin resistance (IR) and assessment of glucose homeostasis. HOMA-IR was calculated as follows[216,217]: HOMA-IR = (FPI x FPG) / 405.

4.13.1.3. Lipid Profile

Total cholesterol (T-Chol), triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C) were measured by homogenous enzymatic colorimetric method with Roche/Hitachi Cobas C systems (Cobas C 311/501), supplied by Roche Diagnostics (Indianapolis, USA). Low-density lipoprotein cholesterol (LDL-C) was determined using the Friedewald formula[250]: LDL-C = (T-Chol) – (HDL-C) – (TG/5).

4.13.1.4. Adiponectin and Lipopolysaccharide binding protein (LBP)

Enzyme linked immunosorbent assay was used to measure plasma LBP concentration by human LBP ELISA kit (catalog number E-EL-H2289-96T) and serum adiponectin by human ADP/Acrp 30 ELISA kit (catalog number E-EL-H5811-96T), from Elab Science (Texas, USA). The specifications of LBP ELISA kit include the following: sensitivity of 1.88 μg/mL, detection range of 3.13–200 μg/mL and <10% coefficient of variation (Intra-assay CV, low: 3.52%; high: 6.49%; Inter-assay CV, low: 4.66%; high: 5.32%). Adiponectin ELISA kit specifications are: sensitivity of 0.10 μg/mL, detection range was 0.16–10 μg/mL and <10% coefficient of variation (Intra-assay CV, low: 11.04%; high: 12.77%; Inter-assay CV, low: 7.81%; high: 8.40%). Optical density was measured by spectrophotometer at a wavelength of 450 ± 2 nm (optical density value is proportional to concentration of human LBP and to concentration of human ADP/Acrp 30).

4.13.2. Statistical Analyses

4.13.2.1. Effects of IVF Therapy

Data analysis was performed with the Statistical Package for the Social Sciences (SPSS) software version 21.0 for Windows (SPSS, Chicago, IL). Parameters normality was first visually tested with the histogram configuration, and confirmed with the Shapiro-Wilk test. Most of the measured parameters were not normally distributed. For non-normally distributed parameters, data for the pregnant and non-pregnant groups (baseline and final) are summarised

using median and interquartile range (IQR). IQR represents the difference between 75^{th} and 25^{th} percentiles or between upper and lower quartiles ($IQR = Q_3 - Q_1$).

Non-parametric 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. Non-parametric Wilcoxon test for two related samples was conducted to assess changes at baseline vs. post-IVF treatment (12 weeks) within groups (pregnant or non-pregnant). Bivariate correlation was used to determine correlations between the different anthropometric and metabolic parameters at week 12. Data from the two groups were analysed separately. Linear regression was used for assessment of association of change in glucose level at 12 weeks (dependent variable) with change in other anthropometric and biochemical parameters, separately for each group. Changes in glucose and insulin levels (and the resulting HOMA-IR) throughout IVF therapy (baseline, 2, 4 and 12 weeks) were determined with the mixed model for repeated measures test.

4.13.2.2. Anthropometrics and Biomarkers of GDM

In the pregnant group, the Mann-Whitney U test for two independent samples was used to compare data at baseline with 12 weeks, for comparison between women who later developed GDM to the non-GDM group. Changes at baseline, 4 and at 12 weeks were assessed by non-parametric Wilcoxon test (two related samples) within groups (future GDM or non-future GDM women). Data are summarised using median and interquartile range (IQR).

Chi-square and fisher's exact tests were used to assess the association of anthropometric characteristics and pregnancy outcomes (categorical) with GDM. Ethnicity was stratified into seven groups: Middle East, Gulf, Europe, North America, South Asia, East Asia and Africa. Predictors of GDM were assessed using binary logistic regression, adjusting for the following variables: age and history of PCOS. In addition, chi-square was used to test the validity of documented and selected appropriate anthropometric and metabolic cut-offs (levels at 12 weeks) to predict GDM: age ≥35 years, BMI ≥30 kg/m^2, BMI ≥35 kg/m^2, HbA1c ≥5.7% (39 mmol/mol, TSH ≥2.5 μIU/mL, TG ≥137 mg/dL (≥1.55 mmol/L), HDL-C ≤85.5

mg/dL (≤2.21 mmol/L), log TG/HDL-C ≥0.099, adiponectin ≤6.4 μg/mL and belonging to the Arab ethnicity. The significance level was set at $p<0.05$ with 95% confidence interval (CI).

4.13.2.3. Pregnancy and Foetal Outcomes

Chi-square and fisher's exact tests were also used to measure the association of pregnancy and foetal outcomes (categorical) with delivery by caesarean section.

Predicting delivery by caesarean section with maternal and foetal characteristics were assessed using binary logistic regression, adjusted for BMI at 12 weeks, with significance level of $p<0.05$ and 95% confidence interval (CI).

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Section 3: Results and Discussion

Chapter 5A: Results

5.1. Participants Enrolment

A total of 702 women were pre-screened, 693 screened of whom 673 participants were eligible for enrolment in the study. Out of the 673 only 354 participants had embryo transfer, with the remaining 221 cycles were converted to freezing, 98 were cancelled due to poor quality, low number of eggs and/or genetic abnormalities. Post-embryo transfer, 191 participants presented with a clinically confirmed pregnancy, 153 with a negative β-HCG and 10 experienced either a biochemical or ectopic pregnancy. At week 12, there were 52 drop-outs whereby some of participants had already left the country before the 12 weeks tests and others withdrew consent due to failed pregnancy (Figure 3). Overall, 275 participants completed the study, with 158 pregnant and 117 non-pregnant women. Ultimately, pregnancy outcomes included 34% multiple, 6% biochemical and 3% ectopic pregnancy, and 8% miscarriage.

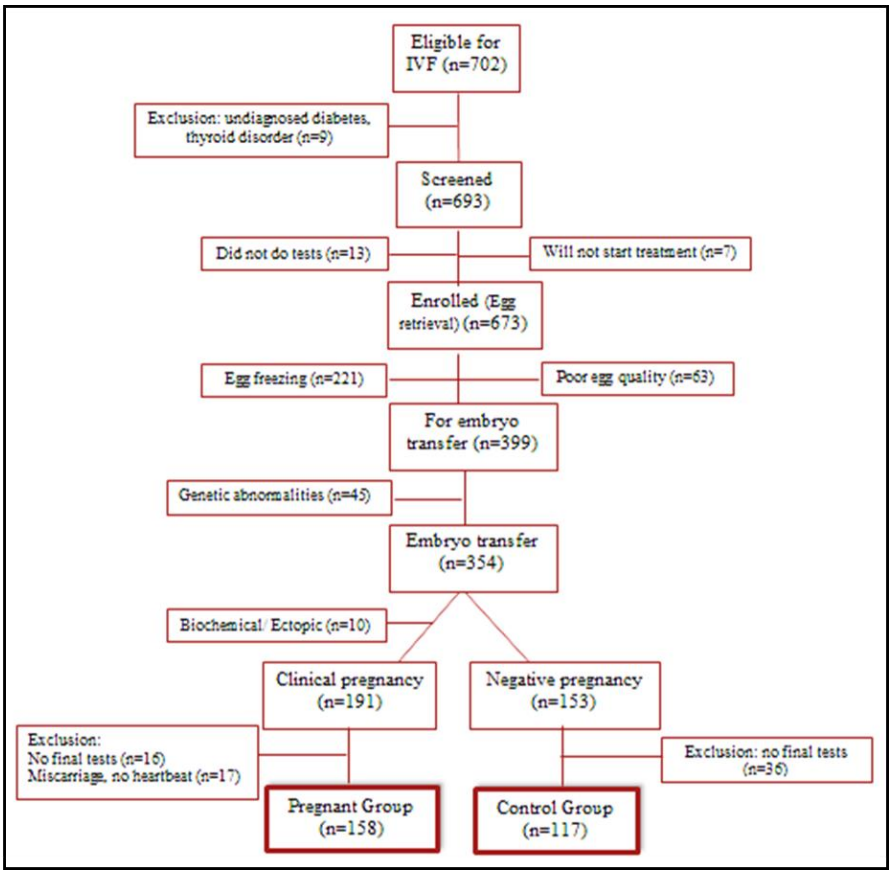


Figure 3: Flowchart of Participants' Recruitment and Enrolment in the Study

5.2. Metabolic, Endocrine and Inflammatory Outcomes

Phenotypic characteristics of participants are shown in Tables 8, 9 and 10. Data are summarised as median (IQR). At baseline, there was no significant difference between the two groups (pregnant and non-pregnant following IVF) in relation to anthropometric, endocrine (including female hormonal profile), and metabolic parameters (Table 10). At preconception, participants had a median age of 32 (6) years, BMI of 25.4 (6.9) kg/m^2, HbA1c of 5.2 (0.52) % (33 mmol/mol) and TSH of 1.82 (1.4) µIU/mL. Age (>35 years) did not affect IVF outcome.

Phenotypic characteristics of pregnant women are presented in Table 8. Compared with baseline values, there were significant reductions at 12 weeks in fasting glucose (86.15 to 82.19 mg/dL or the equivalent to 4.8 to 4.6 mmol/L), HbA1c (5.3 to 5.08% or the equivalent to 34 to 32 mmol/moL) and TSH (1.7 to 1.4 µIU/mL), with $p<0.001$ for all parameters; whilst serum insulin level was unchanged ($p=0.23$). Lipid profile parameters increased significantly at 12 weeks compared with baseline values by: 12% in T-Chol (177.5 to 199.5 mg/dL or the equivalent to 4.60 to 5.15 mmol/L), 72% in TG (73.5 to 126.78 mg/dL or the equivalent to 0.83 to 1.43 mmol/L) and 18% in HDL-C levels (55.3 to 65.1 mg/dL or the equivalent to 1.43 to 1.68 mmol/L). BMI also increased significantly (24.8 to 25.7 kg/m^2, $p<0.001$) at 12-weeks compared with baseline. Compared to baseline concentrations, insulin, adiponectin, LBP, and HOMA-IR all remained unchanged when measured at 12 weeks of pregnancy.

Phenotypic characteristics of non-pregnant women are presented in Table 9. Compared with baseline values, at 12 weeks fasting plasma glucose level increased by 2% (86.04 to 87.62 mg/dL or the equivalent to 4.77 to 4.86 mmol/L), serum insulin by 7% (8.72 to 9.37 µIU/mL) and HOMA-IR by 10% (1.9 to 2.1); with all $p<0.01$. Lipid profile parameters also increased significantly at 12-weeks: T-Chol by 3% (169.5 to 174.9 mg/dL 4.38 to 4.52 mmol/L), TG by 18% (71.0 to 83.7 mg/dL or the equivalent to 0.81 to 0.95 mmol/L) and HDL-C by 4% (52.0 to 54.11 mg/L or the equivalent to 1.34 to 1.40 mmol/L); with $p<0.001$ for all lipid parameters. Adiponectin, LBP and TSH levels remained unchanged at 12-weeks compared with baseline values. There was small but significant increase in BMI by

0.7% (25.6 to 25.8 kg/m^2; p=0.002) at 12-weeks compared to baseline value.

When comparing both pregnant and non-pregnant groups, the small increment in weight (2%) was similar in both groups (Table 10). Fasting glucose and HbA1c varied significantly between the two groups (p<0.001), with lowered glucose level in clinically-confirmed (positive) pregnancy and increased with negative pregnancy (Graph 1A). Insulin level and HOMA-IR did not differ between groups at 12 weeks (Table 8; Graph 1B and 1C). The increment in lipid parameters occurred regardless of pregnancy status, but with a higher increase from baseline in positive pregnancy (p<0.001). TSH level was only significantly altered during pregnancy and which is likely associated with the observed difference between the two groups (p<0.0001). Regardless of pregnancy status, adiponectin and LBP levels remained the same.

Table 8. Anthropometrics, Metabolic and Endocrine Parameters at Baseline and 12 Weeks of IVF Therapy for Pregnant Women

Variables	(n=158)		*p* value
	Baseline	**12 Weeks**	
Age (years)	32.0 (7.0)		
Weight (kg)	65.5 (18.95)	66.9 (15.9)	<0.001
Body mass index (kg/m^2)	24.8 (7.30)	25.57 (6.90	<0.001
Female Hormones			
FSH (IU/L)	6.46 (2.51)		
LH (IU/L)	5.99 (3.16)		
Ratio FSH/LH	1.10 (0.60)		
Estrogen (pg/mL)	41.9 (24.2)	*412.15 (857.10)	<0.001
Progesterone (ng/mL)	0.23 (0.23)	*41.07 (37.61)	<0.001
Metabolic and Endocrine			
Fasting glucose (mg/dL)	86.15 (8.0)	82.19 (7.19)	<0.001
Fasting insulin (μIU/mL)	8.84 (6.81)	9.45 (6.95)	0.23
HbA1c (%)	5.3 (0.58)	5.08 (0.53)	<0.001
HOMA-IR	1.95 (1.52)	2.00 (1.60)	0.75
T-Chol (mg/dL)	177.5 (44.95)	199.5 (44.35)	<0.001
TG (mg/dL)	73.5 (44.0)	126.78 (60.3)	<0.001
LDL-C (mg/dL)	103.0 (38.95)	103.2 (32.43)	0.82
HDL-C (mg/dL)	55.3 (15.94)	65.1 (18.3)	<0.001
TSH (μIU/mL)	1.71 (1.29)	1.36 (1.10)	<0.001
ŦAdiponectin (μg/mL)	8.87 (1.86)	8.66 (2.41)	0.29
ŦLBP (μg/mL)	62.96 (78.83)	45.18 (71.82)	0.71

Data presented in median and interquartile range (IQR; IQR=Q3-Q1); Ŧn=42 pregnant; $p<0.05$ vs. at 12 weeks of IVF therapy, by two-related-samples 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 9. Anthropometrics, Metabolic and Endocrine Parameters at Baseline and 12 Weeks of IVF Therapy for Non-pregnant Women

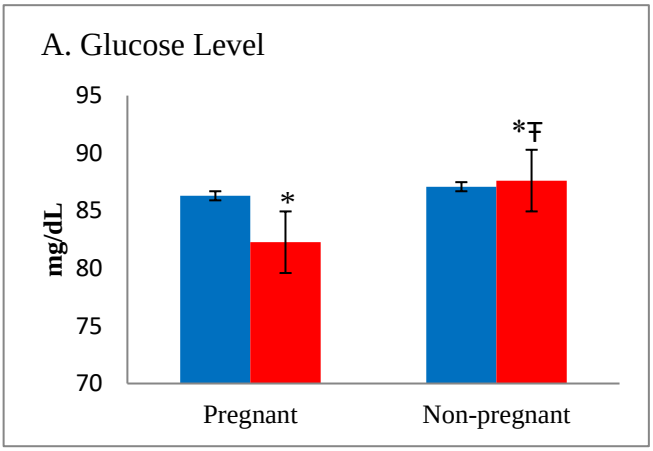
Variables	(n=117)		*p* value
	Baseline	**12 Weeks**	
Age (years)	32.5 (7.00)		
Weight (kg)	64.0 (13.97)	64.7 (15.05)	0.003
Body mass index (kg/m^2)	25.55 (6.15)	25.75 (5.73)	0.002
Female Hormones			
FSH (IU/L)	6.65 (2.47)		
LH (IU/L)	5.75 (2.70)		
Ratio FSH/LH	1.10 (0.50)		
Estrogen (pg/mL)	41.04 (19.15)	*220.5 (197.90)	<0.001
Progesterone (ng/mL)	0.24 (0.20)	*20.96 (23.95)	<0.001
Metabolic and Endocrine			
Fasting glucose (mg/dL)	86.04 (10.0)	87.62 (8.34)	<0.001
Fasting insulin (μIU/mL)	8.72 (6.41)	9.37 (5.4)	0.008
HbA1c (%)	5.2 (0.50)	5.19 (0.47)	0.16
HOMA-IR	1.9 (1.50)	2.1 (1.5)	0.003
T-Chol (mg/dL)	169.5 (39.33)	174.9 (48.03)	<0.001
TG (mg/dL)	71.0 (41.98)	83.7 (35.15)	<0.001
LDL-C (mg/dL)	101.3 (44.0)	102.57 (38.83)	0.49
HDL-C (mg/dL)	52.0 (18.82)	54.11 (14.30)	<0.001
TSH (μIU/mL)	1.95 (1.46)	1.8 (1.05)	0.17
ŦAdiponectin (μg/mL)	8.47 (2.17)	8.46 (1.94)	0.53
ŦLBP (μg/mL)	55.60 (70.70)	41.29 (88.16)	0.29

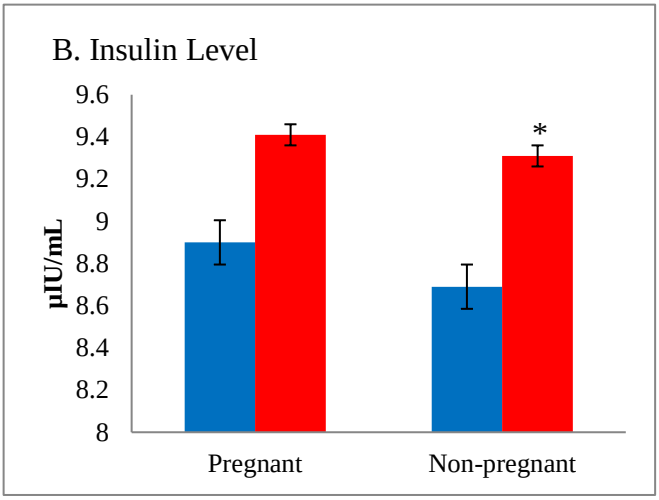
Data presented in median and interquartile range (IQR; IQR=Q3-Q1); Ŧn=42 pregnant; *Levels at 4 weeks; $p<0.05$ vs. at 12 weeks of IVF therapy, by two-related-samples test; FSH: follicle-stimulating hormone; LH: luteinizing hormone; HbA1c: glycated haemoglobin A1c; HOMA-IR: homeostatic model assessment 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

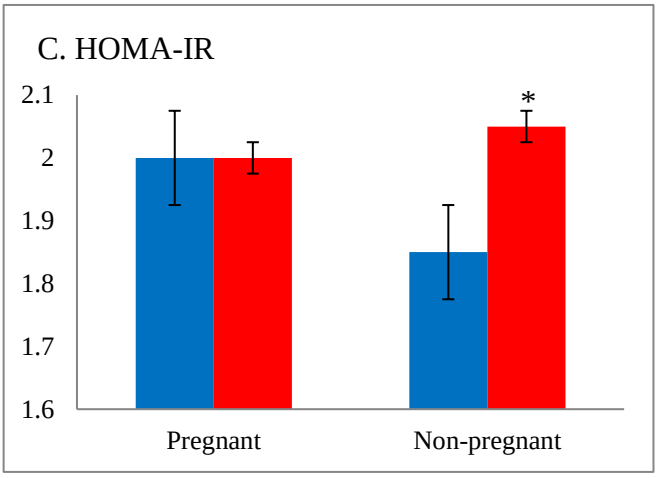
Table 10: Comparison of Anthropometrics, Metabolic and Endocrine Parameters at Baseline and 12 Weeks of IVF Therapy for Pregnant and Non-pregnant Women

Variables	Baseline		*p* value	12 Weeks		*p* value
	Pregnant (n=158)	Non-pregnant (n=117)		Pregnant (n=158)	Non-pregnant (n=117)	
Age (years)	32.0 (7.0)	32.5 (7.00)	0.32			
Weight (kg)	65.5 (18.95)	64.0 (13.97)	0.58	66.9 (15.9)	64.7 (15.05)	0.21
Body mass index (kg/m^2)	24.8 (7.30)	25.55 (6.15)	0.62	25.7 (6.90)	25.75 (5.73)	0.86
Female Hormones						
FSH (IU/L)	6.46 (2.51)	6.65 (2.47)	0.25			
LH (IU/L)	5.99 (3.16)	5.75 (2.70)	0.39			
Ratio FSH/LH	1.10 (0.60)	1.10 (0.50)	0.14			
Estrogen (pg/mL)	41.9 (24.2)	41.04 (19.15)	0.41	*412.15 (857.10)	*220.5 (197.90)	<0.001
Progesterone (ng/mL)	0.23 (0.23)	0.24 (0.20)	0.84	*41.07 (37.61)	*20.96 (23.95)	<0.001
Metabolic and Endocrine						
Fasting glucose (mg/dL)	86.15 (8.0)	86.04 (10.0)	0.73	82.19 (7.19)	87.62 (8.34)	<0.001
Fasting insulin (µIU/mL)	8.84 (6.81)	8.72 (6.41)	0.93	9.45 (6.95)	9.37 (5.4)	0.86
HbA1c (%)	5.3 (0.58)	5.2 (0.50)	0.77	5.08 (0.53)	5.19 (0.47)	0.003
HOMA-IR	1.95 (1.52)	1.9 (1.50)	0.99	2.00 (1.60)	2.1 (1.5)	0.17
T-Chol (mg/dL)	177.5 (44.95)	169.5 (39.33)	0.15	199.5 (44.35)	174.9 (48.03)	<0.001
TG (mg/dL)	73.5 (44.0)	71.0 (41.98)	0.94	126.78 (60.3)	83.7 (35.15)	<0.001
LDL-C (mg/dL)	103.0 (38.95)	101.3 (44.0)	0.61	103.2 (32.43)	102.57 (38.83)	0.47
HDL-C (mg/dL)	55.3 (15.94)	52.0 (18.82)	0.12	65.1 (18.3)	54.11 (14.30)	<0.001
TSH (µIU/mL)	1.71 (1.29)	1.95 (1.46)	0.34	1.36 (1.10)	1.8 (1.05)	<0.001
ŦAdiponectin (µg/mL)	8.87 (1.86)	8.47 (2.17)	0.17	8.66 (2.41)	8.46 (1.94)	0.82
ŦLBP (µg/mL)	62.96 (78.83)	55.60 (70.70)	0.97	45.18 (71.82)	41.29 (88.16)	0.65

Data presented in median and interquartile range (IQR; IQR= Q3-Q1); Ŧn=73 (42 pregnant, 31 non-pregnant); *Levels at 4 weeks; [a]p<0.05 vs. pregnancy by independent test; [b]p<0.05 vs. at 12 weeks within subgroups by two-related-samples 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







Graph 1. Comparison of Glucose Homeostasis at Baseline and 12 Weeks of IVF Therapy between Pregnant and Non-pregnant Women (Graph A. Fasting Glucose Level; B. Fasting Insulin Level; C. HOMA-IR)

*p<0.05 at 12 weeks of IVF therapy; Ŧp<0.05 vs. pregnant; HOMA-IR: homeostatic model assessment of insulin resistance

5.2.1. Glucose and Insulin Homeostasis

Glucose and insulin homeostasis results are summarised in Table 11 for the pregnant, Table 12 for non-pregnant women and comparing both groups in Table 13 and Graph 2.

In the pregnant group, compared to baseline values, glucose level measured at 2 (OPU), 4 (β-HCG) and 12 weeks (final) were signficanly lower (respectively, p=0.001, p=0.007 and p=0.001) (Table 11). Plasma glucose level did not differ between weeks 2 and 12 and between weeks 4 and 12. However, plasma glucose level at 4 weeks was significantly higher than at 2 weeks (p=0.004); hence glucose went down at 2 weeks, and then increased at 4 weeks and remained the same at 12 weeks. The greatest difference in glucose level was between baseline and at 12 weeks, which corresponds to a significant drop in glucose level by -4.40 mg/dL (0.2 mmol/L). In the pregnant group, serum insulin level increased significantly at 4 weeks compared to values at OPU (delta change: 3.93 µIU/mL, p=0.01) and at baseline (delta change: 3.49 µIU/mL, p=0.03). Serum insulin level dropped by -3.03 µIU/mL (p=0.06) at 12 weeks compared to 4 weeks. Serum insulin level differed the most at 4 weeks compared to baseline, and at 12 weeks it seems that the level goes back to baseline level. In the pregnant group, at 4 weeks HOMA-IR increased significantly compared to baseline (delta change: 0.76, p=0.03) and OPU (delta change: 0.91, p=0.01) but then decreased significantly at 12 weeks (delta change: -0.79, p=0.03).

In the non-pregnant group, there was an initial drop in plasma glucose level at OPU by -4.01 mg/dL (-0.2 mmol/L) (p<0.001), followed by a progressive rise at 4 weeks to baseline level, and reaching a significantly higher level at 12 weeks (delta change: 1.88 mg/dL or the equivalent to 0.1 mmol/L; p<0.001) (Table 12). Serum insulin level appeared to follow a reciprocal relationship to plasma glucose levels, with an initial increase in serum insulin level at 4 weeks by 2.85 µIU/mL (p<0.001) compared to baseline, and subsequent reduction at 12 weeks by -2.41 µIU/mL (p<0.001). It seems that the OPU stage marked the point in IVF thereapy where serum insulin level started to decrease in the non-pregnant group. The

increment in HOMA-IR at 4 weeks is significant compared to OPU and baseline values (respectively, p=0.82 and p=0.60) and drops at 12 weeks to lower level than baseline (delta change: -0.14, p=0.03).

Table 11: Mean Difference in Glucose and Insulin Homeostasis at Each Stage of IVF Therapy: Baseline, 2 weeks (OPU), 4 weeks (β-HCG test) and 12 weeks (Final) for Pregnant Women (n=158) using Mixed Model for Repeated Measures

Stages	Glucose		Insulin		HOMA-IR	
	Mean Difference	*p value*	Mean Difference	*p value*	Mean Difference	*p value*
Baseline_2 weeks	-3.96	<0.001	-0.04	0.35	-0.14	0.22
Baseline_4 weeks	-1.83	0.007	3.48	0.02	0.76	0.03
Baseline_12 weeks	-4.40	<0.001	0.45	0.25	-0.02	0.80
2 weeks_4 weeks	2.12	0.004	3.92	0.01	0.91	0.01
2 weeks_12 weeks	-0.44	0.73	0.89	0.04	0.12	0.29
4 weeks_12 weeks	-2.56	0.64	-3.03	0.06	-0.79	0.03

OPU: oocyte pick-up; β-HCG: beta-human chorionic gonadotrophin pregnancy test; HOMA-IR: homeostatic model assessment of insulin resistance

Table 12: Mean Difference in Glucose and Insulin Homeostasis at Each Stage of IVF Therapy: Baseline, 2 weeks (OPU), 4 weeks (β-HCG test) and 12 weeks (Final) for Non-pregnant Women (n=117) using Mixed Model for Repeated Measures

Stages	Glucose		Insulin		HOMA-IR	
	Mean Difference	*p value*	Mean Difference	*p value*	Mean Difference	*p value*
Baseline_2 weeks	-4.01	<0.001	-0.54	0.27	-0.21	0.06
Baseline_4 weeks	-0.54	0.35	2.85	<0.001	0.60	<0.001
Baseline_12 weeks	1.88	<0.001	0.44	0.11	0.14	0.03
2 weeks_4 weeks	3.46	<0.001	3.40	<0.001	0.82	<0.001
2 weeks_12 weeks	5.89	<0.001	0.99	0.03	0.36	0.001
4 weeks_12 weeks	2.42	<0.001	-2.40	<0.001	-0.45	<0.001

OPU: oocyte pick-up; β-HCG: beta-human chorionic gonadotrophin pregnancy test; HOMA-IR: homeostatic model assessment of insulin resistance

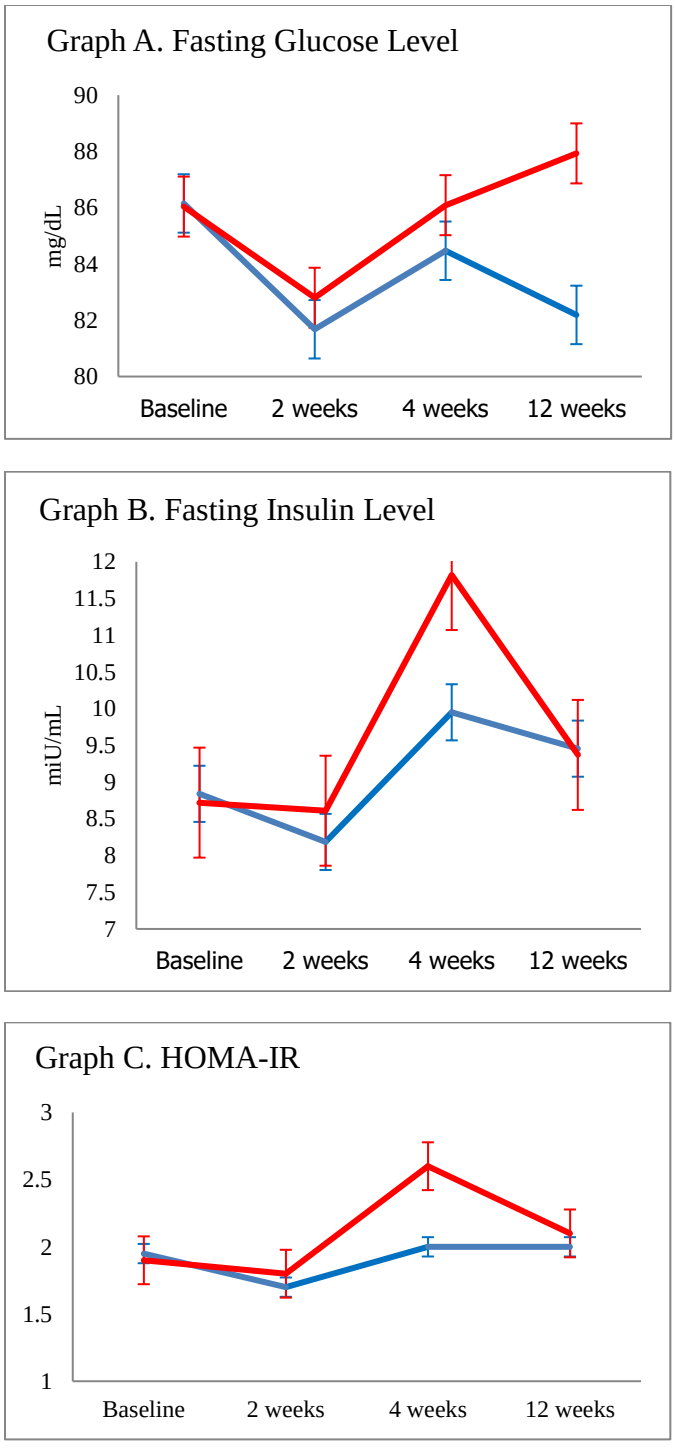
When comparing the pregnant and non-pregnant groups, significant

changes in glucose and insulin homeostasis (including HOMA-IR) occur after 2 weeks of IVF therapy (Table 13). At 4 weeks, the increment in glucose level was significantly higher in non-pregnant women (4%) compared to the pregnant group (3%) (p=0.01). Fasting glucose was also different between the two groups at 12 weeks but changed in divergent directions depending on IVF outcome (pregnant or non-pregnant) (p<0.001) (Graph 2A). Insulin levels only differ between the two groups at 4 weeks of IVF hormonal therapy, with a much higher rise in insulin concentration for non-pregnant women (37% vs. 21%, p=0.01) and reverts back to baseline levels for both groups at 12 weeks (Graph 2B). Similarly, HOMA-IR only varied at 4 weeks between the two groups, with 44% increment in non-pregnancy and 18% in pregnancy (p=0.01). Overall change in glucose homeostasis followed similar pattern throughout the IVF stages for the two groups of pregnant vs. non-pregnant up until 4 weeks and the magnitude of change was different (Graph 2C). Therefore, significant differences in glucose and insulin homeostasis between the pregnant and non-pregnant groups occured after week 2 of IVF hormonal therapy, were observed at week 4 and depended on IVF-pregnancy outcome (positive vs negative pregnancy).

Table 13: Trend of Glucose and Insulin Homeostasis at Each Stage of IVF Therapy Between Pregnant (n=158) and Non-pregnant Women (n=117)

Parameters	Baseline			2 weeks (OPU procedure)			4 weeks (β-HCG test)			Final (12 weeks)		
	Pregnant	Non-pregnant	*p value*	Pregnant	Non-pregnant	*p value*	Pregnant	Non-pregnant	*p value*	Pregnant	Non-pregnant	*p value*
Glucose (mg/dL)	86.15	86.04	0.73	81.68	82.8	0.42	84.47	86.09	0.01	82.19	87.62	<0.001
Insulin (µIU/mL)	8.84	8.72	0.93	8.19	8.61	0.62	9.95	11.82	0.01	9.46	9.31	0.86
HOMA-IR	1.95	1.85	0.99	1.7	1.8	0.58	2.0	2.6	0.01	2.0	2.1	0.17

$p<0.05$, by independent test; values are medians; OPU: oocyte pick-up; HOMA-IR: homeostatic model assessment of insulin resistance; βHCG: beta-human chorionic gonadotrophin pregnancy test



Graph 2. Changes in Glucose Homeostasis Throughout IVF Therapy between Pregnant and Non-pregnant Women (Graph A. Fasting Glucose Level; B. Fasting Insulin Level; C. HOMA-IR); 2 weeks (OPU: oocyte pick-up); 4 weeks (β-HCG pregnancy test); 12 weeks (Final) HOMA-IR: homeostatic model assessment of insulin resistance.

5.2.2. Anthropometrics, Metabolic and Endocrine Parameters Correlations

5.2.2.1. Pregnant Women

Correlations of the different parameters levels at 12 weeks are presented in Table 14A and B; oestrogen and progesterone levels were measured at 4 weeks and only FSH/LH ratio refers to baseline value.

For the pregnant group (Table 14A), age showed a positive weak but highly significant correlation with ratio FSH/LH (r=0.29, *p*=0.01) and T-Chol (r=0.24, *p*=0.01), but negatively correlated with insulin level at 12 weeks (r=-0.17, *p*=0.05). At 12 weeks, BMI positively corrrelated with levels of plasma glucose, serum insulin, and HOMA-IR (r=0.22, r=0.48 and r=0.49 respectively, with *p*=0.01). BMI correlated less strongly with FSH/LH ratio (at baseline) and TG (respectively, r=0.20 and r=0.19, *p*=0.05). Body weight positively correlated with glucose, insulin and HOMA-IR (respectively, r=0.23, r=0.44 and r=0.46, *p*=0.01), as well as TG (r=0.18, *p*=0.05), and HbA1c (r=0.16, *p*=0.05). Insulin and HOMA-IR positively correlated with HbA1c (r=0.25 and r=0.26, *p*=0.01), and TG level (r=0.36 and r=0.35, *p*=0.05) but negatively associated with HDL-C (respectively, r=-0.28 and r=-0.21, *p*=0.05). There was a positive correlation between FSH/LH ratio and T-Chol (r=-0.17, *p*=0.05), and with LDL-C (r=-0.19, *p*=0.05). HbA1c inversly and significantly correlated with T-Chol (r=-0.21, *p*=0.01) and with LDL-C (r=-0.19, *p*=0.05). At 12 weeks, serum TSH correlated positively with body weight (r=0.21, *p*=0.01), BMI (r=0.22, *p*=0.01), insulin (r=0.24, *p*=0.01) and HOMA-IR (0.24, *p*=0.01). Serum TSH also correlated with serum progesterone at 4 weeks (r=0.18, *p*=0.05). Adiponectin level correlated positively with plasma glucose (r=0.32, *p*=0.05) and insulin (r=0.35, *p*=0.05), but no correlation was found between serum adiponectin and age, BMI and lipid paramters. LBP did not correlate with any of the metabolic and endocrine parameters.

Table 14A: Correlations between Anthropometrics and Metabolic Parameters for Pregnant Women (n=158) using Spearman's Correlation Coefficient Test

Variables	Age	Ratio FSH/ LH	Weight _F	BMI_ F	Oesto_ 4week	Prog_ 4week	HbA1c _F	Gluco _F	Insulin _F	HOMA _F	T-Chol_F	TG_F	LDL-C_F	HDL-C_F	TSH _F	Adip_ F
Ratio FSH/LH	0.29**															
Weight_F	0.03	0.15														
BMI F	0.02	0.20*	0.89**													
Estro_4weeks	0.07	0.15	-0.02	0.03												
Prog_ 4weeks	0.06	0.05	-0.06	-0.05	0.56**											
HbA1c_F	0.03	0.02	0.16*	0.13	0.01	0.01										
Gluco_F	0.04	0.10	0.23**	0.22**	-0.07	-0.15	0.18*									
Insulin_F	-0.17*	-0.02	0.44**	0.48**	-0.05	-0.12	0.24**	0.36**								
HOMA_F	-0.15	-0.01	0.46**	0.49**	-0.04	-0.12	0.25**	0.46**	0.99**							
T-Chol_F	0.24**	0.19*	0.15	0.12	-0.03	-0.03	-0.21**	0.08	-0.01	0.01						
TG_F	0.05	-0.03	0.17*	0.19*	-0.06	-0.02	-0.09	0.02	0.36**	0.35**	0.43**					
LDL-C_F	0.15	0.17*	0.10	0.13	-0.03	0.01	-0.19*	0.09	-0.03	-0.01	0.78**	0.32**				
HDL-C_F	0.12	-0.06	-0.03	-0.14	-0.07	-0.14	-0.10	-0.04	-0.23**	-0.21**	0.33**	-0.10	0.07			
TSH_F	0.06	0.09	0.21**	0.22**	0.14	0.18*	-0.02	0.02	0.24**	0.24**	0.05	0.10	0.05	-0.03		
ŦAdip_F	-0.18	-0.04	0.23	0.20	-0.06	-0.11	0.26	0.32*	0.35*	0.02	0.22	-0.08	0.14	-0.02	-0.03	
ŦLBP_F	-0.05	0.16	-0.02	-0.07	-0.04	0.22	-0.04	0.01	-0.01	-0.01	-0.15	-0.10	0.01	-0.05	-0.05	-0.25

**p<0.001, *p<0.05; ŦN=42 pregnant; F: final (12 weeks); FSH: follicle-stimulating hormone; LH: luteinizing hormone; Oestro: oestrogen; Prog: progesterone; Gluco: glucose; HbA1c: glycated haemoglobin; 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; Adip: adiponectin; LBP: lipopolysaccharide binding protein

5.2.2.2. Non-pregnant Women

For the non-pregnant group (Table 14B), there was a weak but significant negative correlation between age and 12-week levels of glucose (r=-0.28, *p*=0.01) and insulin (r=-0.23, *p*=0.05) but positive correlation with LDL-C (r=0.22, *p*=0.05). Age and baseline ratio FSH/LH also correlated positively (r=0.32, *p*=0.05). Similarly to the pregnant group, weight and BMI were independently and significantly correlated with glucose (respectively, r=0.37 and r=0.42, *p*=0.01) and with insulin (respectively, r=0.36 and r=0.42, *p*=0.005); while negatively with LDL-C (r=-0.25, *p*=0.01 and r=-0.25, *p*=0.05). Oestrogen at 4 weeks positively correlated with TSH level at 12 weeks (r=0.28, *p*=0.01), while level of progesterone at 4 weeks correlated with final (12 weeks) HbA1c (r=0.19, *p*=0.05) and insulin level (r=0.27, *p*=0.01). Glucose and insulin positively correlated with T-Chol (respectively, r=0.38 and r=0.36, *p*=0.01) but negatively correlated with LDL-C levels (respectively, r=-0.32 and r=-0.29, *p*=0.01); insulin also negatively correlated with TSH value (r=-0.19, *p*=0.05). HOMA-IR positively associated with TG (r=0.93, *p*=0.01), T-Chol and LDL-C (respectively, r=0.35 and r=0.22, *p*=0.05), and negatively correlated with HDL-C (r=-0.23, *p*=0.05). As for pregnant women, LBP did not correlate with any of the metabolic and endocrine parameters.

Table 14B: Correlations between Anthropometrics and Metabolic Parameters for Pregnant Women (n=158) using Spearman's Correlation Coefficient Test

Variables	Age	Ratio FSH/ LH	Weight _F	BMI_ F	Oesto_ 4week	Prog_ 4week	HbA1 c_F	Gluco_ F	Insulin _F	HOMA _F	T-Chol_F	TG_F	LDL-C_F	HDL-C_F	TSH _F	Adip_ F
Ratio FSH/LH	0.32**															
Weight_F	0.01	0.06														
BMI F	-0.01	0.07	0.86**													
Estro_4weeks	0.15	0.03	-0.17	-0.21*												
Prog_ 4weeks	0.05	0.03	0.10	0.15	0.08											
HbA1c_F	0.08	-0.07	0.15	0.17	0.05	0.27**										
Gluco_F	-0.27**	-0.07	0.36**	0.42**	-0.17	0.14	0.34**									
Insulin_F	-0.23*	-0.08	0.36**	0.42**	-0.13	0.19*	0.14	0.98**								
HOMA_F	0.13	0.03	-0.03	0.07	-0.11	0.04	-0.02	0.03	0.02							
T-Chol_F	0.02	-0.02	0.06	0.15	-0.08	0.14	0.10	0.38**	0.36**	0.34**						
TG_F	0.08	0.01	0.06	0.15	-0.12	-0.01	-0.05	0.09	0.08	0.92**	0.27**					
LDL-C_F	0.22*	0.14	-0.25**	-0.23*	-0.01	0.13	0.04	-0.32**	-0.29**	0.21*	-0.24**	-0.06				
HDL-C_F	0.07	-0.12	0.01	0.02	-0.01	-0.03	-0.02	0.05	0.04	-0.22*	0.01	-0.19*	-0.19*			
TSH_F	0.04	0.03	-0.07	-0.09	0.27**	0.12	-0.09	-0.18	-0.19*	0.10	0.17	0.06	-0.03	0.01		
₸Adip_F	0.14	0.22	0.29	0.13	-0.03	-0.2	-0.01	0.03	0.01	-0.05	-0.21	-0.03	0.29	-0.03	-0.3	
₸LBP_F	-0.22	-0.12	-0.01	-0.12	-0.08	-0.05	0.05	0.27	0.25	-0.14	-0.18	-0.10	-0.20	0.01	0.08	0.17

**$p<0.001$, *$p<0.05$; ₸N=42 pregnant; F: final (12 weeks); FSH: follicle-stimulating hormone; LH: luteinizing hormone; Oestro: oestrogen; Prog: progesterone; Gluco: glucose; HbA1c: glycated haemoglobin; 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; Adip: adiponectin; LBP: lipopolysaccharide binding protein

5.2.2.3. Correlation of Change in Glucose Level and in Other Parameters

The association of change in glucose level from baseline to 12 weeks and compared to the other parameters (delta levels) for both groups is presented in Table 15. In the pregnant group, the only two delta parameters values which were significantly associated with the change in glucose were HOMA-IR and isnulin. There was an inverse relationship between change in glucose level (12 weeks vs. baseline) and change in insulin level (B=-4.83; 95% CI=[-5.43,-4.24]; $p<0.001$); there was however a positive relationship between change in glucose level (12 weeks vs. baseline) and change in HOMA-IR (B=8.99; 95% CI=[5.32,12.67]; $p<0.001$). Similar associations were observed in the non-pregnant group, with a negative relationship between change in glucose and insulin levels (B=-5.59; 95% CI=[-6.52,-4.67]; $p<0.001$) between measures at baseline and 12 weeks, and a positive relationship between change in glucose and HOMA-IR for the same time-points (B=15.32; 95% CI=[6.34,24.29]; $p<0.001$). In addition, in the non-pregnant group, change in TSH between baseline and 12-week time-points negatively associated with change in glucose level. Overall, change in HOMA-IR level was shown to be the best predictor of change in glucose level at 12 weeks regardless of the pregnancy status of participants.

Table 15: Difference in Glucose Level at Baseline vs. 12 Weeks and Compared to Changes in Other Anthropometrics, Metabolic and Endocrine Parameters for Pregnant and Non-pregnant Women using Linear Regression Analysis

Variables	Pregnant (n=158)			Non-Pregnant (n=117)		
	B	**95% CI**	***p* value**	**B**	**95% CI**	***p* value**
Weight	-0.09	-0.34, 0.15	0.45	-0.19	-0.71, 0.33	0.47
BMI	0.01	-0.70, 0.71	0.99	0.15	-0.67, 0.96	0.41
Oestrogen_4 weeks	0.001	0.0001, 0.001	0.25	0.001	-0.001, 0.003	0.28
Progesterone_4 weeks	-0.03	-0.07, 0.01	0.20	-0.002	-0.01, 0.004	0.53
HbA1c	0.76	-1.17, 2.69	0.44	0.61	-1.77, 2.98	0.61
Insulin	-4.83	-5.43, -4.24	<0.001	-5.59	-6.52, -4.67	<0.001
HOMA-IR	8.99	5.32, 12.67	<0.001	15.32	6.34, 24.29	<0.001
T-Chol	-0.002	-0.03, 0.29	0.92	-0.04	-0.11, 0.03	0.29
TG	0.001	-0.01, 0.01	0.84	0.003	-0.02, 0.02	0.79
LDL-C	-0.02	-0.06, 0.02	0.26	0.02	-0.06, 0.09	0.64
HDL-C	0.03	-0.03, 0.09	0.30	0.001	-0.10, 0.10	0.99
TSH	0.09	-0.77, 0.95	0.84	-0.98	-1.98, 0.01	0.05
ŦAdipo	0.002	-0.01, 0.01	0.69	-0.003	-0.01, 0.002	0.18
ŦLBP	0.004	-0.02, 0.02	0.73	0.02	-0.02, 0.05	0.38

Ŧn=73(42 pregnant, 31 non-pregnant); HbA1c: glycated haemoglobin; 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; Adipo: adiponectin; LBP: lipopolysaccharide binding protein

Section 3: Results and Discussion

Chapter 5B: Results

5.3. Early Predictors of Gestational Diabetes Mellitus

5.3.1. Participants Characteristics

The prevalence of GDM accounted for 22% in the pregnant group (n=158). In relation to the known anthropometric and medical predictors of GDM (including obesity, ethnicity, age, presence of PCOS and history of GDM), counting from the entire pregnant group: 23% had a BMI ≥30 kg/m^2, 46% with PCOS, 5% with HbA1c ranging between 5.7–6.1% (39–42 mmol/mol) and 15% with a past history of GDM. Ethnicity of participants was multicultural: 53% Gulf nationals, 20% from Far East (South and East Asia), 15% Middle Eastern, 8% Europeans and 4% with African origins. Anthropometrics, metabolic and endocrine parameters of future GDM and non-GDM women are shown in Table 16; data are summarised as median (interquartile range). Compared to non-GDM pregnant women, at baseline participants 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 significant higher baseline levels of the following parameters: ratio FSH/LH by 17% (1.20 vs. 1.0), HbA1c by 5% (5.50 vs 5.20% or the equivalent to 37 vs. 33 mmol/mol), insulin by 33% (10.60 vs. 7.14 µIU/mL) and HOMA-IR by 23% (2.20 vs. 1.70); with p<0.05 for all parameters. Women who later developed GDM also had higher baseline T-Chol (199.0 vs. 171.0 mg/dL or the equivalent to 5.17 vs. 4.42 mmol/L; p=0.002) and LDL-C (123.0 vs. 104.8 mg/dL or the equivalent to 3.18 vs. 2.71 mmol/L; p=0.003), and lower TG levels (74.0 vs. 76.0 mg/dL or the equivalent to 1.91 vs. 2.0 mmol/L; p=0.005) compared to non-GDM women. At 4 weeks, pregnant women who later developed GDM had a significantly lower 4-week glucose level compared to non-future GDM (83.5 vs. 85.30 mg/dL or the equivalent to 4.6 vs. 4.7 mmol/L; p=0.004), but significantly higher 4-week insulin (11.94 vs. 9.73 µIU/mL; p=0.02) and HOMA-IR values (2.40 vs. 2.10; p=0.01).

At 12 weeks, compared to baseline, women with future GDM experienced significant body weight gain (delta change: 3.4kg, p=0.003), and reduction in fasting plasma glucose (88.38 to 80.0 mg/dL or the equivalent to 4.9 to 4.4 mmol/L; p=0.007) and in TSH levels (1.33 to 1.08 µIU/mL; p=0.05). In relation to 12-week lipid parameters in the non-future GDM group, there was a significant increase in

TG (74.0 to 177.9 mg/dL, or the equivalent to 0.84 to 2.01 mmol/L), T-Chol (199.0 to 211.4 mg/dL, or the equivalent to 5.15 to 5.47 mmol/L) and HDL-C (55.0 to 65.0 mg/dL, or the equivalent to 1.42 to 1.68 mmol/L) levels compared to baseline, with all $p<0.001$. Comparable figures were found in non-GDM women at 12 weeks with significant increase in body weight (delta change: 1.5kg; $p<0.001$), reduction in fasting plasma glucose (85.3 to 81.0 mg/dL or the equivalent to 4.7 to 4.5 mmol/L; $p<0.001$), and in TSH levels (1.58 to 1.39 μIU/mL; $p<0.001$). Lipid profile was significantly increased at 12 weeks: TG (76.0 to 120.0 mg/dL or the equivalent to 2.0 to 3.1 mmol/L), T-Chol (171.0 to 198.4 mg/dL or the equivalent to 4.42 to 5.13 mmol/L) and HDL-C (57.0 to 63.0 mg/dL or the equivalent to 1.7 to 1.63 mmol/L), with all $p<0.001$. There were no significant changes in insulin, HOMA-IR, adiponectin and LBP levels at 12 weeks for both subsequent GDM and non-GDM groups compared to their baseline levels.

In addition to the 14% higher preconception weight (75.9 vs. 65.0 kg; $p=0.01$), at 12 weeks, GDM women presented with greater weight gain (delta change: 3.4 vs. 1.5 kg), and higher levels of: insulin (11.33 vs. 7.57 μIU/mL; $p=0.02$) by 33%, TG (177.9 vs. 120.0 mg/dL or the equivalent to 2.01 vs. 1.35; $p=0.003$) by 33% and HOMA-IR (2.30 vs. 1.50; $p=0.01$) by 35% compared to non-GDM group. The significant decrease in glucose and HbA1c levels have similarly occurred in the two groups (non-GDM and future GDM pregnant women) at 12 weeks; values remain within normal range. Additionally, adiponectin and LBP levels did not differ between the two groups at 12 weeks. Amongst the parameters measured, the greatest difference between the two groups of pregnant women was a higher serum insulin level at baseline and at 12 weeks in pregnant women who later developed GDM, compared with the non-GDM group.

Table 16: Comparison of Anthropometrics, Metabolic and Endocrine Parameters at Baseline, 4 and 12 Weeks of IVF Therapy for Pregnant Women with and without Gestational Diabetes Mellitus (GDM)

Variables	Baseline		*p* value	4 Weeks		*p* value	12 Weeks		*p* value
	GDM (n=34)	Non-GDM (n=124)		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)	65.5 (16.30)	0.01				75.9 (15.0)	65.0 (15.0)	**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						
Estrogen (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	83.50 (9.80)	85.30 (8.45)	0.004	80.00 (8.4)	81.0 (8.44)	0.16
Fasting insulin (µIU/mL)	10.60 (10.53)	7.14 (6.86)	0.01	11.94 (9.18)	9.73 (6.29)	0.02	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	2.40 (2.50)	2.10 (1.40)	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; IQR= Q3-Q1); Ŧn= 42 pregnant; $p<0.05$ 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

5.3.2. Anthropometrics, Metabolic and Endocrine Correlations and Predictors of GDM

Preconception and prenatal BMI (12-week) positively correlated with development of GDM (p=0.001) (Table 17). Interestingly, ethnicity, history of PCOS and of GDM did not associate with development of GDM (respectively, p=0.15, p=0.33 and p=0.88). Additionally, there was no relationship between number and gender of babies and GDM risk (p=0.80 and p=0.82).

Table 17: Association of Women Characteristics and Pregnancy Outcomes with the Development of Gestational Diabetes Mellitus (GDM)

Variables		**GDM** (n=34)	**Non-GDM** (n=124)	***p* value**
		% (n)	% (n)	
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)	0.15*
	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)	
Number of Babies	Single	67.6 (23)	65.3 (81)	0.80**
	Twin	32.4 (11)	34.7 (43)	
Gender of Baby	Male	41.2 (14)	45.2 (56)	0.82**
	Female	38.2 (13)	38.7 (48)	
	Mix	20.6 (7)	16.1 (20)	
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)	

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

The anthropometric, metabolic and endocrine predictors of GDM are summarised in Table 18. Regression analyses revealed baseline FSH/LH ratio as a predictor of GDM (OR=2.05; 95% CI=[1.12,3.75]; p=0.02). Other predictors of GDM at 12 weeks include: 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), age (OR=1.12; 95% CI=[1.01,1.23]; p=0.03) and insulin (OR=1.11; 95% CI=[1.03,1.18]; p=0.004). One unit increase in the ratio FSH/LH doubles the risk of development of GDM. Although preconception BMI associated with onset of GDM (OR=1.01; 95% CI=[0.73,1.39]; p=0.001), other baseline metabolic parameters (including lipid profile, glucose, HbA1c, LBP and adiponectin) did not associate with onset of GDM. After adjustment for maternal age and PCOS history, prenatal BMI (12-weeks) was the only significant predictor of GDM (OR=1.11; 95% CI=[0.98,1.20]; p=0.03). Ratio FSH/LH was no longer a significant predictor of GDM, but approaching significance (p=0.08). Ethnicity and history of GDM were not adjusted for this analysis, given that they were shown to have a non-signifcant impact on GDM predisposition (Table 17).

Table 18: Anthropometric and Metabolic Predictors of Gestational Diabetes Mellitus (as dependent variable) in Pregnant Women (n=158), adjusted for Age and PCOS using Binary Logistic Regression

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	0.68	0.31, 1.48	0.33			
Ratio FSH/LH	2.05	1.12, 3.75	0.02	1.61	0.94, 2.78	0.08
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_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			
Oestrogen_4weeks	1.00	1.00, 1.00	0.39			
Progesterone_4weeks	0.99	0.97, 1.01	0.34			
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; OR: odds ratio; C.I.: confidence interval

The validity of documented cut-off levels of anthropometrics, metabolic and endocrine predictors of GDM is presented in Table 19. Prenatal BMI ≥35 kg/m^2 presented the highest impact factor on GDM with a six-fold (95% CI=[1.7,24.32]; *p*=0.002) increase in risk. Other predictors of GDM included 12-week levels of HbA1c ≥5.7% (39 mmol/mol) (OR=4.05; 95% CI=[0.95,16.93]; *p*=0.04), TG ≥137 mg/dL (≥1.55 mmol/L) (OR=2.79; 95% CI=[1.28,6.07]; *p*=0.01), and Arab ethnicity (OR=0.33; 95% CI=[0.18,0.90]; *p*=0.03). There is a trend toward

statistical significance in relation to 12-week TSH ≥2.5 µIU/mL and increased predisposition to GDM (OR=2.42; 95% CI=[0.92,6.37]; *p*=0.07). Age ≥35 years, HDL-C ≤85.5 mg/dL (≤2.20 mmol/L), log TG/HDL ≥0.099 and adiponectin ≤6.4 µg/mL did not predict onset of GDM.

Table 19: Levels of Anthropometrics, Metabolic and Endocrine Predictors of Gestational Diabetes Mellitus (GDM) at 12 Weeks using Evidence-based Cut-off Levels for High Risk

Parameters		GDM (n=34)	Non-GDM (n=124)	OR	95% CI	*p* value
		% (n)	% (n)			
Age (years)	<35 ≥35	67.6 (23) 32.4 (11)	75.8 (94) 24.2 (30)	1.50	0.66, 3.43	0.34
BMI (kg/m^2)	<30 ≥30	52.9 (18) 47.1 (16)	83.1 (103) 16.9 (21)	4.37	1.92, 9.91	<0.001
	<35 ≥35	82.4 (28) 17.6 (6)	96.8 (120) 3.2 (4)	6.43	1.7, 24.32	0.002
HbA1c (%)	<5.7 ≥5.7	88.2 (30) 11.8 (4)	96.8 (120) 3.2 (4)	4.05	0.95, 16.93	0.04
Ethnicity	Arab Non-Arab	85.3 (29) 14.7 (5)	65.3 (81) 34.7 (43)	0.33	0.18, 0.90	0.025
TSH (µIU/mL)	<2.5 ≥2.5	76.5 (26) 23.5 (8)	88.7 (110) 11.3 (14)	2.42	0.92, 6.37	0.07
TG (mg/dL)	<137 ≥137	41.2 (14) 58.8 (20)	66.1 (82) 33.9 (42)	2.79	1.28, 6.07	0.008
HDL-C (mg/dL)	<85.5 ≥85.5	88.2 (30) 11.8 (4)	92.7 (115) 7.3 (9)	0.59	0.17, 2.04	0.40
Log TG/HDL	<0.099 ≥0.099	20.6 (7) 79.4 (27)	16.1 (20) 83.9 (104)	0.74	0.28, 1.94	0.54
ŦAdiponectin (µg/mL)	<6.4 ≥6.4	9.1 (1) 90.9 (10)	9.7 (3) 90.3 (28)	1.07	0.10, 11.53	0.96

Ŧn=75; *p*<0.05 vs. GDM, by Chi-square test; OR: odds ratio; C.I.: confidence interval; HbA1c: glycated haemoglobin A1c; TSH: thyroid-stimulating hormones; TG: triglycerides; HDL-C: high-density lipoprotein cholesterol

Section 3: Results and Discussion

Chapter 5C: Results

5.4. Pregnancy and Foetal Outcomes

Delivery by caesarean section accounted for 65% in the pregnant group (n=158). Two participants were excluded from the final pregnant data (delivery point), since they experienced a miscarriage around mid-gestation. The present data account for n=156. In singleton, birth weight was 2.8 (0.58) kg; and in multiple pregnancy, 2.3 (0.51) kg for first baby and 2.2 (0.56) kg for the second.

Multiple pregnancy (i.e twin pregnancies) positively correlated with delivery by caesarean section (p=0.002) (Table 20). Interestingly, presence of GDM did not associate with caesarean section (p=0.41).

Table 20: Association of Pregnancy and Foetal Outcomes with Delivery by Caesarean Section

Variables		**Natural** (n=53)	**Caesarean** (n=103)	***p* value**
		% (n)	% (n)	
GDM	Yes	26.4 (14)	19.4 (20)	0.41
	No	73.6 (39)	80.6 (83)	
Number of Babies	Single	83.0 (44)	58.3 (60)	0.002
	Twin	17.0 (9)	41.7 (43)	

p<0.05 vs. caesarean section, by Chi-square test; GDM: gestational diabetes mellitus

The assessment of maternal and foetal outcomes prediction of caesarean section is summarised in Table 21. Regression analyses revealed that maternal age, preconception BMI and BMI at 12 weeks of women did not predict delivering by caesarean section. In regards to foetal outcome, in singleton pregnancy, the weight of the baby did not predict a delivery by caesarean section but in multiple pregnancy only the weight of the first baby did (OR=0.03; 95% CI=[0.001,0.83]; p=0.038). There is a trend toward statistical significance in relation to weight of the second baby and its possibility in predicting a caesarean section (OR=19.96; 95% CI=[0.98,405.5]; p=0.051). When adjusting for 12-week BMI of pregnant women, the weight of first baby in twin pregnancies remained a significant predictor of delivery by caesarean section (OR=0.03; 95% CI=[0.001,0.91]; p=0.04).

Table 21: Maternal and Foetal Characteristics Predicting Delivery by Caesarean Section (as dependent variable) in Pregnant Women (n=158), adjusted for 12-week BMI using Binary Logistic Regression

Variables	Unajusted Analysis			Adjusted Analysis		
	OR	**95% CI**	***p value***	**OR**	**95% CI**	***p value***
Age	1.03	0.95, 1.11	0.49			
BMI_Baseline	1.06	0.98, 1.14	0.16			
BMI_12 weeks	1.06	0.99, 1.14	0.10			
Weight_Singelton	1.13	0.58, 2.21	0.73			
Weight_Twin1	0.03	0.001, 0.83	0.038	0.03	0.001, 0.91	0.044
Weight_Twin2	19.96	0.98, 405.5	0.051	17.48	0.80, 381.9	0.07

OR: odds ratio; C.I.: confidence interval

Section 3: Results and Discussion

Chapter 6A: Discussion

6.1. IVF-related Maternal Characteristics and Outcomes

Overall, the age of participants was within the optimal range for reproductive age (<35 years) and also favourable for IVF and pregnancy outcomes[6–8]. Advanced age, known as one of the important factors of female infertility, did not affect IVF pregnancy rate. A subgroup of participants was older, partly explained perhaps that they were seeking IVF therapy for gender selection. Despite the high rate of obesity and PCOS in the UAE[27], percentages of participants with a preconception (or baseline) BMI in the obese range and/or history of PCOS were not as high as expected. The difference may be explained by the ethnic diversity in the UAE[205] and hence of participants in this study. Overall, participants' preconception BMI was within the overweight range (BMI 25–29 kg/m^2) and remained so when measured at 12 weeks. Even though weight gain was statistically significant during the first trimester of pregnancy, it did not exceed the recommended weight-gain of 0.5–2.0kg[251]. At baseline, 17% of women had pre-diabetes (HbA1c 5.7–6.4% or the equivalent to 39–47 mmol/mol)[199], 4% presented with high insulin level (>24 µIU/mL)[252] and 31% were insulin resistant based on HOMA-IR >2.5[218]. Lipid profile and TSH levels were within normal range at preconception stage[179,180,253].

6.2. IVF-related Foetal Outcomes

Controversies have not yet been resolved as to whether adverse foetal and neonatal outcomes are more prevalent with IVF-conceived pregnancy[56,59,71,72], due to a lack of relevant published data after the first trimester; especially that in some cases pregnant women may be administered exogenous hormones (mainly progesterone) until late gestation. Longitudinal prospective studies on IVF-conceived children will be helpful to assess their development and predisposition to obesity, T2DM and cardiovascular diseases.

6.3. Effects of IVF Therapy on Metabolic, Inflammation and Endocrine Systems

6.3.1. Metabolic Profile

IVF hormonal therapy raised glucose and insulin levels, and reduced insulin sensitivity (increased HOMA-IR), evidenced in failed IVF cycle at 4 and 12 weeks measurements. These metabolic excursions likely reflect changes in serum progesterone levels. In compliance with the theory, a negative correlation was shown between change in glucose level and that of insulin between baseline and 12-weeks, and positive correlation between plasma glucose and HOMA-IR. In addition, change in HOMA-IR level predicted best the change in plasma glucose level between baseline and 12 weeks regardless of pregnancy status. A similar relationship between insulin sensitivity and glucose intolerance was reported with long-term use of oral contraceptives[99–101]. Oestrogen therapy at a dose of 1.25 mg/day for a three year period was previously suggested to be associated with a 25% decrease in insulin sensitivity[102]. In this study, a reduction of 10% in insulin sensitivity (based on HOMA-IR) was found with 6 mg/day of exogenous oestrogen. It is possible that longer duration of hormonal therapies may have a greater impact on glucose and insulin homeostasis than a high hormonal dose for a shorter duration.

During early gestation, dogma states that glucose homeostasis (including glucose and insulin levels, and insulin sensitivity) remains similar to that of non-pregnant women[79–81]. However, some studies have shown a drop in plasma glucose level during early pregnancy[254] and/or 20% increase in insulin synthesis to maintain euglycaemic levels[81,255]. The effect of IVF hormones on glucose homeostasis was down-regulated by pregnancy, whereby, no change in insulin level and sensitivity (HOMA-IR) were observed, while glucose level was reduced (still remained within normal range). The drop in plasma glucose during pregnancy has different explanations. In response to the increased foetoplacental energy requirements in early pregnancy, a physiological adaptation triggers focus on carbohydrates (i.e. glucose) instead of lipids as a source of energy[87,88,256]. The drop in glucose level in early pregnancy may also be partially related to a dilutional effect as maternal blood

volume increases[254]. Later in pregnancy, increased glucose level is related to impairment in glucose tolerance, which is in accordance with excessive increase in insulin level and reduced insulin sensitivity[84,257]. Controversies exist in relation to the change of HbA1c in pregnancy, with most studies reporting a decrease in the first trimester[258], concordant with the findings from this IVF study. The diabetogenic state from increased insulin resistance and hyperinsulinemia usually manifests during mid-pregnancy[79–81]. Accordingly, such changes did not occur by 12 weeks of gestation. The findings from this study suggest that IVF-related hormonal therapy does not hasten the diabetogenic effect of pregnancy during the first trimester. However, when planning repeated cycles of IVF therapy (e.g. egg banking cycles and post-failed IVF cycles), it is important to monitor plasma glucose and serum insulin levels.

6.3.1.1. Glucose and Insulin Homeostasis

At week 2 of IVF therapy, the effect of exogenous FSH and LH on glucose and insulin were assessed compared to baseline levels; week 2 to 4 represented the effect of oestrogen and progesterone, and additional pregnancy effect after week 4. The significant decrease in glucose level at week 2 in both pregnant and non-pregnant groups may have different possible explanations: 1) impact of exogenous FSH and LH hormones, 2) longer than 10 hours fast of participants, 3) participants were instructed by the IVF educator at the clinic to reduce carbohydrates intake (as proven to be beneficial for the treatment) and they ended up eating less the night before the procedure. The role of FSH on glucose metabolism remains incompletely understood. A study by Wang et al. reported lower FSH levels in prediabetes and diabetes in post-menopausal women compared to controls, associated with adiposity and insulin resistance[199,259]. This promotes the notion of FSH as a novel biomarker of GDM risk. The association between serum FSH and GDM lacks verification in this IVF study due to adjustments of the doses of IVF hormones (FSH and LH) according to baseline levels. Additionally, FSH level was not measured at 2 weeks to verify if there was any correlation between FSH level and that of glucose, insulin and HOMA-IR. However, FSH and LH-related IVF

hormones did not seem to have affected insulin level and insulin sensitivity. More studies are required to clarify the effect of FSH and LH hormones on glucose homeostasis. GnRH hormone (administered a few days before OPU procedure) did not influence glucose or insulin levels[260], this may have been better confirmed if glucose and insulin levels were also measured just before the GnRH injection was initiated.

At week 2 of IVF therapy, exogenous oestrogen and progesterone were initiated. Measurement at week 4 marks not only the difference in pregnancy status but also distinguishes pattern of change in glucose and insulin levels between the two groups. Compared to week 2, exogenous reproductive hormones (oestrogen and progesterone) increased glucose and insulin levels, and insulin resistance (HOMA-IR) in both pregnant and non-pregnant groups, with a much greater increase in insulin and insulin resistance in the non-pregnant women at 4 weeks. The lower increments observed in the pregnant group may be due to pregnancy effect. The effect of IVF hormones on raising glucose level at 12 weeks was down-regulated by pregnancy, while insulin level and sensitivity were fully reverted to baseline levels. The increased in glucose level post-failed IVF (at 12 weeks) may have different possible explanations. As mentioned earlier, non-pregnant women presented with a significant weight gain (and possibly of adiposity) at 12 weeks, which may have affected glucose level. In addition, post-failed IVF may elucidate poor mental well-being, with a risk of depression and anxiety[261–263]. Mental distress is associated with elevated plasma cortisol level and in which is thought to affect glucose metabolism[264,265]. Mental stress of participants was not measured and hence this remains a speculative explanation.

6.3.2. Inflammation and Gut Microflora

6.3.2.1. Gut Microflora

Female reproductive hormones (oestrogen and progesterone) stimulate synthesis of inflammatory markers[110]. A similar response is expected to occur with IVF hormonal therapy. Gut dysbiosis has been previously linked to insufficient or overload of female hormones (oestrogen and progesterone), in which the latter are

thought to affect LPS signalling and may trigger an inflammatory response[149,156]. Interestingly, the exposure to IVF hormonal therapy did not provoke any change in LBP level at 12 weeks for both groups of women. Consequently, it can be speculated that no related impairment in LPS level or in gut microflora occurred. One possible explanation of unchanging LBP levels relates to diet, an important modulator of intestinal microbiota diversity and richness[132]. Women undergoing IVF therapy might have been more conscious in their food choices by avoiding high fat/energy dense diets. Unlike a high fibre diet, high fat/calories diet may induce micro-inflammation and increased endotoxin to appear in the circulation, resulting from changes in gut permeability and microflora diversity[114,117]. Physiological and hormonal changes during pregnancy also mediate an inflammatory response[109]. In the presented IVF study, LBP level did not correlate with any endocrine and metabolic parameters, indicating that IVF-conceived pregnancy did not hasten the inflammatory-related effect of pregnancy.

Gut microflora composition has been linked to preconception BMI and gestational weight gain in pregnancy[266], but no such link was detected at 12 weeks of pregnancy. In addition, obesity-related gut microflora disturbances may result from gestational inflammation, increased body fat and decreased insulin sensitivity during pregnancy[152]. This physiological change was not observed in this IVF study (with unchanged HOMA-IR level), possibly because parameters were tested too early in pregnancy to see any effect. Therefore, in early gestation, gut microflora remains intact and uninfluenced by hormonal changes of glucose and lipids homeostasis, and thyroid profile.

6.3.2.2. Adiponectin Level

Adiponectin, a useful marker of inflammation and insulin sensitivity, is thought to gradually decrease during pregnancy, secondary to hormonal fluctuation or in response to stress[113,116]. Low maternal adiponectin level during early pregnancy predicted an increased risk of GDM[113]. At 12 weeks of IVF-conceived pregnancy, adiponectin level was identical to baseline and to levels in the non-pregnant group. Surprisingly, adiponectin level did not correlate with insulin or HOMA-IR. As previously mentioned, adiponectin has a glucose lowering effect;

however in this IVF study a positive correlation was found between adiponectin and glucose levels at 12 weeks and both parameters can be reduced in early pregnancy. In relation to adiponectin anti-inflammatory properties and lipid metabolism[113,228], lipid parameters were increased at 12 weeks (TG, T-Chol, and HDL-C) regardless of pregnancy status, but likely not adiponectin related since no positive correlation was detected between adiponectin or any of the lipid components. Adiponectin impairs LPS activation of the inflammatory cascade and insulin resistance[156]. However, there was no correlation between LBP and adiponectin, which may have also expectedly resulted in no association with LPS in this presented study. Regardless of pregnancy, exposure to IVF hormonal therapy did not mediate an inflammatory response, consistent with unchanging adiponectin levels. Furthermore, IVF-conceived pregnancy within the first trimester does not seem to be more predisposed to an inflammatory environment compared to a spontaneous pregnancy.

6.3.2.3. Lipid Profile

The interplay between inflammation and lipid metabolism is well documented, and the two play an important role in the pathophysiology of metabolic conditions, such as insulin resistance. T-Chol and lipoprotein may trigger the inflammatory system, and inter-relatedly, pro-inflammatory cytokines may impair lipid metabolism[267]. Insulin resistance and hyperinsulinemia are often featured with obesity and/or PCOS and are associated with hyperlipidemia[140]. Despite the high prevalence of PCOS and obesity in the participants of the presented IVF study, lipid profile was normal at baseline and remained so at 12 weeks. However, there was an excursion of lipid components between baseline and 12 weeks. Deterioration in insulin sensitivity following IVF hormones positively associated with changes in TG, T-Chol and LDL-C levels, but negatively with HDL-C. Similarly to oral contraceptives[101,141–143], IVF hormones augmented lipid parameters, but values remained within normal range possibly due to the short duration of the treatment.

Metabolic and hormonal changes of pregnancy impose changes on lipid homeostasis and lipoprotein levels; reference guidelines of lipid parameters are not

yet conclusive. Lipid metabolism plays a role in pregnancy and ensures sufficient nutrients for the foetus. While TG serves as energy storage, T-Chol is implicated in normal foetal development. During early gestation, an anabolic phase predominates, whereby lipid synthesis and maternal fat stores are increased to prepare for future higher energy needs of both pregnancy and foetus later in pregnancy (characterised as a catabolic phase); this effect is assisted by increased insulin sensitivity[136,256]. Mixed reports exist regarding changes in lipid metabolism during pregnancy. Pregnancy-related hyperlipidemia usually manifests in mid-gestation and onwards with higher TG, T-Chol, LDL-C and HDL-C levels[134]. At 12 weeks of IVF-conceived pregnancy, lipid profile complied with normal changes of pregnancy and similar to previous studies, showed an increase in TG, T-Chol and HDL-C levels[134,135,138,139]. In the present study, increased lipid profile combined with decreased glucose level (described earlier) confirm the suggested notion of enhanced fat accretion and use of fat as a source energy by the mother in early pregnancy, to ensure sufficient glucose supply to the foetus[88,256]. The attributable effect of IVF hormones on lipids in pregnancy cannot be determined because of disparities in duration of IVF hormonal therapy exist between the two groups (4 weeks for non-pregnant vs. until 12 weeks of pregnancy). Furthermore, a cumulative effect would have been identified if lipid parameters exceeded the reference ranges in pregnancy[253]. Finally, inclusion of a well-matched spontaneous-pregnant group would have allowed determining the magnitude of change in lipid profile as an effect of pregnancy alone. Increased gestational hormones, mainly progesterone, are implicated in increased fat storage[136]; however, no correlation was found between progesterone and TG levels.

As previously mentioned, hyperoestrogenemia stimulates hepatic synthesis of lipids[138], and possibly influences changes in TG, T-Chol and HDL-C levels at 12 weeks. In accordance with the effect of oral contraceptives on lipids[88,101,141–143], increased TG level was the principal observed change for both pregnant and non-pregnant women following IVF therapy. Changes in TG level are thought to be oestrogen-dose-related[142]; however, there was no correlation found between TG and level of oestrogen, but probably it would have been seen if the gestational

hormones were measured at 12 weeks. The same reasoning can also be extended to the potential correlation that would have been seen at 12 weeks between oestrogen and T-Chol levels. The increase in TG level may impair insulin sensitivity and vice versa[101]. Hypertriglyceridemia may result from increased body fat and reduced lipolytic activity[134,139], evidenced in the presented IVF study by a positive correlation between weight and TG level at 12 weeks. Reduced lipolysis may occur as a result of impaired insulin sensitivity, with reduced ability of insulin to suppress lipolysis[116]. The significant positive correlation between HOMA-IR (as a marker of insulin resistance) and insulin level, independently with TG level was shown in both groups. However, although hypertriglyceridemia was not observed during the first trimester in participants of this study, there was also a positive correlation between TG level and BMI at 12 weeks of pregnancy, which is expected to be intensified with increased adiposity later in pregnancy. Taken together, it can be speculated that IVF hormonal-related deterioration in insulin sensitivity observed with failed IVF may have reduced lipid oxidation[113]. Therefore, caution should be exercised when planning several consecutive IVF cycles or when extending the duration of the therapy (as in the case of poorly responding women). On another note, the absence of change in LDL-C level with IVF hormones regardless of pregnancy status does not explain the known hormonal association with increased risk of coronary heart diseases with oral contraceptives[19].

6.3.3. Endocrine Profile

Participants had normal TSH level at baseline (0.4–4.0 μIU/mL), which eliminated the possible effect of TSH on impairing IVF and pregnancy outcome[176,177]. The positive correlation between oestrogen and TSH levels (at 12 weeks) was only observed in negative pregnancy, confirming that oestrogen therapy of IVF has an effect on TSH; this effect was masked in clinically confirmed pregnancy[159,164]. In addition, an inverse association was found between the change in glucose level and that of TSH at 12 weeks for non-pregnant women, which endorses the well-known relation between thyroid hormone, insulin secretion and glucose homeostasis[192,249]. However, while IVF hormonal therapy induced increase in glucose and insulin levels, this was not combined with any change in TSH level

(at 12 weeks) with absence of clinical pregnancy. Taken together two possible explanations may be proposed. Firstly, IVF hormones were stopped at 4 weeks and oestrogen therapy has already been cleared out from the body, or that duration of IVF hormones administration was too short to induce changes in TSH level. Conversely, the effect of oestrogen therapy on raising TSH level would have possibly been seen at 4 weeks of treatment, but it was not measured at that time. In pregnancy, TSH level decreased at 12 weeks, and complied with previous investigators reporting 20–50% suppression due to sharp increase in hCG concentrations[174]. Level of TSH complied with the American Thyroid Association recommendation of TSH range of 0.1–2.5 μIU/mL in the first trimester[171]. In early gestation, TSH measurement is not a good indicator for diagnosing thyroid dysfunction, and instead T4 and T3 hormones should be tested for a better assessment of thyroid function; TSH level is more reflective of thyroid status later in gestation (>16 weeks)[171]. There was a positive correlation, albeit weak, between progesterone and TSH levels. This observation is explained by the normal metabolic-related suppression of TSH throughout pregnancy, with the lower TSH level happening in the first trimester[169,170], and expected to increase with increased progesterone level. Prenatal weight and BMI were also found to be positively correlated with TSH level, suggesting that abnormal maternal weight gain may predispose the most to the change in thyroid level during pregnancy, and emphasises the well-known correlation between thyroid function and obesity[268]. Correspondingly, an association between thyroid hormones and adiposity-related cytokines (e.g. adiponectin) was proposed in the literature, but findings remain inconclusive[268]. Additionally, insulin and TSH levels were positively correlated at 12 weeks of pregnancy, and this is explained by the interrelation between the two parameters, whereby, both hypo- and hyperthyroidism impair insulin sensitivity. Conversely, hyperinsulinemia may block the conversion of T4 to its active form T3, and therefore impairs thyroid profile[175,176]. Consequently, a longer exposure to IVF therapy during pregnancy would have probably evidenced an increase in insulin level and resulted in such observations. IVF-conceived pregnancy appears protected from IVF hormonal effects on thyroid function in the short-term. More

attention should be geared towards thyroid levels post-failed IVF with repeated cycles, or if IVF-related hormones are provided for a longer gestational period.

Section 3: Results and Discussion

Chapter 6B: Discussion

6.4. Early Predictors of Gestational Diabetes Mellitus

6.4.1. Characteristics of Women with GDM

Unrecognised diabetes during pregnancy is associated with increased adverse outcomes and risks for the mother, foetus and neonate. IVF-conceived pregnancy has previously been considered a 'high-risk' intervention with increased risk for maternal and obstetric complications, such as GDM[6,52,59–62]. The prevalence of GDM in this study was expected to be higher given the "high-risk" intervention and the fact that participants presented with strong GDM predisposing factors: obesity (BMI >30 kg/m^2), advanced age (>35 years), high incidence of PCOS, predisposed ethnicities and/or communities (South and East Asia, Gulf and Middle East) and exaggerated maternal weight gain[55,88,180,189–191,193,201,269]. GDM rate from this study was almost comparable with the latest national statistics on spontaneous pregnancies, whereby one in every three pregnant women in the UAE develops GDM[187,188]. Accordingly, this may reject considering IVF-conceived pregnancy as a powerful risk factor for GDM.

Women who developed GDM were older (still below the high-risk age group, i.e. <35 years) and more overweight (closer to obesity range) compared to those who did not develop GDM. They also presented at baseline higher levels (still within normal reference) of the well-known glucose, insulin and lipids-related markers of GDM[196,200]: HbA1c, insulin, HOMA-IR, T-Chol and TG. In addition, baseline FSH/LH ratio was also significantly higher for this group, which hints the possibility of being an early predictor and/or risk factor of GDM; the mechanism remains unclear. Ratio of FSH/LH measurement during the first trimester should be further investigated in predicting onset of GDM.

At 12 weeks, pregnant women who went on to develop GDM experienced more weight gain (from baseline) compared to non-GDM women, and this exceeded the recommended weight gain of less than 2.0 kg during the first trimester[251]. Initially, plasma glucose of future GDM was not different from non-GDM women levels and remained so at 12 weeks (taking into account the significant similar drop in glucose level in both groups of women from baseline to 12 weeks); it seems that only later in pregnancy that glucose level deteriorates in

GDM. Hence, another important point for early metabolic changes in pregnancy is related to maintaining normoglycaemia in the first trimester, and only later in the second trimester does GDM develop. In relation to screening for early predictors of GDM, neither glucose nor HOMA-IR levels at 12 weeks revealed to be good predictors in this study. Findings from this study also supports that assessment for GDM with OGTT later in the second trimester may be more accurate rather than in the first trimester.

Compared to the other aforementioned predictors of GDM at baseline, TG level was the only one to increase significantly at 12 weeks (and significantly differ from non-GDM 12-week levels), which in turn highlights its importance as a strong risk factor of GDM. Baseline levels of adiponectin and LBP did not predict GDM risk. Furthermore, no change in levels of these parameters occurred at 12 weeks in either group. Insulin level remained higher at 12 weeks in future GDM pregnant women, but the level was not different from baseline. Insulin should hence be considered in assessment of GDM risk in addition to the well-recognised strong predictors including TG level, preconception and prenatal BMI, as well as weight gain during pregnancy.

6.4.2. Anthropometric and Medical Predictors

Numerous studies have emphasised the association between preconception BMI, gestational weight gain and GDM risk[201,270,271]. The presented study has evidenced that increased GDM risk was strongly and equally associated with both pregravid and prenatal obesity. However, when adjusting for age and PCOS history, only BMI at 12 weeks was a significant determinant of GDM risk. Hence, the higher the BMI at the first trimester (i.e. 12 weeks), the greater the risk of GDM in mid-gestation, with four-fold increase for BMI ≥30 kg/m^2 and six-fold for a BMI ≥35 kg/m^2. This finding complies with previous studies' reporting that being in the overweight (BMI: 25–29.5 kg/m^2) or obesity category (BMI >30 kg/m^2) is the most important predisposing factor for GDM[272]. Advanced age, essentially considered a powerful risk to adverse obstetric outcomes in pregnancy, was also effectively an important predictor of GDM in the population of this study; but the threshold of 35

years was not a significant age-predictor level for higher GDM risk. Previous history of GDM and PCOS did not predict the onset of GDM in participants of this study.

The number of pregnant women with a history of GDM was small given that a large proportion of participants referred for primary infertility, and this may have affected the impact of GDM history. In addition, ethnicity was not associated with increased GDM risk when classifying participants into seven groups (Middle Eastern, Gulf, African origins, South Asia, East Asia, Europeans and North Americans). However, when categorizing participants into two groups (Arab vs. non-Arabs), the Arab ethnic group was more predisposed to develop GDM. It is in fact well evidenced that the Arab communities (including the Gulf, Levant and Middle East) are more at risk of GDM[192,249].

Pregnancy outcome and foetus characteristics did not serve as early markers of GDM. Multiple pregnancy has long been considered as a predisposing risk for complications and adverse medical outcomes (such as GDM)[41,53]. There was no detected association between gender and number of babies with GDM risk in IVF-conceived pregnancy. A well-matched group of spontaneously-conceived pregnancy, including those with multiple pregnancy would have helped in the assessment of the impact of IVF-conceived pregnancy on obstetric outcomes.

The well-studied glucose and lipid markers of GDM have poorly served this purpose in this IVF study (i.e. glucose, HbA1c and TG levels). Baseline ratio of FSH/LH was the best predictor of GDM risk followed by levels of the following in decreasing order at 12 weeks: HOMA-IR, prenatal BMI, age and insulin level. However, when adjusting for age and history of PCOS, the FSH/LH ratio no longer showed a significant early predictor of GDM risk, and prenatal BMI was the strongest predictor. This may be caused by the higher prevalence of PCOS, advanced age and/or poor ovarian reserve in the population of this presented study; these conditions affect FSH level. PCOS women have lower FSH level secondary to reproductive hormone imbalance, and not related to any kind of glucose homeostasis disparities[273]. Elevated FSH in older women indicates poor ovarian reserve[41], and as a matter of fact, age was positively correlated with baseline

FSH/LH ratio in participants of this IVF study. Lower FSH level was previously reported in prediabetes and diabetes post-menopausal women[199,259]; however, FSH level at 12 weeks of pregnancy was not measured to assess the possibility of considering FSH as an additional predictor for GDM. Overall, in addition to the known risk factors of GDM, there should be an emphasis on preconception FSH/LH ratio and BMI in clinical practice as early high-risk markers, and close surveillance of early gestational gain of weight should be a focus as a predictor for onset of GDM.

Prevention trials have not yet confirmed optimal lifestyle intervention and macronutrients distribution to prevent the onset of GDM[274–276], and neither guarantee that gestational-related adiposity can be prevented by one specific intervention[277]. Preconception care seems to be an ideal window of opportunity to prepare women who are planning to get pregnant. In line with this, the American Diabetes Association and American College of Obstetrics and Gynaecologists recommend management at the preconception stage, during which modifiable risk factors like high BMI can be improved[278]. Preconception weight loss cannot be emphasised enough as a mean to improve fertility and IVF success rate, as well as reduce risks for both mother and foetus[277]. A BMI <35 kg/m^2 is recommended before commencing any fertility treatment[21–24], given that pregnancy is associated with weight and fat gain, which in turn will be more problematic if preconception BMI exceeds the normal range. In the presented IVF study, high risk of GDM was experienced at a lower BMI cut-off (≥30 kg/m^2), which hence suggests aiming for a lower preconception BMI and enforces the NICE guidelines[25] for a BMI <29 kg/m^2 before commencing any ART treatment. Several studies have affirmed that as little as a 5% and ideally 10% weight loss were sufficient to improve reproductive hormonal profile and menstrual cyclicity, as well as insulin sensitivity and risk of GDM, in obese women with and without PCOS[15,279,280]. A study by Stubert et al. reported that 10% reduction in BMI associated with about 10% reduction in risk of preeclampsia and GDM[277]. Healthy lifestyle intervention, comprising a balanced dietary plan and regular physical activity, may promote 10 to 15% weight loss within one year. Pursuant to these findings, the upper limit BMI

of 38 kg/m^2 in advanced age recommended by HAAD (UAE) may in fact be worth delaying by a reasonable period to achieve some weight loss; this will improve IVF success rate (including quality of eggs) and pregnancy outcome.

6.4.3. Glucose Homeostasis Markers

During pregnancy, insulin maintains normoglycaemia. Insulin secretion increases in response to elevated plasma glucose level and to counteract reduction in insulin sensitivity, the latter corresponding to pre-GDM situation[207]. In this study, a significant reduction in plasma glucose level at the first trimester of IVF-conceived pregnancy (at 12 weeks) was found with no change in insulin level, which the latter may be related to increased metabolic demand at this time for glucose. Hence, glucose level was not reliable enough to predict GDM risk in this study. The decrease in glucose level can be related to different factors, including longer than 10 hours fast, poor nutritional intake secondary to pregnancy-related nausea and more importantly, it can result from a physiological adaptation where the body uses mainly glucose as a source of energy to the foetus[87,88]. The use of HbA1c (average of glucose for three months) for predicting, diagnosing and managing GDM remains inconclusive[200,255,281]. Additionally, HbA1c is thought to be a weak surrogate of insulin sensitivity and secretion[204]. Level of HbA1c can be influenced by different factors: anaemia, physiological hydraemia, gastrointestinal disorder (such as hyperemesis gravidarum) and dietary intake[204,255]. Unfortunately, information in relation to participants' dietary intake and gastrointestinal disorders were not collected. Given that HbA1c was also significantly lower at 12 weeks (unlike with the presence of pre-GDM), may reject its possibility as an early marker for GDM in this IVF study. When narrowed down, an HbA1c ≥5.7% at 12 weeks associated with a four-fold higher risk of developing GDM. Altogether, even though the literature does not support relying on HbA1c to predict GDM, it can be used to identify those at high risk of adverse pregnancy outcomes and who may benefit from early intervention, especially if presenting with HbA1c above 5.7% at <20 weeks of gestation[200,202,203,206].

In relation to insulin, high serum insulin level may predispose to a higher

risk of GDM, which is often the case for women with PCOS[11,12]. In addition, the risk of GDM was documented in the literature to be higher with increased HOMA-IR at the second trimester[282]. This observation is compatible with findings from the presented IVF study, whereby even with unchanged levels in insulin and HOMA-IR at 12 weeks, these parameters successfully predicted GDM risk at an even earlier stage. However, when adjusting for history of PCOS, HOMA-IR and insulin level were no longer significant valid predictors of GDM. This may suggest that early gestation insulin and HOMA-IR levels of participants may be related to their PCOS condition rather than a pregnancy effect. Additionally, decreased insulin sensitivity (based on HOMA-IR) positively correlated with significant maternal weight gain and BMI during the first trimester; which in turn emphasises further the well-known role of obesity in the aetiology of insulin resistance and GDM[256].

The OGTT test for GDM is conducted around 24–28 weeks of gestation[195,196,198], whereby, many changes in glucose homeostasis have already happened with possible sequelae on mother and foetus. Measuring routinely insulin parameters in the first trimester may predict GDM and protect mother and foetus from related adverse events.

6.4.4. Other Endocrine and Metabolic Markers

This presented IVF study corroborates previous findings in relation to thyroid-mediated regulation of glucose metabolism and that thyroid impairment may be a risk factor for the development of GDM[221]. Low TSH level decreases insulin sensitivity; correspondingly in the presented study, TSH level inversely correlated with (unchanged) levels of insulin and HOMA-IR at 12 weeks. Change in glucose level did not however associate with change in TSH suggesting that the change in glucose homeostasis was more likely a physiological adaptation, rather than related to thyroid status. It should be noted that reduced TSH level remained within the normal range[180]; and this might have affected power to show any association between TSH and glucose. In contrast to other studies' findings[222], only few participants (n=6) experienced a low TSH level (<0.04 μIU/mL) at 12 weeks, but did not develop GDM. However, the number of participants was too small to

make meaningful conclusions, and it would have been interesting to measure other thyroid-related hormones (T3 and T4) to confirm diagnosis of thyroid impairment. In sum, TSH level at 12 weeks poorly predicted GDM risk, especially when the change in level remains in the normal range. In relation to the American Thyroid Association TSH cut-off of <2.5 μIU/mL associated with lower maternal adverse outcomes[170,181,182], exceeding this level during the first trimester doubled GDM risk in the pregnant group in this presented study, with a trend toward statistical significance.

In relation to lipid profile and risk of GDM, unlike insulin the observed decrease in glucose level at 12 weeks did not associate with change in lipids (TG, T-Chol, LDL-C and HDL-C). Insulin regulates lipid metabolism, and triggers hepatic and fat tissue TG synthesis[218]. Even with unchanged insulin homeostasis, there was a positive correlation between levels of insulin and TG and negative with HDL-C at 12 weeks. Enquobahrie et al. highlighted the positive association between elevated TG (>137 mg/dL or the equivalent to 1.55 mmol/L) and GDM risk with 3.5-fold increased risk even after adjusting for pre-pregnancy adiposity, and found that each 20 mg/dL increase in TG promoted a 10% increase in GDM risk[223]. Independently of obesity, TG ≥140 mg/dL (1.58 mmol/L) was characterised as an important risk factor for GDM, with a 1.8-fold higher GDM risk in lean and 2.7-fold in the obese group[225]. Contrary to other studies, the potential predisposition to GDM when considering median TG level was not observed in the presented IVF study[223–225], possibly because levels of lipid parameters remained within normal ranges at 12 weeks despite their significant increase from baseline. However, with TG ≥137 mg/dL, pregnant women from this study were 2.8 times more predisposed to GDM, likely related to the effect of insulin. 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 control groups; pointing out the possibility of HDL-C as a risk factor of GDM[225]. Abell et al. expanded on this finding noting that GDM women presented with lower HDL-C concentration (60 mg/dL) during the first trimester of their pregnancy, and HDL-C ≥85.5 mg/dL reduced GDM risk by 50% in lean women[119]. Participants in the study had an increase in HDL-C at 12 weeks,

excluding its possibility in predicting GDM. Additionally, 12-week HDL-C ≥85.5 mg/dL did not protect from GDM risk for participants in the presented IVF study.

The logarithm of TG/HDL-C ratio, commonly used as an atherogenic marker (Log TG/HDL-C >0.099) and to identify pregnant women with higher risk of GDM before 24 weeks of gestation[226]. When tested on participants in the study, log TG/HDL of more than 0.099 did not predict a higher risk of GDM. Early pregnancy lipids were hence not sufficient in predicting GDM, especially given that concentrations remained within the guideline ranges.

Future studies assessing GDM predisposition from lipid precursor hormones, which are associated with obesity, PCOS and reduced insulin sensitivity are needed. Vitamin D, derived from cholesterol and commonly deficient in pregnancy, affects glycaemic control and is thought to have a strong implication in GDM pathophysiology[119,283]; the related mechanism is worth further investigation. The Gulf region (including UAE) and certain ethnicities are more prone to vitamin D deficiency (e.g. South Asia, the Middle East and Africa), which characterises a large portion of the participants in this study[284–286]. Additionally, given the association of vitamin B 12 with insulin resistance and obesity, it would be interesting to explore its role in GDM. Vitamin B 12 level was shown to be inversely associated with fasting glucose level and negatively with BMI in early pregnancy[287]; highlighting its potential role as a novel biomarker of GDM.

6.4.5. Inflammatory Markers

Low grade inflammation is associated with increased risk of insulin resistance and T2DM, while limited data is available in relation to inflammatory predictors of GDM; a condition which is pathophysiologically similar to T2DM[288]. Increased inflammatory profile in the first trimester has been previously reported in women who later develop GDM[289]. Adiponectin and LBP levels were measured to assess the inflammatory status of pregnancy and related predisposition to GDM. Strong evidence has reported lower adiponectin levels in obesity, pregnancy and GDM; its role in the pathophysiology of GDM crosses different mechanisms[146]. There was a positive but weak correlation between levels of glucose and

adiponectin at 12 weeks, but the decrease in glucose level was not paired with any change in adiponectin. Consequently, the observed change in glucose level may not be related to the anti-glycaemic properties of adiponectin and rather a pregnancy effect[116]. Additionally, unchanged levels of insulin and adiponectin levels confirm previous findings that changes mainly occur after the second trimester[207].

As previously mentioned, GDM is characterised by inflammation and insulin resistance, and early signs of inflammation precedes the condition[116]. Given that adiponectin level did not differ between women who later developed GDM and non-GDM group, this suggests that either IVF-conceived pregnancy does not promote an inflammatory response in pregnancy, or that it was too early to see any inflammatory response within the first trimester. In line with this finding, hypoadiponectinemia (adiponectin level <6.4 ug/mL) at 12 weeks of pregnancy did not associate with increased risk of developing GDM in this study[113,227]. Measuring adiponectin and insulin-related parameters later in pregnancy would have helped confirming these speculations, as adiponectin secretion is expected to decrease with the increase in insulin resistance[113].

In addition, maternal adiposity is another risk factor for the development of GDM, and provokes increased circulation of cytokines[119,120,227]. There was no correlation between gestational BMI and adiponectin level, possibly reflecting little change in inflammatory profile within the first trimester. Taken together, the potential role of adiponectin as an inflammatory marker and/or diabetogenic predictor from the first trimester of pregnancy was not conclusive. High CRP level is another commonly used marker of inflammation and is positively associated with increased GDM risk; however, it was not measured in this study[288,289].

Gut flora dysbiosis is associated with pregnancy-related complications, such as insulin resistance, and nowadays, evidence supports strong involvement of dysbiosis of the gut in the pathogenesis of GDM[151,153,154]. Unlike with oral contraceptives long-term use, LBP level did not differ at 12 weeks between pregnant women who later developed GDM compared to those who did not. Possibly, the first trimester is too early to show LBP-related LPS or gut microflora changes. Such changes may possibly occur later in pregnancy, or if IVF hormones

were administered for a longer period. In addition, reduced microbiota richness was reported in the first trimester in those who later developed GDM[132]. The data from the presented IVF study is inconclusive in relation to the change in diversity of the microflora, since no stool samples were collected from participants, and reasoning of microbiota change is based on LBP, which in turn might not be sufficient to determine changes in gut microflora. Unlike previous studies showing a powerful positive correlation with obesity (independently predisposes to GDM) and negative correlation with insulin sensitivity[117,230,290], LBP was not associated with any of these parameters in the presented study and hence could not predict insulin resistance and risk of GDM.

GDM-related inflammation and insulin resistance are also partially modulated by other placental hormones (refer to Section 2.3.2.1), including HPL, placental growth hormone, relaxin and kisspeptin[89,291]. Measurement of these hormones and any existing correlations with glucose homeostasis may have enriched findings of novel early biomarkers of GDM. Accelerated foetal growth (i.e. abdominal circumference) may precede the diagnosis of GDM. By the time the OGTT test is conducted (around 28 weeks of gestation), Sovio et al. suggested that foetal growth is already abnormal for those who are subsequently diagnosed with GDM[292]. Therefore, foetal development may also serve as an early predictor to GDM, highlighting the importance of close surveillance and earlier intervention.

Thus, the observed metabolic and endocrine changes, as well as inflammatory and gut microflora profiles were not distinguishable from a spontaneously-conceived pregnancy. This raises the question whether IVF-conceived pregnancy should still be considered as a “high-risk” pregnancy. Preconception care increases the likelihood of a successful and healthy IVF-conceived pregnancy, and may help identify conditions that have adverse effects on both mother and foetus. In addition, prenatal monitoring and surveillance of glucose and lipid metabolisms stratify pregnant women with higher risk and remain as key surrogates for early screening and diagnosis of GDM, especially for women with advanced age and higher BMI.

Section 3: Results and Discussion

Chapter 6C: Discussion

6.5. Pregnancy and Foetal Outcomes

In single pregnancy, birth weight was within the references range of 2.5–4.0 kg, but slightly below in multiple pregnancy (<2.5 kg for each of the babies)[293]. This finding is comparable to previous studies, which reported a higher birth weight in singleton preganncies[57].

As mentioned earlier, caesarean section is more common in IVF-conceived pregnancies[63] and even more in multiple pregnancies, which the latter is not considered as an indicator for a caesarean delivery[294–296]. Results from this study revealed similar findings, in which a significant association was found between twin pregnancies and delivering by caesarean section. In regards to babies' weight, unlike in singleton pregnancy, first baby in twin pregnancy significantly predicted a caesarean delivery, and which remained significant even after adjusting for weight of mother at 12-week of gestation. In addition, caesarean section is usually recommended in foetal macrosomia (> 5.0 kg)[295]. Overall, babies' birth weight did not exceed 4.0 kg in the present study, and this may suggests that birth weight of babies was not likely the only reason to undergo a caesarean section.

In regards to mother's characteristics and type of delivery, preconception obesity (BMI >29 kg/m^2) and greater gestational weight gain (> 11 kg recommended by the Institute of Medicine in obese women), are documented to be associated with higher rate of caesarean delivery[297]. Preconception BMI and BMI at 12 weeks did not predict a caesarean section. Measuring late gestational weight gain may have provided more understanding on gestational weight gain and indication for a caesarean section. Advanced maternal age (≥ 35 years) relates to a higher rate of a caesarean delivery, possibly explained by the physician and pregnant women concerns over pregnancy outcome in older age[298–301]. The present study did not find any association between age of pregnant women (≤39 years) and a specific type of delivery. Furthermore, diabetes in pregnancy is associated with a higher rate of a caesarean delivery, partially to reduce the incidence of unexpected intrauterine death during delivery and foetal trauma related to macrosomia[295]. The presence of GDM in participants of this study did not however predict a caesarean section.

Women from this study may have presented other obstetric complications or conditions (not reported in this study) that urged a caesarean section (e.g. repeated caesarean, failure to progress in natural labour, position of baby and cord prolapse)[294,295], and less likely IVF or GDM-related. Another possible explanation for the high rate of caesarean section which was described earlier, is that women may consider IVF-conceived pregnancy as "precious" after many years of infertility and may have requested to have a delivery by elective caesarean section to prevent perceived complications and not necessarily clinically indicated[64].

6.6. Study Strengths and Limitations

The main strength of this study consists of accessing information from a multicultural population. It is important to mention that this study is a prospective study where women are followed from baseline (including preconception assessment) to the first trimester. Consequently, assessment of the outcomes is better than basing it on a retrospective data. Repeated measures of glucose and insulin throughout the study have allowed assessing the effect of different exogenous hormones (FSH and LH, followed by oestrogen and progesterone). Estimates of GDM risks tested a wide range of well-documented factors and predictors simultaneously rather than measuring them only in isolation, and tested the possibility of novel markers. Adjusted for risk factors when assessing the biochemical potential predictors of GDM with a regression model.

There are several limitations to this study, including misreporting of participants ethnicity and history of GDM. Using HOMA-IR to estimate insulin sensitivity is not as precise as the euglycaemic clamp protocol, but it is certainly more practical and non-invasive for pregnant women. In the non-pregnant group, measuring anthropometrics (weight) and biomarkers at 4 weeks may have likely showed higher levels of glucose, insulin, lipids and thyroid profile than those reported in the results section at 12 weeks. In addition, measurement of oestrogen, progesterone levels, and FSH/LH ratio at 12 weeks may have allowed a better assessment of their possibilities as novel markers for GDM. Furthermore, assessing

placental hormones at week 12, may have allowed observing early changes in glucose homeostasis (normally occurs mid-gestation) and identifying other possible novel markers of GDM. Dietary intake and stool sample collection would have enabled more accurate assessment of changes in microflora. The inclusion of a spontaneous-conceived pregnant group would have allowed comparing the usual gestational physiological changes compared to IVF-conceived pregnancy and the magnitude of change induced by IVF hormonal therapies on the different parameters. Finally, testing metabolic and endocrine parameters during mid-second trimester may have provided additional insight on GDM predictors. It will be also important to evaluate long-term effects of IVF hormones on foetal outcomes in case they are administered until delivery. Despite these limitations, the findings of this study support further research into the IVF hormonal therapy domain and its outcome.

Section 4: Conclusion, Publications, Appendices and References

Chapter 7: Conclusion and Future Directions

7.1. Conclusion

This thesis enriches the literature with insight on the safety of IVF hormonal therapies and their effect on maternal metabolic, endocrine and inflammatory status. IVF therapy induces some weight-gain and impairment in glucose, insulin and lipid homeostasis, but not to the extent of diabetogenic, atherogenic and inflammatory levels. IVF hormones combined with gestational hormones did not hasten or aggravate the metabolic, endocrine and inflammatory changes of pregnancy. Pregnancy masks the metabolic-related effects of IVF therapy, and instead normal gestational physiological adaptations become manifest during the first trimester with minimal changes in gut microflora. This may reassure IVF seekers of the safety of the IVF procedure, with comparison with a spontaneously-conceived pregnancy, at least until the first trimester.

Early prediction remains more promising than prevention of GDM, given that current measures are not yet powerful enough in preventing onset of GDM. Consequently, identifying preconception and early gestational markers of GDM are key preventive measures. A combination of preconception and prenatal metabolic, endocrine and inflammatory biomarkers enables a better estimation of GDM risk, until identification of optimal markers for subsequent GDM development. In addition to the well-documented preconception predictors of GDM development including higher BMI and advanced age, there should also be emphasis on ratio of FSH/LH. Weight gain during the first trimester and maternal BMI predicted GDM development. In relation to glucose-related GDM markers, early assessment of insulin level and sensitivity remain the best predictors of future change in glucose during later stages of pregnancy and onset of GDM. The exciting predictive role of gut microflora in GDM was uneventful during the first trimester of gestation. Prospective studies testing these biomarkers in mid-gestation will grant more information in relation to early pathogenesis of GDM.

Preconception maternal characteristics (age and BMI) did not predict delivery by caesarean section, nor did prenatal BMI (at 12 weeks) and presence of GDM. In regards to foetal outcome, multiple pregnancies strongly correlated with a caesarean delivery, and unexpectedly, only the first baby's birth weight in twin

pregnancies predicted this type of delivery. In this study, caesarean section was possibly urged by obstetric complications or more likely electively requested by the mother as a precaution of unpredicted complications harming her “precious” baby. Given the similarities between spontaneous and IVF-conceived pregnancy in regards to the normal physiological effect of pregnancy, this may ensure IVF pregnant women of the safety of a natural delivery, unless clinically indicated otherwise.

7.2. Future Directions

Findings from this study therefore provide new evidence, in relation to optimal preconception and preventive measures of IVF therapy, which can be considered for future updated guidelines. Firstly, monitoring of glucose, lipids and thyroid functioning during IVF therapy should be performed particularly with failed and repeated IVF attempts. Secondarily, in addition to the confirmed preconception cut-offs from this study (e.g. BMI and ratio FSH/LH), further updated preconception preventive measures and stratification of high-risk women are needed to manage controllable factors and prevent possible obstetric complications. Such measures will also improve IVF success rate, and both pregnancy and foetal outcomes. Furthermore, accurate GDM biomarkers will optimise screening and potentially reduce cost of unnecessary tests and implications of missing GDM cases. Finally, following participants post-delivery will convey with greater certainty longer-term impact of IVF hormones on mother and predisposition to future chronic medical conditions in both mother and offspring.

Section 4: Conclusion, Publications, Appendices and References

Chapter 8: Publications

Abstracts arising from Thesis

1. Coussa, A., Hasan, H.A., Barber, T.M. Effects of fertility medications on glucose homeostasis and other metabolic parameters in women undergoing in vitro fertilization (IVF). Presented as a poster at the Warwick Medical School Postgraduate Research *Symposium, University of Warwick, Coventry, UK, May 2019.*

2. Coussa, A., Hasan, H.A., Barber, T.M. Effects of fertility drugs on glucose homeostasis and other metabolic parameters in women undergoing in vitro fertilization (IVF). Oral Presentation at the Seventh Annual Congress of the *Gulf Chapter* of the American Association of Clinical Endocrinologists (*AACE*), Muscat, Oman, October 2019. (Awarded 4th best abstract speaker).

3. Coussa, A., Barber, T.M., Hasan, H.A. Unresolved concerns in gestational diabetes…prevention or prediction? Oral Presentation at the Dubai International Nutrition Congress organized by Dubai Health Authority (DHA), Dubai, United Arab Emirates, October 2019.

4. Coussa, A., Hasan, H.A., Fakih, M., Barber, T.M.Effects of fertility drugs on glucose homeostasis and other metabolic parameters in women undergoing in vitro fertilization (IVF). Oral Presentation at the Middle East Fertility Society (MEFS), Cairo, Egypt, November 2019.

Publications arising from Thesis

1. Coussa, A., Hasan, H.A., Barber, T.M. Impact of contraception and IVF hormones on metabolic, endocrine and inflammatory status. Accepted for publication in *Journal of Assisted Reproduction and Genetics* (March 12th, 2020).

2. Coussa, A., Hasan, H.A., Barber, T.M. (Submitted for review on February 28th, 2020). Effect of IVF therapy on metabolic, endocrine and inflammatory status in IVF-conceived pregnancy. *European Journal of obstetrics & Gynecology and reproduction biology*

3. Coussa, A., Hasan, H.A., Barber, T.M. (Submitted for review on April 10th, 2020). Is the diabetogesnic and atherogenic effect of pregnancy hastened in IVF-conceived pregnancy? A prospective study. *Diabetes and Metabolism.*

Appendices

Appendix 1. BSREC Ethics Approval

PRIVATE
Miss Ayla Melanie Coussa
WMS
University of Warwick
Coventry
CV4 7AL

03 September 2018

Dear Miss Coussa

Study Title and BSREC Reference: *Effects of fertility drugs on glucose homeostasis and other metabolic parameters in women undergoing In Vitro Fertilization* REGO-2018-2232

Thank you for submitting the revisions to the above-named study to the University of Warwick's Biomedical and Scientific Research Ethics Sub-Committee for approval.

I am pleased to confirm that approval is granted.

In undertaking your study, you are required to comply with the University of Warwick's *Research Data Management Policy*, details of which may be found on the Research and Impact Services' webpages, under "Codes of Practice & Policies" » "Research Code of Practice" » "Data & Records" » "Research Data Management Policy", at:
http://www2.warwick.ac.uk/services/ris/research_integrity/code_of_practice_and_policies/research_code_of_practice/datacollection_retention/research_data_mgt_policy

You are also required to comply with the University of Warwick's *Information Classification and Handling Procedure*, details of which may be found on the University's Governance webpages, under "Governance" » "Information Security" » "Information Classification and Handling Procedure", at:
http://www2.warwick.ac.uk/services/gov/informationsecurity/handling.
Investigators should familiarise themselves with the classifications of information defined therein, and the requirements for the storage and transportation of information within the different classifications:

> *Information Classifications:*
> http://www2.warwick.ac.uk/services/gov/informationsecurity/handling/classifications
> *Handling Electronic Information:*
> http://www2.warwick.ac.uk/services/gov/informationsecurity/handling/electronic/
> *Handling Paper or other media*
> http://www2.warwick.ac.uk/services/gov/informationsecurity/handling/paper/.

Please also be aware that BSREC grants ethical approval for studies. The seeking and obtaining of *all* other necessary approvals is the responsibility of the investigator.

These other approvals may include, but are not limited to:

1. Any necessary agreements, approvals, or permissions required in order to comply with the University of Warwick's Financial Regulations and Procedures.
2. Any necessary approval or permission required in order to comply with the University of Warwick's Quality Management System and Standard Operating Procedures for the governance, acquisition, storage, use, and disposal of human samples for research.
3. All relevant University, Faculty, and Divisional/Departmental approvals, if an employee or student of the University of Warwick.
4. Approval from the applicant's academic supervisor and course/module leader (as appropriate), if a student of the University of Warwick.
5. NHS Trust R&D Management Approval, for research studies undertaken in NHS Trusts.
6. NHS Trust Clinical Audit Approval, for clinical audit studies undertaken in NHS Trusts.
7. Approval from Departmental or Divisional Heads, as required under local procedures, within Health and Social Care organisations hosting the study.
8. Local ethical approval for studies undertaken overseas, or in other HE Institutions in the UK.
9. Approval from Heads (or delegates thereof) of UK Medical Schools, for studies involving medical students as participants.
10. Permission from Warwick Medical School to access medical students or medical student data for research or evaluation purposes.
11. NHS Trust Caldicott Guardian Approval, for studies where identifiable data is being transferred outside of the direct clinical care team. Individual NHS Trust procedures vary in their implementation of Caldicott guidance, and local guidance must be sought.
12. Any other approval required by the institution hosting the study, or by the applicant's employer.

There is no requirement to supply documentary evidence of any of the above to BSREC, but applicants should hold such evidence in their Study Master File for University of Warwick auditing and monitoring purposes. You may be required to supply evidence of any necessary approvals to other University functions, e.g. The Finance Office, Research & Impact Services (RIS), or your Department/School.

May I take this opportunity to wish you success with your study, and to remind you that any Substantial Amendments to your study require approval from BSREC before they may be implemented.

Yours sincerely

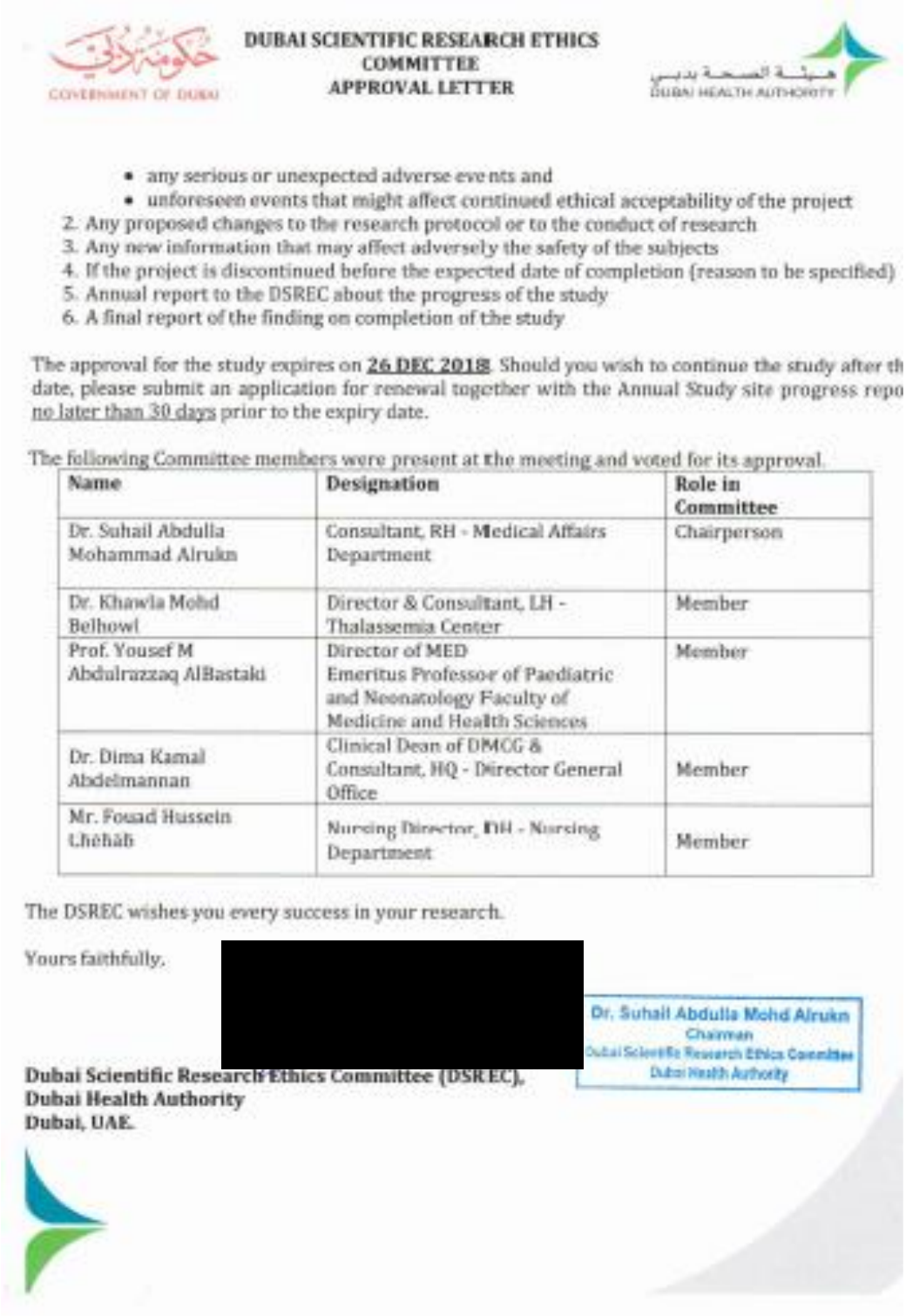
pp. [redacted]

Dr David Ellard
Chair
Biomedical and Scientific
Research Ethics Sub-Committee

Biomedical and Scientific Research Ethics Sub-Committee
Research & Impact Services
University of Warwick
Coventry, CV4 8UW.
E: BSREC@Warwick.ac.uk

http://www2.warwick.ac.uk/services/ris/research_integrity/researchethics committees/biomed

Appendix 2. Dubai Health Authority Ethical Approval



حكومة دبي
GOVERNMENT OF DUBAI

DUBAI SCIENTIFIC RESEARCH ETHICS COMMITTEE APPROVAL LETTER

هيئة الصحة بدبي
DUBAI HEALTH AUTHORITY

- any serious or unexpected adverse events and
- unforeseen events that might affect continued ethical acceptability of the project

2. Any proposed changes to the research protocol or to the conduct of research
3. Any new information that may affect adversely the safety of the subjects
4. If the project is discontinued before the expected date of completion (reason to be specified)
5. Annual report to the DSREC about the progress of the study
6. A final report of the finding on completion of the study

The approval for the study expires on **26 DEC 2018**. Should you wish to continue the study after th date, please submit an application for renewal together with the Annual Study site progress repo no later than 30 days prior to the expiry date.

The following Committee members were present at the meeting and voted for its approval.

Name	Designation	Role in Committee
Dr. Suhail Abdulla Mohammad Alrukn	Consultant, RH - Medical Affairs Department	Chairperson
Dr. Khawla Mohd Belhowl	Director & Consultant, LH - Thalassemia Center	Member
Prof. Yousef M Abdulrazzaq AlBastaki	Director of MED Emeritus Professor of Paediatric and Neonatology Faculty of Medicine and Health Sciences	Member
Dr. Dima Kamal Abdelmannan	Clinical Dean of DMCG & Consultant, HQ - Director General Office	Member
Mr. Fouad Hussein Lhéhâb	Nursing Director, RH - Nursing Department	Member

The DSREC wishes you every success in your research.

Yours faithfully,

[redacted]

Dr. Suhail Abdulla Mohd Alrukn
Chairman
Dubai Scientific Research Ethics Committee
Dubai Health Authority

Dubai Scientific Research Ethics Committee (DSREC),
Dubai Health Authority
Dubai, UAE.

DUBAI SCIENTIFIC RESEARCH ETHICS COMMITTEE
APPROVAL LETTER

From :	Dubai Scientific Research Ethics Committee (DSREC), Dubai Health Authority	**Date :**	26 DEC 2017
To :	Dr. Hayder A. Hasan M.D, MSc., PhD, Clinical Nutrition & Dietetics, College of Health Sciences, University of Sharjah	**Ref :**	DSREC-11/2017_09
Study Site:	Fakih IVF Clinic, Dubai		

Subject: Approval for the research proposal: ***"Effects of fertility drugs on glucose homeostasis and other metabolic parameters on patients undergoing In Vitro Fertilization (IVF)"***

Short Title: ***IVF impact on metabolic parameters***

Dear Dr. Hayder A. Hasan,

Thank you for submitting the above mentioned research proposal to Dubai Scientific Research Ethics Committee, DHA. The Dubai Scientific Research Ethics Committee has been organized and operates in accordance with the ICH/GCP guidelines and the committee is registered with the Office for Human Research Protection (OHRP).

Your request was initially discussed during the committee meeting held on 11 DEC 2017. On review and discussion, the committee issued some queries that were communicated to you for further clarifications.

As the response-addressed points by points fulfil DSREC Member's queries, we are pleased to advice you that the committee has granted ethical approval for the below mentioned documents:

No	Study Documents
1	Application form for the Ethical Approval of a Research Project
2	Study Proposal
3	Participant Information sheet and Consent form_English and Arabic-***Amended***
4	Data Collection Tool
7	Resume of Principal Investigator and Co-Investigators

Please note that it is DSREC's policy that the principal investigator should report to the committee of the following:

1. Anything which might warrant review of ethical approval of the project in the specified format, including:

Appendix 3. Health Authority of Abu Dhabi Ethical Approval

دائـــرة الـصـحـة
DEPARTMENT OF HEALTH

Date: 3rd, September, 2018
Ref: HCQ/١٩٥/١٨

Subject: **DOH Approval for the research proposal "*Effects of fertility drugs on glucose homeostasis, and other metabolic parameters on patients undergoing In Vitro Fertilization (IVF)*"**

Dear Dr. Kalthum Abuelnas,

Thank you for completing Department of Health "Application for Authorization to Conduct Human Subjects Research."

Please be informed that we are in the transitional period of changing the current process of Medical Research Authorization. Therefore, DOH approval will be exclusively given for the research proposal.

After reviewing the below documents, Medical Research Department grant your facility the approval to go ahead with the above-mentioned research in your institute.

- ADREC Form
- Consent Form
- Questionnaire Data Collection
- Fakih Approval letter

Compliance with all Department of Health policies and consistently operating the Fakih IVF Fertility Center Research Ethics Committee by Department of Health *Standard Operating Procedures for Research Ethics Committees* and the *Integrated Addendum to the International Conference on Harmonisation Good Clinical Practice (ICH GCP) E6(R2)* guideline will ensure that your facility maintains good implementation to conduct human subjects research.

This approval is issued under the Fakih IVF Fertility Center L.L.C. Health Facility License #MF3000, and is subject to Department of Health facility audit and license renewal procedures.

We appreciate your commitment to fostering productive health research at Fakih IVF Fertility Center L.L.C.. Please contact us if we can provide further guidance or assistance.

[redacted]

Dr. Rasheed Ahmed Al Hammadi
Manager of Medical Research Department
Healthcare Quality Division

● PUBLIC / عـــام

Appendix 4. Anthropometrics and Medical History Questionnaire

Effects of fertility drugs on glucose homeostasis, and other metabolic parameters on patients undergoing In Vitro Fertilisation (IVF)

Subject Name:
Telephone No:
E-mail:

Age	
Height	
Weight	
BMI	
Nationality	
Smoking	
Parity	

Past Illness and Surgeries

Drug History

Family History

Women Health

Cycle: Regular	Irregular
History of GDM:	
Previous IVF trials:	

Blood Analysis

Type	Level/ Unit	Frequency
Fasting glucose		4 times
Serum insulin		4 times
Lipid profile		Twice
FSH, LH		4 times
Oestrogen, Progesterone		4 times
Beta-HCG		Once

Appendix 5. Consent Form

Informed Consent Form

Project: Effects of fertility drugs on glucose homeostasis and other metabolic parameters on patients undergoing In Vitro Fertilisation (IVF)

مشروع دراسة :تأثير أدوية الإخصاب على توازن الجلوكوز ومقاومة الانسولين وغيرها من المؤشرات الأيضية لدى المرضى الذين يخضعون لعلاج أطفال الانابيب

The United Arab Emirates (U.A.E) are listed among the top 10 countries worldwide in term of obesity and have one of the highest rates of polycystic ovary syndrome (PCOS), whereby 60 % of Gulf women and 30 % of Indian origins women living in the U.A.E have PCOS. Obesity has increased along with increasing related abnormalities in the reproductive system, such as an ovulation and infertility. In fact, 20% of couples worldwide are infertile; this corresponds to 50% of women in the UAE are facing infertility issues. Obesity is considered as a major risk factor for developing pregnancy-related complications such as gestational diabetes (GDM); as well as PCOS independently of the obesity factor. More scientific-based studies are required to assess whether Assisted Reproductive Technology (ART) is another predisposing factor to GDM compared to normal pregnancy.

وتعد دولة الإمارات العربية المتحدة من بين الدول العشرة الأولى في العالم من حيث السمنة ولديها أعلى معدلات متلازمة تكيس المبايض حيث تبلغ نسبتها 60٪ من النساء الخليجيات و30٪ من النساء الهنديات اللواتي يعشن في دولة الإمارات العربية المتحدة. وقد زادت السمنة جنبا إلى جنب مع زيادة تشوهات ذات الصلة في الجهاز التناسلي، مثل الإباضة والعقم. في الواقع، 1 من كل 5 أزواج في جميع أنحاء العالم يعانون من العقم، وهذا يتوافق مع 50٪ من النساء في دولة الإمارات العربية المتحدة حيث تواجه قضايا تتعلق العقم. تعتبر السمنة عامل خطر رئيسي لتطوير مرض السكري الحملي وكذلك تكيس المبايض وبشكل مستقل عن عامل السمنة. المزيد من الدراسات العلمية مطلوبة لتقييم ما إذا كانت التكنولوجيا المساعدة على الإنجاب عامل مؤهب آخر لمرض السكري الحملي بالمقارنة مع الحمل الطبيعي.

You are being asked to participate in study of "Effects of fertility drugs on glucose homeostasis and other metabolic parameters on patients undergoing In Vitro Fertilisation"**.** Up to 192 patients will be participating in this study.

فأنت مدعوة للمشاركة في مشروع دراسة "تأثير أدوية الإخصاب على توازن الجلوكوز ومقاومة الانسولين وغيرها من المؤشرات الأيضية على المرضى الذين يخضعون لعلاج أطفال الانابيب "حيث من المتوقع أن يكون عدد المشاركين 192 شخصا.

This study will be done by: القائمون على هذه الدراسة:

- Dr. Hayder A. Hasan, principal investigator, College of Health Sciences, University of Sharjah
- الدكتورحيدر عباس حسن - الباحث الرئسي، كلية العلوم الصحية، جامعة الشارقة
- Dr. Marikinti Karunakar, Co-investigator, Fakih IVF
- الدكتورماريكينتي كاروناكار- باحث، مركز فقيه للإخصاب وعلاج أطفال الانابيب في دبي
- Mrs Ayla Coussa, Co-investigator, Fakih IVF
- السيدة آيلا كوسا - باحث، مركز فقيه للإخصاب وعلاج أطفال الانابيب بدبي

Study Details

تفاصيل الدراسة

As part of this study, the researchers will measure your: كجزء من هذه الدراسة، فإن الباحثين سيقومون بقياس

- Height
- Weight
- Blood pressure
- الطول
- الوزن
- ضغط الدم

A blood sample will be collected from you at 4 episodes during your IVF treatment, which will be sent to a laboratory for the following tests:
سيتم اخذ عينة من دمك في 4 حلقات أثناء العلاج، وسيتم إرسالها إلى المختبر لإجراء الفحوصات المختبرية التالية:

- Oral Glucose Tolerance Test (OGTT)
- Insulin level
- Women hormonal levels
- Lipid profile
- اختبار تحمل الجلوكوز
- مستوى الأنسولين في الدم
- تحليل هرمونات النساء للدورة الشهرية والحمل
- معدل الدهون

Neither the patients nor their medical insurances will be paying for these tests; but instead the ethic will be covering the costs of the tests.
It will take around two hours to complete the tests at Fakih IVF Clinic in Dubai. There are no known risks to participate in the study and you may not benefit directly from taking part in this study. However, this study will help us assess if IVF drugs impair metabolic parameters such as glucose tolerance, insulin sensitivity and lipid

profile. The study will also help screening and managing patients who are more at risk of pregnancy-related complications.

- وسوف يستغرق ساعتين لإكمال الاختبارات في مركز فقيه للإخصاب في دبي.لا توجد مخاطر معروفة وقد لا تستفيد بشكل مباشر من المشاركة في هذه الدراسة . ومع ذلك، فقد تساعدنا هذه الدراسة على توضيح ما إذا كانت أدوية الإخصاب لها تأثير على المؤشرات الأيضية توازن الجلوكوز ومقاومة الانسولين ومعدل الدهون. وستساعد الدراسة أيضا في فحص و رعاية المرضى الذين هم أكثر عرضة للمضاعفات المرتبطة بالحمل.

All the study information will be kept confidential. You will not be identified in any publication or presentation of the study findings. Only groups' results will be reported. Blood samples (with your Fakih IVF code) will be preserved and stored, and can be used in the future to test other parameters. Samples will also be locked and only accessible by the PI and co-investigators.
All documents from this study will be kept confidential at Fakih IVF Clinic, locked with Dr Karunakar Marikinti (Tel: [redacted]), and only accessible by the co- investigators of the study.

- وستبقى جميع معلومات هذه الدراسة سرية .ولن يتم التعرف عليكم في أي منشور أو عرض لنتائج هذه الدراسة. سيتم حفظ وتخزين عينات الدم (مع رمز فقيه IVF الخاص بك)، ويمكن استخدامها في المستقبل لاختبار معلمات أخرى. كما يمكن مطالعتها من قبل الباحثون في الدراسة فقط.
وسيتم إبلاغ النتائج كمجموعات فقط .وسيتم الاحتفاظ بجميع الوثائق بشكل سري مع الدكتورماريكينتي كاروناكار- (هاتف:[redacted]) في مركز فقيه للإخصاب وعلاج أطفال الانابيب في دبي ويمكن مطالعتها من قبل الباحثون في الدراسة فقط.

Your decision whether or not to participate will not affect your current or future relationship with the investigator, nor your IVF treatment. If you decide to participate you are free to withdraw at any time without affecting this relationship. The researchers also may choose to withdraw you from this study if this is in your interest.

- إن قرار المشاركة أو عدمه سوف لن يؤثرعلى علاقتك الحاليةٌ أو المستقبلية مع الباحث أو على علاجك في المركز.
إذا قررت المشاركة فأنت حر في الانسحاب في أي وقت دون التأثيرعلى هذه العلاقة وقد يكون الخيار للباحث بانسحابكم من هذه الدراسة إذا كان ذلك من مصلحتكم.

You are encouraged to ask any questions regarding this project and if you wish to find out the results of this study you may contact Dr Karunakar Marikinti or Mrs Ayla Coussa (contact details above) at Fakih IVF Clinic in Dubai, six months from today.
If for any ethical concern arises, you may contact the Dubai Scientific Research Ethics Committee directly (042191961/65) or by email (DSREC@dha.gov.ae).

- أنت مدعو لطرح أي سؤال حول هذا المشروع، وإذا كنت ترغب في معرفة نتائج هذه الدراسة عليك الاتصال بالدكتورماريكينتي كاروناكار أوالسيدة آيلا كوسا (تفصيل الاتصال مذكورة أعلاه) في مركز فقيه للإخصاب وعلاج أطفال الانابيب في دبي بعد ستة أشهر من هذا البحث .

في حال نشوء أي مخاوف أخلاقية، يمكنك التواصل مع لجنة أخلاقيات البحث العلمي في دبي مباشرة (04219161/65) أو عن طريق البريد الإلكتروني (DSREC@dha.gov.ae

Signed Agreement:

I understand that;

a. My signature indicates that I voluntarily agree to be a part of this research study

b. I will receive a copy of this form

Signature of Subject:

Date:

Signature of Investigator:

Date:

توقيع الاتفاق:

أنا أتفهم بان؛

أ .توقيعي يشير إلى أني أوافق طوعا على أن أكون جزءا من هذه الدراسة البحثية

ب .وسوف احصل على نسخة من هذا النموذج

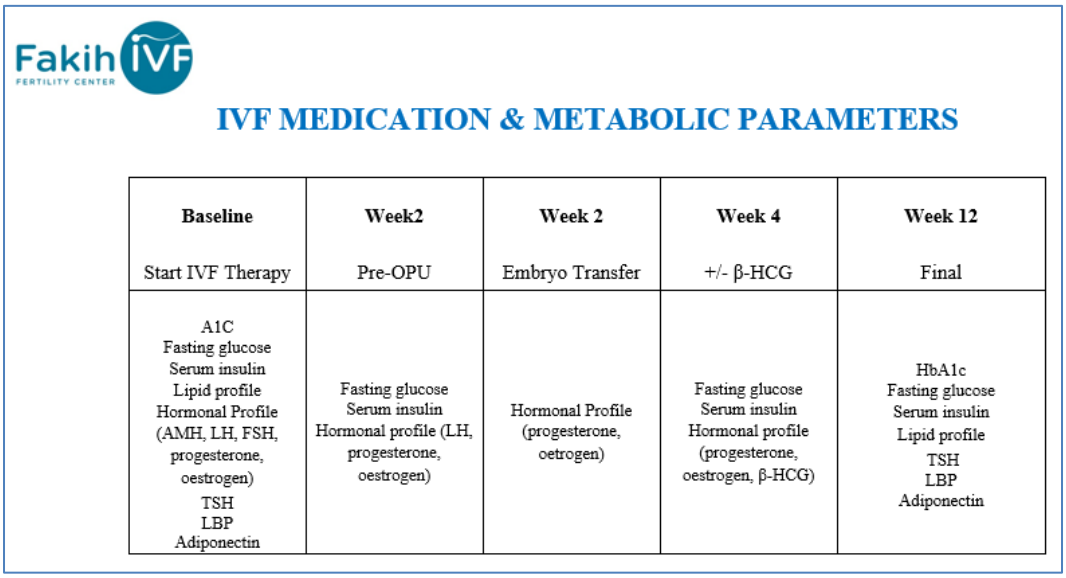
توقعُ المشارك:

التاريخ:

توقيع الباحث:

التاريخ:

Appendix 6. Participants Study Tests



Fakih IVF
FERTILITY CENTER

IVF MEDICATION & METABOLIC PARAMETERS

Baseline Start IVF Therapy	**Week2** Pre-OPU	**Week 2** Embryo Transfer	**Week 4** +/- β-HCG	**Week 12** Final
A1C Fasting glucose Serum insulin Lipid profile Hormonal Profile (AMH, LH, FSH, progesterone, oestrogen) TSH LBP Adiponectin	Fasting glucose Serum insulin Hormonal profile (LH, progesterone, oestrogen)	Hormonal Profile (progesterone, oetrogen)	Fasting glucose Serum insulin Hormonal profile (progesterone, oestrogen, β-HCG)	HbA1c Fasting glucose Serum insulin Lipid profile TSH LBP Adiponectin

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