Effect of Preconception Impaired Glucose Tolerance on Pregnancy Outcomes in Women With Polycystic Ovary Syndrome

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Context: Women with polycystic ovary syndrome (PCOS) commonly have intrinsic insulin resistance and are recommended to undergo an oral glucose tolerance test (OGTT) for diabetes screening. However, the effect of preconception impaired glucose tolerance (IGT) on pregnancy is still unclear.

Objective: To prospectively assess the effect of preconception IGT on pregnancy outcomes.

Design, Setting, Patients, Interventions, and Main Outcome Measures: This was a secondary analysis of a multicenter randomized trial in 1508 women with PCOS comparing live birth and obstetric complications between fresh and frozen embryo transfer. At baseline, fasting and 2-hour glucose and insulin levels after 75-g OGTT were measured.

Results: Women with preconception IGT had higher risks of gestational diabetes in both singleton pregnancy [9.5% vs 3.2%; odds ratio (OR) 3.13; 95% confidence interval (CI) 1.23to 7.69] and twin pregnancy (20.0% vs 3.2%; OR 7.69; 95% CI 2.78 to 20.00) than women with normoglycemia. Preconception IGT was associated with a higher risk of large for gestational age in singleton newborns compared with normoglycemia (34.7% vs 19.8%; OR 2.13; 95% CI 1.19 to 3.85) or isolated impaired fasting glucose (i-IFG) (34.7% vs 15.4%; OR 2.94; 95% CI 1.33 to 6.25). Women with preconception IGT had a higher singleton pregnancy loss rate than women with i-IFG (31.4% vs 17.5%; OR 2.17; 95% CI 1.11 to 4.17). After adjusting for age, body mass index, duration of infertility, total testosterone level, and treatment groups (frozen vs fresh embryo transfer), these associations remained.

Conclusions: Preconception IGT, independent from BMI, was associated with adverse pregnancy outcome compared with i-IFG and normoglycemia. **(*J Clin Endocrinol Metab* 102: 3822–3829, 2017)**

Polycystic ovary syndrome (PCOS) is a common endocrine-metabolic disorder in reproductive-aged women (1), with a prevalence ranging from 5% to 20% depending on the diagnostic criteria used (2). The common features of PCOS include oligomenorrhea, hyperandrogenism, and polycystic ovary morphology on

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Abbreviations: BMI, body mass index; CI, confidence interval; hCG, human chorionic gonadotropin; HOMA-IR, homeostasis model assessment of insulin resistance; IFG, impaired fasting glucose; i-IFG, isolated impaired fasting glucose; IGT, impaired glucose tolerance; IVF, *in vitro* fertilization; LGA, large for gestational age; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; OR, odds ratio; PCOS, polycystic ovary syndrome; SGA, small for gestational age.

ultrasonography (1). Women with PCOS also frequently manifest metabolic alterations such as insulin resistance, obesity, and metabolic syndrome. Insulin resistance is suggested to be intrinsic of PCOS and play an important role in its pathogenesis (3), independent of obesity (4). Such intrinsic insulin resistance places women with PCOS at increased risk of dysglycemia, type 2 diabetes, and cardiovascular diseases (5, 6). When women with PCOS become pregnant, the pre-existing insulin resistance and glucose intolerance is exacerbated and results in an increased risk of gestational diabetes (7–9). Preconception hyperinsulinemia and dysglycemia may also affect endometrial receptivity (10) and place women with PCOS at increased risk of poor fecundity (10) and pregnancy loss (11, 12). Moreover, *in utero* exposure to maternal hyperglycemia may increase offspring risk of abnormal glucose intolerance, obesity, and higher blood pressure (13).

In women with PCOS, diabetes risk may be better detected by oral glucose tolerance test (OGTT) measuring 2-hour glucose level after 75-g glucose load than fasting glucose level alone (14, 15). Screening with fasting glucose alone may miss >50% of prediabetes and >10% of diabetes as determined by OGTT (15). OGTT is recommended for diabetes and prediabetes screening in women with PCOS (16). For those who are seeking fertility, preconceptual OGTT is recommended for all by the Endocrine Society (17). However, there is still no consensus on whether preconception prediabetes status, such as impaired glucose tolerance (IGT) and impaired fasting glucose (IFG), should be corrected before starting infertility treatment, as the effects on later pregnancy are uncertain. We hypothesized that preconception IGT defined by OGTT would be associated with adverse pregnancy outcomes independent of obesity.

We recently completed a multicenter randomized trial in 1508 women with PCOS comparing fresh vs frozen embryo transfer (FreFro-PCOS) (18, 19). At baseline, glucose levels at fasting and 2 hours following 75-g OGTT were measured. The pregnancy outcomes were prospectively documented. In the current study, we performed secondary analyses aiming to assess the association between preconception IGT and pregnancy outcomes such as live birth, pregnancy loss, and incidences of gestational diabetes, pre-eclampsia, small for gestational age (SGA), and large for gestational age (LGA).

Materials and Methods

Research design

The FreFro-PCOS study was conducted in 14 academic centers for reproductive medicine in China during June 2013 and July 2015 (ClinicalTrials.gov, NCT01841528). This study was approved by the institutional review boards of all study sites. Written informed consent was obtained from every couple. The design, conduct, and main outcomes of FreFro-PCOS trial have been previously reported in detail (18, 19). Briefly, a total of 1508 women with PCOS who were undergoing their first cycle of *in vitro* fertilization (IVF) with or without intracytoplasmic sperm injection were enrolled. PCOS was diagnosed by the presence of menstrual disturbance combined with either hyperandrogenism (hyperandrogenemia and/or hirsutism) or a polycystic ovary on ultrasonography (defined as either an ovary that contains ≥12 antral follicles or ovarian volume >10 cm^3) and exclusion of other causes of hyperandrogenism and ovulation dysfunction. The cutoff value for diagnosis of hyperandrogenemia was based on local laboratory reference of total testosterone, which had been determined before the start of study. Hirsutism was defined by modified Ferriman–Gallwey score >6 (20). The other exclusion criteria were abnormal intrauterine cavity, a history of unilateral oophorectomy, a history of recurrent spontaneous abortion, and abnormal karyotype. Women who developed a high risk of ovarian hyperstimulation syndrome during ovarian stimulation were also excluded.

At baseline, history of reproduction and infertility and results of physical examination were systematically collected by standardized case report forms. Levels of sex hormones and glucose were measured at local sites as part of clinical evaluation of PCOS. A 75-g OGTT was performed to determine fasting and 2-hour glucose and insulin levels. Glucose levels were measured by hexokinase method and insulin levels by electrochemiluminescence method in all sites. Categories of glucose metabolism were defined according to the 2012 criteria of the American Diabetes Association (21). For fasting glucose results, normal fasting glucose was defined as fasting glucose level <100 mg/dL (5.6 mmol/L), IFG as fasting glucose level 100 to 125 mg/dL (5.6 to 6.9 mmol/L), and diabetes as fasting glucose level ≥126 mg/dL (≥7.0 mmol/L). For 2-hour OGTT results, normal glucose tolerance (NGT) was defined as 2-hour glucose level <140 mg/dL (7.8 mmol/L), IGT as 2-hour glucose level 140 to 199 mg/dL (7.8 to 11.0 mmol/L), and diabetes as 2-hour glucose level ≥200 mg/dL (≥11.1 mmol/L). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as: (fasting insulin mIU/L × fasting glucose mmol/L)/22.5. A total of 34 women (2.3%) were diagnosed with diabetes. These patients were referred to a diabetes clinic for glycemic control before IVF procedure and excluded from this analysis. Normoglycemia was defined as normal fasting glucose and NGT; isolated IFG (i-IFG) was defined as IFG and NGT.

The detailed procedures of ovarian stimulation, oocyte retrieval, embryo culture, and endometrial preparation were performed as previously described (19). Briefly, a standard gonadotropin-releasing hormone antagonist protocol was used for ovarian stimulation. Human chorionic gonadotropin (hCG) was administered to induce final oocyte maturation when at least two follicles measured ≥18 mm. Subjects were randomized to receiving fresh or frozen embryo transfer. For women in the fresh embryo transfer group, embryos were transferred 3 days after oocyte pickup. For women in the frozen embryo transfer, all embryos were frozen after *in vitro* culture. Frozen-thawed embryos were transferred in the subsequent menstrual cycles after the recovery of ovaries and steroid hormone levels. Up to

two day-3 embryos were transferred in both fresh and frozen embryo transfer groups as per American Society of Reproductive Medicine and Chinese guidelines (19, 22). Luteal phase support with intramuscular progesterone was administered 3 days before embryo transfer until 10 weeks' gestation. All pregnancies were followed up until delivery or termination.

Conception was defined as serum hCG level of >10 mIU/mL at 14 days after embryo transfer. Pregnancy loss among conception was defined as conceptions that end up with miscarriage or induced abortion due to fetal congenital malformation before 28 weeks of gestation. Live birth was defined as delivery of any viable infant at ≥28 weeks' gestation. The diagnoses of gestational diabetes and pre-eclampsia as well as birth weight were obtained from obstetrical and neonatal medical records. SGA and LGA were determined according to gender and gestational weeks adjusted birth weight reference for Chinese (23). SGA was defined as birth weight lower than the 10th percentile of referential birth weight. LGA was defined as birth weight higher than the 90th percentile of referential birth weight.

Data analysis

Normally distributed continuous variables were described as mean ± standard deviation, and between-group differences were compared by one-way analysis of variance test. For nonnormally distributed continuous variables, medians (25th to 75th percentiles) were presented, and comparisons were done by Kruskal-Wallis test. *Post hoc* comparisons among groups were performed, and the significance levels were corrected by the Bonferroni method. Categorical variables were described as frequency and percentage and compared by χ^2 test among groups. Multivariable logistic regression was performed to adjust for the effect of age, body mass index (BMI), duration of infertility, total testosterone level, and treatment groups (frozen vs fresh embryo transfer). Multivariable logistic regression was performed to adjust for the effect of age, BMI, duration of infertility, total testosterone level, and treatment groups (frozen vs fresh embryo transfer). When we chose the covariates to be adjusted in the multivariable models, we only considered those known and important confounders that are clinically routinely collected. Forward approach with forced enter of the groups of glucose status (*i.e.*, normoglycemia, i-IFG, and IGT) was used in the regression analysis. The *P* value for covariates entering final models was set at 0.05. The adjusted odds ratio (OR) and 95% confidence intervals (CIs) were presented. The statistically noteworthy level was set at 0.05. All analyses were performed using SPSS (Version 21.0; SPSS Inc., Chicago, IL).

Results

Data on the 2-hour glucose level were missing in 43 women, and data on the fasting glucose level were missing in another 2 women. After excluding the 34 women who were diagnosed with diabetes at baseline, 1429 women were included in this study. Baseline characteristics are listed in Table 1. A total of 289 women (20.2%) were diagnosed with IGT, 251 women (17.6%) with i-IFG, and 889 women (62.2%) with normoglycemia. Patients with IGT were older ($P = 0.02$ for IGT vs normoglycemia and $P = 0.001$ for IGT vs i-IFG) and had a longer duration of infertility ($P < 0.001$ for IGT vs normoglycemia and $P = 0.016$ for IGT vs i-IFG)

Table 1. Baseline Characteristics

	Normoglycemia (n = 889)	Isolated IFG (n = 251)	IGT (n = 289)	*P* Value
Age (y)[a,b]	28.1 ± 3.0[c]	27.7 ± 3.0	28.7 ± 2.9	0.001
BMI[a,d]	23.2 ± 3.5	24.5 ± 3.6	24.9 ± 3.8	<0.001
Duration of infertility (y)[a,b]	3.0 (2.0–5.0)[e]	3.0 (2.0–5.0)	4.0 (2.0–5.0)	<0.001
Total testosterone level (ng/dL)[a,b]	38.3 (28.0–51.5)	39.0 (26.5–53.2)	41.7 (31.8–55.7)	0.018
Fasting glucose level (ng/dL)[a,b,d]	90.0 ± 7.6	106.2 ± 4.1	96.7 ± 11.0	<0.001
Fasting insulin (mIU/L)[a,d]	9.9 (7.1–14.3)	12.9 (10.1–18.2)	13.9 (9.1–20.0)	<0.001
HOMA-IR[a,d]	2.2 (1.5–3.2)	3.3 (2.6–4.8)	3.3 (2.1–4.6)	<0.001
2-h glucose level (ng/dL)[a,b,d]	106.4 ± 18.4	116.1 ± 16.6	158.2 ± 14.8	<0.001
2-h insulin (mIU/L)[a,b]	49.6 (30.1–78.8)	56.1 (35.7–87.0)	107.1 (69.3–151.6)	<0.001
Number of oocytes retrieved	14.5 ± 6.0	13.7 ± 5.7	14.3 ± 5.8	0.150
Treatment group[f]				0.480
Fresh embryo transfer [n (%)]	437/866 (50.5)	114/247 (46.2)	142/283 (50.2)	
Frozen embryo transfer [n (%)]	429/866 (49.5)	133/247 (53.8)	141/283 (49.8)	
No. of embryo transferred				0.165
Two embryos [n (%)]	834/866 (96.3)	231/247 (93.5)	270/283 (95.4)	
One embryo [n (%)]	32/866 (3.7)	16/247 (6.5)	13/283 (4.6)	
Endometrial thickness on day of hCG administration (mm)[b,d]	10.5 ± 2.1	10.9 ± 2.0	10.2 ± 2.0	0.001
Endometrial thickness before frozen embryo transfer (mm)[b,d]	9.1 ± 1.4	9.6 ± 1.3	9.2 ±1.4	<0.001

[a] $P < 0.05$ for the comparison between IGT and normoglycemia.

[b] $P < 0.05$ for the comparison between IGT and i-IFG.

[c] Data are mean ± standard deviation.

[d] $P < 0.05$ for the comparison between i-IFG and normoglycemia.

[e] Data are median (25th percentile to 75th percentile).

[f] A total of 23 women with normoglycemia (2.6%), 4 women with i-IFG (1.6%), and 6 women with IGT (2.1%) did not undergo embryo transfer.

and higher levels of total testosterone ($P = 0.005$ for IGT vs normoglycemia and $P = 0.02$ for IGT vs i-IFG) and 2-hour insulin ($P < 0.001$ for both IGT vs normoglycemia and IGT vs i-IFG) than women with normoglycemia or i-IFG. Women with normoglycemia had lower BMI ($P < 0.001$ for both normoglycemia vs IGT and normoglycemia vs i-IFG), lower fasting insulin ($P < 0.001$ for both normoglycemia vs IGT and normoglycemia vs i-IFG), and lower HOMA-IR ($P < 0.001$ for both normoglycemia vs IGT and normoglycemia vs i-IFG) than women with IGT or i-IFG. The number of oocytes retrieved, the number of embryo transferred, and the proportion of frozen embryo transfer were comparable among three groups.

Conception rate and proportion of twin pregnancies

The conception rates in women with IGT, i-IFG, and normoglycemia were 60.6%, 72.9%, and 65.4%, respectively ($P = 0.010$) (Table 2). The conception rate in women with IGT was lower than that in women with i-IFG (OR 0.57; 95% CI 0.40 to 0.85) even after adjusting for age, BMI, duration of infertility, total testosterone level, and treatment groups (OR 0.58; 95% CI 0.40 to 0.85) (Table 3). Women with i-IFG had a higher rate of conception than women with normoglycemia (72.9% vs 65.4%; OR 1.43; 95% CI 1.05 to 1.95) even after adjustment (OR 1.38; 95% CI 1.001 to 1.91). The conception rate between women with IGT and women with normoglycemia was not statistically noteworthy. Although the number of embryos transferred was comparable among these three groups, the proportions of twin pregnancies among clinical pregnancies in women with preconception IGT were lower than those in women with normoglycemia (31.8% vs 44.4%; OR 0.59; 95% CI 0.40 to 0.85), even after adjustment (OR 0.65; 95% CI 0.44 to 0.95). There were no statistical differences in the proportion of twin pregnancy between women with i-IFG and women with normoglycemia or IGT.

Outcome of singleton pregnancies among women with preconception normoglycemia, i-IFG, and IGT

Compared with women with preconception normoglycemia, women with preconception IGT had a higher rate of pregnancy loss (31.4% vs 19.8%; OR 1.85; 95% CI 1.12 to 3.13) and a lower rate of live birth (68.6% vs 80.1%; OR 0.54; 95% CI 0.33 to 0.90) (Tables 2 and 3). However, after adjusting for age, BMI, duration of infertility, total testosterone level, and treatment groups, these differences became statistically nonsignificant (Table 3). Women with IGT had higher risks of gestational diabetes (9.5% vs 3.2%; OR 3.13; 95% CI 1.23 to 7.69) and LGA (34.7% vs 19.8%; OR 2.13; 95% CI 1.19 to 3.85) than women with normoglycemia, even after adjustment of age, BMI, duration of infertility, total testosterone level, and treatment groups. The risk of SGA was comparable between women with IGT and normoglycemia. None of the women with preconception

Table 2. Pregnancy Outcome in Women With Preconception Normoglycemia, i-IFG, and IGT

	Normoglycemia (n = 889)	Isolated IFG (n = 251)	IGT (n = 289)	*P* Value
Conception [n (%)][a]	581/889 (65.4)	183/251 (72.9)	175/289 (60.6)	0.010
Proportion of twin pregnancy [n (%)][b]	223/502 (44.4)	69/166 (41.6)	50/157 (31.8)	0.020
Singleton pregnancies [n (%)]				
Pregnancy loss[b]	55/278 (19.8)	17/97 (17.5)	33/105 (31.4)	0.025
Live birth[b]	222/277 (80.1)[c]	78/96 (81.3)[c]	72/105 (68.6)	0.035
GDM[b,d]	9/277 (3.2)[c]	9/96 (9.4)[c]	10/105 (9.5)	0.017
Pre-eclampsia	4/277 (1.4)	4/96 (4.2)	0	0.066
SGA	15/222 (6.8)	5/78 (6.4)	4/72 (5.6)	0.937
LGA[a,b]	44/222 (19.8)	12/78 (15.4)	25/72 (34.7)	0.009
Twin pregnancies [n (%)]				
Pregnancy loss	37/223 (16.6)	11/69 (15.9)	10/50 (20.0)	0.819
Live birth	183/221 (82.8)[c]	58/69 (84.1)[c]	40/50 (80.0)	0.842
GDM[b]	7/221 (3.2)[c]	4/69 (5.8)[c]	10/50 (20.0)	<0.001
Pre-eclampsia	11/221 (5.0)	5/69 (7.2)	1/50 (2.0)	0.497
SGA[e]	79/319 (24.8)	30/106 (28.3)	17/71 (23.9)	0.734
LGA[e]	10/319 (3.1)	4/106 (3.8)	5/71 (7.0)	0.302

Abbreviation: GDM, gestational diabetes mellitus.

[a] $P < 0.05$ for the comparison between IGT and i-IFG.

[b] $P < 0.05$ for the comparison between IGT and normoglycemia.

[c] In singleton pregnancies, one woman with normoglycemia and one woman with i-IFG were lost to follow-up. In twin pregnancies, two women with normoglycemia were lost of follow-up.

[d] $P < 0.05$ for the comparison between i-IFG and normoglycemia.

[e] In the 221 women with IGT and twin pregnancies, 138 women delivered twins and 45 women delivered singleton. The birth weights of a pair of twins were missing. In the 69 women with i-IFG and twin pregnancies, 48 women delivered twins and 10 women delivered singleton. In the 50 women with IGT and twin pregnancies, 31 women delivered twins, and 9 women delivered singleton.

Table 3. Univariate and Multivariable Logistic Regression Analyses of Pregnancy Outcome Between Women With Preconception IGT and Women With Preconception Normoglycemia and Women With i-IFG, Adjusting for the Effect of Age, BMI, Duration of Infertility, Total Testosterone Level, and Treatment Groups

	IGT vs Normoglycemia		IGT vs i-IFG		i-IFG vs Normoglycemia	
	Crude OR (95% CI)	Adjusted OR (95% CI)	Crude OR (95% CI)	Adjusted OR (95% CI)	Crude OR (95% CI)	Adjusted OR (95% CI)
Conception	0.81 (0.62–1.06)	0.80 (0.60–1.08)	0.57 (0.40–0.82)	0.58 (0.40–0.85)	1.43 (1.05–1.95)	1.38 (1.001–1.91)
Proportion of twin pregnancy	0.59 (0.40–0.85)	0.65 (0.44–0.95)	0.66 (0.42–1.03)	0.69 (0.44–1.11)	0.89 (0.62–1.27)	0.93 (0.65–1.33)
Singleton pregnancies						
Pregnancy loss	1.85 (1.12–3.13)	1.54 (0.89–2.70)	2.17 (1.11–4.17)	2.13 (1.04–4.35)	0.86 (0.47–1.57)	0.73 (0.39–1.37)
Live birth	0.54 (0.33–0.90)	0.65 (0.38–1.14)	0.50 (0.26–0.97)	0.51 (0.25–1.03)	1.07 (0.59–1.94)	1.28 (0.69–2.39)
GDM	3.13 (1.23–7.69)	3.13 (1.22–8.33)	1.02 (0.40–2.63)	1.02 (0.39–2.70)	3.08 (1.19–8.01)	3.10 (1.19–8.05)
SGA	0.81 (0.26–2.50)	1.03 (0.33–3.33)	0.86 (0.22–3.33)	0.96 (0.25–3.70)	0.95 (0.33–2.69)	1.20 (0.41–3.51)
LGA	2.13 (1.19–3.85)	2.04 (1.08–3.85)	2.94 (1.33–6.25)	2.94 (1.32–6.67)	0.74 (0.37–1.48)	0.69 (0.34–1.40)
Twin pregnancies						
Pregnancy loss	1.25 (0.58–2.70)	1.37 (0.62–3.03)	1.32 (0.51–3.45)	1.43 (0.54–3.70)	0.95 (0.46–1.99)	0.97 (0.46–2.04)
Live birth	0.83 (0.38–1.82)	0.76 (0.34–1.69)	0.76 (0.29–1.96)	0.70 (0.27–1.85)	1.10 (0.53–2.28)	1.08 (0.51–2.29)
GDM	7.69 (2.78–20.00)	8.33 (2.70–25.0)	4.00 (1.19–14.29)	3.70 (1.09–12.50)	1.88 (0.53–6.63)	2.14 (0.59–7.83)
Pre-eclampsia	0.39 (0.05–3.13)	0.36 (0.05–2.94)	0.26 (0.03–2.33)	0.25 (0.03–2.33)	1.49 (0.50–4.45)	1.44 (0.47–4.42)
SGA	0.95 (0.52–1.75)	0.88 (0.45–1.72)	0.80 (0.40–1.59)	0.66 (0.31–1.39)	1.20 (0.73–1.96)	1.34 (0.81–2.22)
LGA	2.33 (0.78–7.14)	2.04 (0.67–6.25)	1.92 (0.50–7.69)	1.56 (0.39–6.25)	1.21 (0.37–3.95)	1.31 (0.40–4.32)

Abbreviation: GDM, gestational diabetes mellitus.

IGT, four women (1.4%) with normoglycemia, and four women (4.2%) with i-IFG developed pre-eclampsia ($P = 0.066$).

Compared with women with preconception i-IFG, women with preconception IGT had a higher rate of pregnancy loss (31.4% vs 17.5%; OR 2.17; 95% CI 1.11 to 4.17) and a lower rate of live birth (68.6% vs 81.3%; OR 0.50; 95% CI 0.26 to 0.97). After adjustment for age, BMI, duration of infertility, total testosterone level, and treatment groups, the association between preconception IGT and pregnancy loss remained (OR 2.13; 95% CI 1.04 to 4.35). The risk of LGA was higher in women with IGT than women with i-IFG (34.7% vs 19.8%; OR 2.94; 95% CI 1.33 to 6.25), even after adjustment (OR 2.94; 95% CI 1.32 to 6.67). The risks of gestational diabetes and SGA were comparable between women with preconception IGT and i-IFG.

Compared with women with normoglycemia, women with i-IFG had a higher risk of gestational diabetes (9.4% vs 3.2%; OR 3.08; 95% CI 1.19 to 8.01) even after adjustment (OR 3.10; 95% CI 1.19 to 8.05). There was no statistical difference in the rates of pregnancy loss, live birth, gestational diabetes, pre-eclampsia, SGA, or LGA between women with i-IFG and women with normoglycemia.

Outcome of twin pregnancies among women with preconception normoglycemia, i-IFG, and IGT

The rates of pregnancy loss and live birth and the risks of pre-eclampsia, SGA, and LGA were comparable among women with preconception IGT, i-IFG, and normoglycemia before and after adjusting for age, BMI, duration of infertility, total testosterone level, and treatment groups. Women with IGT had a higher risk of gestational diabetes than women with normoglycemia (20.0% vs 3.2%; OR 7.69; 95% CI 2.78 to 20.00) and women with i-IFG (20.0% vs 5.8%; OR 4.00; 95% CI 1.19 to 14.29), even after adjustment.

Discussion

In women with PCOS, preconception IGT was associated with a higher risk of gestational diabetes in both singleton and twin pregnancies compared with normoglycemia and a higher risk of neonatal LGA in singleton pregnancies compared with normoglycemia or i-IFG. Women with preconception IGT also had a lower rate of conception and a higher rate of singleton pregnancy loss than women with i-IFG. After adjusting for age, BMI, duration of infertility, total testosterone level, and treatment groups (frozen vs fresh embryo transfer), these associations remained.

Gestational diabetes is one of the most common pregnancy complications, with evident effect on mothers and fetus (24). Preconception fasting levels of glucose and insulin are predictors of gestational diabetes (8). PCOS is characterized by intrinsic insulin resistance (25). Women with PCOS and IGT had even higher serum levels of fasting and 2-hour insulin than patients with normoglycemia and had a higher level of 2-hour insulin than women with i-IFG. Women with PCOS have an increased

risk of gestational diabetes compared with non-PCOS controls, independent of BMI (26). However, studies of adequate size are lacking that prospectively evaluated the association between preconception glucose-metabolic status determined by OGTT and the risk of gestational diabetes. In this study, we found preconception IGT and i-IFG had a similar risk of gestational diabetes in singleton pregnancies, which was higher than that in women with normoglycemia even after adjusting for age, BMI and other confounders. In twin pregnancies, the risk of gestational diabetes was dramatically increased in women with preconception IGT compared with women with i-IFG or normoglycemia. The underlying mechanism is unclear. However, IGT is primarily due to systemic insulin resistance coupled with secondary β-cell decompensation, whereas i-IFG is mainly caused by stationary reduced insulin secretion followed by increased hepatic glycogen output (27). Twin pregnancies have increased placenta mass and thus may have elevated hormones that exacerbate insulin resistance compared with singleton pregnancy (28). Patients with IGT may be at greater risk for β-cell decompensation than patients with i-IFG when they are confronted with the larger challenge of twin pregnancy. Observational studies suggested the risk of gestational diabetes in women with PCOS could be reduced by metformin treatment during pregnancy; however, randomized controlled trials have failed to replicate this effect (29, 30). It is possible that metformin may be most efficient in the subgroup of women with preconception dysglycemia (*i.e.*, IGT or IFG).

According to the Pedersen hypothesis, maternal hyperglycemia leads to fetal hyperglycemia, which induces an exaggerated fetal response to insulin and promotes fetal growth (31). Maternal glucose levels even below the threshold of gestational diabetes were continuously associated with increased birth weight (31). Our findings suggested preconception IGT was associated with a higher risk of having an LGA baby compared with women with normoglycemia and i-IFG. The underlying mechanism is likely related to sustained maternal glucose levels after meals during pregnancy, leading to greater fetal integrated glucose levels (9).

Preconception IGT was associated with a lower rate of conception and a higher rate of singleton pregnancy loss compared with i-IFG, independent of age and BMI. Women with i-IFG had a higher rate of conception than women with normoglycemia. The favorable effect of preconception i-IFG on conception and pregnancy loss seemed to be at least partly mediated through improving endometrial receptivity, though the exact mechanism is still unknown. In this study, we found the endometrial thickness before fresh and frozen embryo transfer was higher in women with i-IFG than in women with IGT or normoglycemia. Although both are intermediate states of abnormal glucose regulation between normoglycemia and diabetes, IGT and IFG have different sites of insulin resistance (32). On dynamic tests of insulin action, IGT primarily is characterized by moderate to severe muscle insulin resistance and normal to slightly reduced hepatic insulin sensitivity, whereas IFG mainly has hepatic insulin resistance and thus increased hepatic glucose output and normal muscle insulin sensitivity (27, 33). Insulin promotes cellular mitogenesis and endometrial proliferation during the proliferative phase and accelerates glucose uptake and glycogen synthesis during the secretory phase (34). It is possible that the state of insulin sensitivity at the endometrium may vary between IGT and i-IFG. Perhaps endometrial insulin sensitivity remains normal in i-IFG with increased endometrial proliferation and thickness on ultrasound but decreases in IGT; further studies are warranted. In women with PCOS who underwent *in vitro* maturation (*i.e.*, no ovarian stimulation but removal of the oocyte from the antral follicle and maturing *in vitro*), Chang *et al.* (35) found women with insulin resistance had similar rates of oocyte maturation and blastocyst formation, but lower rates of implantation and clinical pregnancy than women without insulin resistance, suggesting the adverse effect of insulin resistance on endometrial development. However, the rate of pregnancy loss in twin pregnancies was comparable between IGT and i-IFG. Because most of the women in this study received a double embryo transfer, those who obtained twin pregnancies may have a more favorable uterine environment than women who had singleton pregnancy. The adverse effect of IGT on pregnancy may be diluted by the favorable implantation milieu in twin pregnancies.

The strength of this study included a large sample size, prospective design, and the multicenter setting, which enhanced the validity and extrapolation of the results. Additionally, all subjects in this study received uniform infertility treatment (*i.e.*, standardized procedures of ovarian stimulation, embryo selection, embryo transfer, and luteal phase support), which reduced the confounding effect on pregnancy outcome by varied intervention. There were also limitations in this study. First, our study was underpowered to detect the effect of glucose intolerance on pre-eclampsia, as only one woman with preconception IGT developed pre-eclampsia during pregnancy. Second, we did not track the dynamic change of glucose levels before, during, and after pregnancy. The pragmatic design of this study precluded an explanation of the underlying mechanism of our findings, leading us to speculate. Third, we did not collect information on the family history of type 2 diabetes mellitus and the level of sex hormone–binding globulin, which may be

confounders of the effect of IGT on pregnancy outcomes (8). The hyperandrogenemia was assessed by total testosterone run in local laboratories on platform immunoassays in this study; however, free testosterone or free androgen index, especially as measured by liquid chromatography-mass spectrometry, may be more sensitive for the diagnosis of hyperandrogenemia (36). Future studies addressing these issues are needed. Finally, the participants in this study were women with PCOS undergoing their first cycle of IVF. We should be cautious when generalizing the findings to women who are undergoing multiple cycles of IVF because they may have more severe reproductive dysfunction and worsened metabolic profiles. Similarly, we should be cautious when generalizing the findings to women who conceive spontaneously without assistance, as they are less likely to have reproductive or metabolic dysfunction.

However, our finding that preconception IGT was associated with adverse pregnancy outcome supports the guideline by the Endocrine Society regarding screening diabetes and prediabetes with OGTT before fertility treatment in women with PCOS (17). Additionally, HbA_{1c} level has also been recommended as a diagnostic criterion for diabetes and prediabetes (21) and has been suggested as an alternative screening method for diabetes in women with PCOS (17), because HbA_{1c} level is more convenient to measure than OGTT and can reflect the plasma glucose level during 2 to 3 months before measurement. Targeted preconception HbA_{1c} level of <7% or 6.5% is recommended for women with pre-existing diabetes by several guidelines (37). Further studies are warranted to prospectively evaluate the association between preconception HbA_{1c} level and pregnancy outcome in women with PCOS.

In summary, in women with PCOS, preconception IGT was associated with a higher risk of gestational diabetes in both singleton and twin pregnancies and a higher risk of neonatal LGA compared with normoglycemia and also associated with a lower rate conception and a higher rate of singleton pregnancy loss compared with i-IFG, independent from BMI.

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References

1. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril*. 2004;**81**(1):19–25.
2. Azziz R, Carmina E, Chen Z, Dunaif A, Laven JS, Legro RS, Lizneva D, Natterson-Horowtiz B, Teede HJ, Yildiz BO. Polycystic ovary syndrome. *Nat Rev Dis Primers*. 2016;**2**:16057.
3. Ciaraldi TP, el-Roeiy A, Madar Z, Reichart D, Olefsky JM, Yen SS. Cellular mechanisms of insulin resistance in polycystic ovarian syndrome. *J Clin Endocrinol Metab*. 1992;**75**(2):577–583.
4. Dunaif A, Segal KR, Futterweit W, Dobrjansky A. Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. *Diabetes*. 1989;**38**(9):1165–1174.
5. Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. Consensus on women's health aspects of polycystic ovary syndrome (PCOS). *Hum Reprod*. 2012;**27**(1):14–24.
6. Yildiz BO, Bozdag G, Yapici Z, Esinler I, Yarali H. Prevalence, phenotype and cardiometabolic risk of polycystic ovary syndrome under different diagnostic criteria. *Hum Reprod*. 2012;**27**(10):3067–3073.
7. Lanzone A, Fulghesu AM, Cucinelli F, Guido M, Pavone V, Caruso A, Mancuso S. Preconceptional and gestational evaluation of insulin secretion in patients with polycystic ovary syndrome. *Hum Reprod*. 1996;**11**(11):2382–2386.
8. de Wilde MA, Veltman-Verhulst SM, Goverde AJ, Lambalk CB, Laven JS, Franx A, Koster MP, Eijkemans MJ, Fauser BC. Preconception predictors of gestational diabetes: a multicentre prospective cohort study on the predominant complication of pregnancy in polycystic ovary syndrome. *Hum Reprod*. 2014;**29**(6):1327–1336.
9. Dmitrovic R, Katcher HI, Kunselman AR, Legro RS. Continuous glucose monitoring during pregnancy in women with polycystic ovary syndrome. *Obstet Gynecol*. 2011;**118**(4):878–885.
10. Schulte MM, Tsai JH, Moley KH. Obesity and PCOS: the effect of metabolic derangements on endometrial receptivity at the time of implantation. *Reprod Sci*. 2015;**22**(1):6–14.
11. Sutherland HW, Fisher PM. Fetal loss and maternal glucose intolerance. A retrospective study. *Padiatr Padol*. 1982;**17**(2):279–286.
12. Mills JL, Simpson JL, Driscoll SG, Jovanovic-Peterson L, Van Allen M, Aarons JH, Metzger B, Bieber FR, Knopp RH, Holmes LB, Peterson CM, Withiam-Wilson M, Brown Z, Ober C, Harley E, Macpherson TA, Duckles A, Mueller-Heubach E; National Institute of Child Health and Human Development-Diabetes in Early Pregnancy Study. Incidence of spontaneous abortion among normal women and insulin-dependent diabetic women whose pregnancies were identified within 21 days of conception. *N Engl J Med*. 1988;**319**(25):1617–1623.
13. Tam WH, Ma RCW, Ozaki R, Li AM, Chan MHM, Yuen LY, Lao TTH, Yang X, Ho CS, Tutino GE, Chan JCN. In Utero Exposure to Maternal Hyperglycemia Increases Childhood Cardiometabolic Risk in Offspring. *Diabetes Care*. 2017;**40**(5):679–686.
14. Legro RS, Kunselman AR, Dodson WC, Dunaif A. Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective,

controlled study in 254 affected women. *J Clin Endocrinol Metab*. 1999;**84**(1):165–169.
15. Li HW, Lam KS, Tam S, Lee VC, Yeung TW, Cheung PT, Yeung WS, Ho PC, Ng EH. Screening for dysglycaemia by oral glucose tolerance test should be recommended in all women with polycystic ovary syndrome. *Hum Reprod*. 2015;**30**(9):2178–2183.
16. American Diabetes Association. 2. Classification and Diagnosis of Diabetes. *Diabetes Care*. 2016;**39**(Suppl 1):S13–S22.
17. Legro RS, Arslanian SA, Ehrmann DA, Hoeger KM, Murad MH, Pasquali R, Welt CK; Endocrine Society. Diagnosis and treatment of polycystic ovary syndrome: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2013;**98**(12):4565–4592.
18. Chen Z-J, Shi Y, Sun Y, Zhang B, Liang X, Cao Y, Yang J, Liu J, Wei D, Weng N, Tian L, Hao C, Yang D, Zhou F, Shi J, Xu Y, Li J, Yan J, Qin Y, Zhao H, Zhang H, Legro RS. Fresh versus frozen embryos for infertility in the polycystic ovary syndrome. *N Engl J Med*. 2016;**375**(6):523–533.
19. Shi Y, Wei D, Liang X, Sun Y, Liu J, Cao Y, Zhang B, Legro RS, Zhang H, Chen ZJ. Live birth after fresh embryo transfer vs elective embryo cryopreservation/frozen embryo transfer in women with polycystic ovary syndrome undergoing IVF (FreFro-PCOS): study protocol for a multicenter, prospective, randomized controlled clinical trial. *Trials*. 2014;**15**:154.
20. Hatch R, Rosenfield RL, Kim MH, Tredway D. Hirsutism: implications, etiology, and management. *Am J Obstet Gynecol*. 1981; **140**(7):815–830.
21. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2012;**35**(Suppl 1):S64–S71.
22. Practice Committee of American Society for Reproductive Medicine; Practice Committee of Society for Assisted Reproductive Technology. Criteria for number of embryos to transfer: a committee opinion. *Fertil Steril*. 2013;**99**(1):44–46.
23. Dai L, Deng C, Li Y, Zhu J, Mu Y, Deng Y, Mao M, Wang Y, Li Q, Ma S, Ma X, Zhang Y. Birth weight reference percentiles for Chinese. *PLoS One*. 2014;**9**(8):e104779.
24. Catalano PM, Kirwan JP, Haugel-de Mouzon S, King J. Gestational diabetes and insulin resistance: role in short- and long-term implications for mother and fetus. *J Nutr*. 2003;**133**(5, Suppl 2)1674S–1683S.
25. Stepto NK, Cassar S, Joham AE, Hutchison SK, Harrison CL, Goldstein RF, Teede HJ. Women with polycystic ovary syndrome have intrinsic insulin resistance on euglycaemic-hyperinsulaemic clamp. *Hum Reprod*. 2013;**28**(3):777–784.
26. Joham AE, Ranasinha S, Zoungas S, Moran L, Teede HJ. Gestational diabetes and type 2 diabetes in reproductive-aged women with polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2014; **99**(3):E447–E452.
27. Faerch K, Vaag A, Holst JJ, Hansen T, Jørgensen T, Borch-Johnsen K. Natural history of insulin sensitivity and insulin secretion in the progression from normal glucose tolerance to impaired fasting glycemia and impaired glucose tolerance: the Inter99 study. *Diabetes Care*. 2009;**32**(3):439–444.
28. Weissman A, Drugan A. Glucose tolerance in singleton, twin and triplet pregnancies. *J Perinat Med*. 2016;**44**(8):893–897.
29. Zeng XL, Zhang YF, Tian Q, Xue Y, An RF. Effects of metformin on pregnancy outcomes in women with polycystic ovary syndrome: A meta-analysis. *Medicine (Baltimore)*. 2016;**95**(36):e4526.
30. Vanky E, Stridsklev S, Heimstad R, Romundstad P, Skogøy K, Kleggetveit O, Hjelle S, von Brandis P, Eikeland T, Flo K, Berg KF, Bunford G, Lund A, Bjerke C, Almås I, Berg AH, Danielson A, Lahmami G, Carlsen SM. Metformin versus placebo from first trimester to delivery in polycystic ovary syndrome: a randomized, controlled multicenter study. *J Clin Endocrinol Metab*. 2010; **95**(12):E448–E455.
31. Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, Coustan DR, Hadden DR, McCance DR, Hod M, McIntyre HD, Oats JJ, Persson B, Rogers MS, Sacks DA; HAPO Study Cooperative Research Group. Hyperglycemia and adverse pregnancy outcomes. *N Engl J Med*. 2008;**358**(19):1991–2002.
32. Nathan DM, Davidson MB, DeFronzo RA, Heine RJ, Henry RR, Pratley R, Zinman B; American Diabetes Association. Impaired fasting glucose and impaired glucose tolerance: implications for care. *Diabetes Care*. 2007;**30**(3):753–759.
33. Unwin N, Shaw J, Zimmet P, Alberti KG. Impaired glucose tolerance and impaired fasting glycaemia: the current status on definition and intervention. *Diabet Med*. 2002;**19**(9):708–723.
34. Flannery CA, Saleh FL, Choe GH, Selen DJ, Kodaman PH, Kliman HJ, Wood TL, Taylor HS. Differential epression of IR-A, IR-B and IGF-1R in endometrial physiology and distinct signature in adenocarcinoma. *J Clin Endocrinol Metab*. 2016;**101**(7): 2883–2891.
35. Chang EM, Han JE, Seok HH, Lee DR, Yoon TK, Lee WS. Insulin resistance does not affect early embryo development but lowers implantation rate in in vitro maturation-in vitro fertilization-embryo transfer cycle. *Clin Endocrinol (Oxf)*. 2013;**79**(1):93–99.
36. Goodman NF, Cobin RH, Futterweit W, Glueck JS, Legro RS, Carmina E; American Association of Clinical Endocrinologists (AACE); American College of Endocrinology (ACE); Androgen Excess and PCOS Society. American Association of Clinical Endocrinologists, American College of Endocrinology, and Androgen Excess and PCOS Society disease state clinical review: guide to the best practices in the evaluation and treatment of polycystic ovary syndrome–part 2. *Endocr Pract*. 2015;**21**(12):1415–1426.
37. Abell SK, Nankervis A, Khan KS, Teede HJ. Type 1 and type 2 diabetes preconception and in pregnancy: health impacts, influence of obesity and lifestyle, and principles of management. *Semin Reprod Med*. 2016;**34**(2):110–120.