Consistent Role of Insulin Resistance in Recurrent Pregnancy Loss and Recurrent Implantation Failure: A Case-control Study

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Research

Keywords: insulin resistance, recurrent pregnancy loss, recurrent implantation failure

Posted Date: August 23rd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-827067/v1

Abstract

Background: To explore the role of insulin resistance (IR) in patients with recurrent pregnancy loss (RPL) and/or recurrent implantation failure (RIF) treated with assisted reproductive technology (ART).

Methods: We conducted a case-control study in a tertiary hospital from 2012 to 2018, We included 212 cases of simple RPL (only involved in RPL), 123 cases of simple RIF (only involved in RIF), 67 cases involved in both conditions (complicated group). We screened 123 women as the control cohort, who underwent ART due to male infertile, with no adverse pregnant outcomes. We examined the plasm glucose and insulin level in both fasting and postprandial condition after the oral glucose tolerance test (OGTT) and calculated the area under the curve of glucose (AUGG) and insulin (AUCI) as well as the homeostasis model assessment for insulin resistance and β-cell function (HOMA-IR and HOMA-β).

Results: Both the simple RPL group and the complicated group had significantly higher fasting insulin (FINS), HOMA-IR and HOMA-β than the control group. The simple RIF group had the lowest level of FINS, HOMA-IR and HOMA-β. The incidences of IR were significantly higher in both the simple RPL group and the complicated group than the other two groups. After adjusted for age and waist-hip ratio (WHR), the simple RIF group had the highest fasting plasma glucose (FPG) [adjusted-mean (95%CI), 5.20 (5.09-5.33) mmol/L] and lower FINS [adjusted-mean (95%CI), 10.77 (9.25-12.29) mU/L] and HOMA-β [adjusted-mean (95%CI), 127.76 (83.56-171.97)]; the simple RPL group had the highest FINS [adjusted-mean (95%CI), 12.09 (11.21-12.98) mU/L] and HOMA-β [adjusted-mean (95%CI), 189.74 (164.29-215.18)] and a lower FPG [adjusted-mean (95%CI), 5.03 (4.97-5.10) mmol/L]. The FINS tended to increase with times of implantation failure among those patients with implantation failure fewer than six times. However, patients with more than six times implantation failure had extremely low FINS when compared with those with a history of five or six times (P<0.05).

Conclusion: In patients undergoing ART, insulin resistance may be a common etiopathogenesis of RPL and RIF and insulin secretion impairment may be related to RIF.

1 Introduction

Recurrent pregnancy loss (RPL) is defined as two or more failed clinical pregnancies, and up to 50% of cases of RPL do not have an exact etiology[1, 2]. The common reasons for RPL could be genetic disorders, uterine pathologies, endocrine dysfunctions, autoimmune diseases, and adverse effects from environmental factors[2].

Recurrent implantation failure (RIF) is defined as failing implantations that occur in continuous cycles of in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI), or frozen embryo replacement for twice or more with all good-quality embryos[3]. The common reasons for RIF could be the resistance of progesterone, changed receptivity window, declining integrin expression, and immunologic disturbances[4].

In recent years, RIF has been suggested to share many etiopathogenesis and therapies with RPL[5, 6], some autoimmune biomarkers such as antithyroid antibodies and antiphospholipid commonly exist in both RIF and RPL patients[7]. Other immunological parameters like peripheral blood natural killer (NK), cytokines and specific maternal human leukocyte antigen G polymorphisms seem to be associated with both RIF and RPL[8–10]. A recent study found that RPL and RIF had a very similar panel of thrombophilia polymorphisms[11], another study inferred that methylenetetrahydrofolate reductase (MTHFR) gene polymorphism might play a role in the etiology of RPL or RIF[12]. A reasonable inference is that RIF and RPL are different manifestations at different stages but originated from partial common etiopathogenesis.

It has been reported that patients with RPL have a significantly increased prevalence of insulin resistance (IR) than the fertile controls[13, 14]. A recent systematic review and meta-analysis revealed that insulin resistance is a risk factor for an increased risk of spontaneous abortion in polycystic ovary syndrome (PCOS) patients who underwent assisted reproductive technology (ART)[15]. Also, some studies found that ART outcomes such as pregnancy rate, implantation rate, and live birth rate are impaired in IR patients with PCOS[16–18], while using the metformin to release the IR could result in the improvement of the ART outcomes[19, 20].

The role of IR in both RPL and RIF is unclear and whether RPL and RIF share any relation to IR is needed to explore. The purpose of this study was to identify the consistent role of insulin resistance in RPL and RIF in patients undergoing ART.

2 Methods

2.1 Study population

This case-control study was taken from January 2012 to June 2018 in Sun Yat-sen Memorial Hospital, a tertiary and academic hospital in Guangzhou China. The RPL group included women who had twice or more consecutive first-trimester spontaneous abortions. The RIF group included women who had failing implantations in continuous cycles of IVF, ICSI or frozen embryo replacement for twice or more with all good-quality embryos. The control group included women who underwent ART due to male infertile, with no adverse pregnancy outcomes or RIF.

Patients with an explicit cause of abortion were excluded: (1) parental or embryonic chromosomal abnormality; (2) anatomical abnormalities.

Data of all the included cases were collected from medical records.

2.2 Biochemical measurements and calculations

Blood samples were taken after fasting for at least 8 hours for measuring the fasting plasma glucose (FPG) level, fasting plasma insulin (FINS) level; and blood samples were also taken 1h and 2h after ingesting a 75-g glucose solution for the 1-h and 2-h plasma glucose (1hPG and 2hPG) and insulin

(1hINS and 2hINS) level. Homeostasis model assessment of insulin resistance (HOMA-IR), homeostasis model assessment of β-cell function (HOMA-β), the area under the curve of glucose (AUCG), and the area under the curve of insulin (AUCI) were calculated using the following formulas:

HOMA-IR = FINS(U/mL)×FPG(mmol/L)/22.5;

HOMA-β(%) = 20×FINS(U/mL)/[FPG(mmol/L)-3.5];

AUCG = FPG/2 + 1hPG + 2hPG/2; AUCI = FINS/2 + 1hINS + 2hINS/2.

Insulin resistance was defined as HOMA-IR ≥ 2.1, FPG ≥ 6.1 mmol/L, 2hPG ≥ 7.8 mmol/L, and/or 2hINS > 1hINS.

Plasma insulin levels were measured by chemiluminescence using the ACS180 SE autoanalyzer (Bayer Diagnostics; Fernwald, Germany). Glucose levels were measured using the glucose oxidase assay (Tosoh Corp, Tosoh, Japan).

2.3 Anthropometric parameters

The height, weight, waist and hip circumferences were measured in light clothes and without shoes in the fasting state. Waist circumference was measured at the level of halfway between the lower ribs and the superior anterior iliac spine of the pelvis in a standing position. Hip circumference was measured at the level of the pubic symphysis. Body mass index (BMI) and waist to hip ratio (WHR) were calculated according to the follow formulas: BMI = weight (kg) /height^2 (m^2); WHR = waist circumference(cm)/hip circumference(cm).

2.4 Statistical analyses

The distribution of data was determined and outliers were identified using descriptive statistics. Normal distribution continuous variables were expressed as means ± standard deviations and were compared by analysis of variance (ANOVA); Non-normal distribution variables were expressed as medians (interquartile range, IQR) and compared by Kruskal-Wallis H-test. Categorical variables were expressed as relative frequencies and compared by the chi-squared test. To adjust for latent confounding, comparisons of measurements among groups were assessed using analysis of covariance (ANCOVA). Normality was determined by the D'Agostino-Person test, and skewed, non-normally distributed variables were log-transformed before statistical disposal. Statistical analyses were performed using SAS software (version 8.1; SAS Institute Inc, Cary, NC), and the significance level was set at $p < 0.05$.

3 Result

3.1 Characteristics of study subjects

In total, 525 subjects with a mean age of 34 ± 4 (range 22–40) years were included in the analysis. There were 212 (40.4%) patients were with RPL but without RIF and were defined as the simple RPL group; there

were 123 (23.4%) patients were with RIF but without RPL and were defined as the simple RIF group; there were 67 (12.8%) patients with both conditions and were defined as the complicated group. The rest 123 (23.4%) patients without pregnancy loss or implantation failure history were defined as control groups. Compared with the control group, the patients in the other three gourps were older and had higher WHR ($P < 0.05$ for all, Table 1).

Table 1
Age, anthropometry, and laboratory characteristics of the study subjects

		Control (N = 123)	Simple RPL (N = 212)	Complicated (N = 67)	Simple RIF (N = 123)	p-value
Age(y)	M (IQR)	32 (31–35) [bcd]	34 (31–37) [ad]	36 (32–38) [a]	36 (32–39) [ab]	< 0.001
BMI (kg/m^2)	N	118	204	61	68	0.076
	M (IQR)	21.1 (19.8–23.0)	21.8 (20.2–24.0)	22.3 (20.4–24.2)	21.2 (19.9–23.0)	
WHR	N	116	204	61	67	0.018
	M (IQR)	0.80 (0.77–0.84) [bcd]	0.82 (0.78–0.86) [a]	0.83 (0.79–0.87) [a]	0.83 (0.78–0.88) [a]	
FPG (mmol/L)	N	121	209	62	115	0.674
	M (IQR)	5.10 (4.80–5.30)	5.00 (4.80–5.30)	4.70 (5.00-5.30)	5.10 (4.80–5.40)	
1hPG (mmol/L)	N	21	108	36	50	0.157
	M (IQR)	7.10 (5.98–9.55)	8.80 (7.50–10.20)	8.35 (7.00-9.40)	8.55 (7.10–11.00)	
2hPG (mmol/L)	N	22	109	37	52	0.008
	M (IQR)	5.70 (5.10–7.10) [bd]	7.20 (5.80–8.80) [a]	6.60 (5.48–7.70)	6.75 (5.85–8.25) [a]	
AUCG	N	21	108	36	50	0.065
	M (IQR)	12.60 (11.38–15.63)	14.90 (13.00-16.95)	14.30 (12.35–15.40)	14.50 (12.60–18.00)	
FINS (mU/L)	N	120	197	60	111	< 0.001
	M (IQR)	8.72 (5.96–11.42) [bc]	10.65 (7.37–13.78) [ad]	11.01 (7.74–14.07) [ad]	8.37 (6.03–11.26) [bc]	
1hINS (mU/L)	N	18	92	31	49	0.015
	M (IQR)	126.21 (57.87-158.12)	104.10 (71.66-170.39) [d]	123.58 (75.61-173.65) [d]	72.75 (52.50-113.90) [bc]	

		Control (N = 123)	Simple RPL (N = 212)	Complicated (N = 67)	Simple RIF (N = 123)	p-value
2hINS (mU/L)	N	18	92	32	50	0.086
	M (IQR)	79.01 (42.91-117.58)	95.04 (51.89-157.28)	78.00 (53.64–157.50)	66.63 (39.73-113.41)	
AUCI	N	18	91	31	49	0.012
	M (IQR)	176.45 (80.60-256.20)	158.30 (113.63-258.53) [d]	191.70 (111.30–228.00) [d]	110.80 (84.88–163.50) [bc]	
HOMA-IR	N	118	194	58	108	< 0.001
	M (IQR)	1.95 (1.30–2.60) [bc]	2.40 (1.60–3.10) [ad]	2.50 (1.90–3.30) [ad]	1.90 (1.35–2.60) [bc]	
HOMA-β	N	118	194	58	108	< 0.001
	M (IQR)	112.30 (81.00-160.90) [bc]	134.00 (95.30-206.50) [ad]	164.70 (103.10-225.40)[ad]	105.55 (78.45-143.25) [bc]	
Insulin resistance (% (n/N))		40.5 (49/121) [bc]	61.2 (128/209) [ad]	69.4 (43/62) [ad]	47.0 (54/115) [bc]	< 0.001
HOMA-IR ≥ 2.14 (% (n/N))		40.7 (48/118) [bc]	60.3 (117/194) [ad]	67.2 (39/58) [ad]	39.8 (43/108) [bc]	< 0.001
FPG ≥ 6.1 mmol/L (% (n/N))		0.8 (1/121)	1.4 (3/209)	3.2 (2/62)	3.5 (4/115)	0.394
2hPG ≥ 7.8 mmol/L (% (n/N))		13.6 (3/22)	37.6 (41/109)	21.6 (8/37)	28.8 (15/52)	0.072
2hINS > 1hINS (% (n/N))		33.3 (6/18)	40.7 (37/91)	38.7 (12/31)	28.6 (14/49)	0.542

Note: Continuous values with a non-normal distribution were expressed as a median and interquartile range [M (IQR)] and were compared using Kruskal-Wallis H-test; categorical variables were expressed as relative frequencies and were compared using the chi-squared test. N: number of the case recorded; RPL, recurrent pregnancy loss; RIF, repeated implantation failure; BMI, body mass index; WHR, waist-to-hip ratio; FPG, fasting plasma glucose; 1hPG, 1-hour post-load plasma glucose in oral glucose tolerance test (OGTT); 2hPG, 2-hour post-load plasma glucose in OGTT; AUCG, glucose area under the curve during OGTT; FINS, fasting insulin; 1hINS, 1-hour post-load insulin in OGTT; 2hINS, 2-hour post-load insulin in OGTT; AUCI, insulin area under the curve during OGTT; HOMA-IR, homeostasis model assessment for insulin resistance; HOMA-β, homeostasis model assessment for β-cell function; IFG, impaired fasting glucose; IGT, impaired glucose tolerance.

HOMA-IR = FINS×FPG /22.5; HOMA-β = FINS×20/(FPG-3.5); Insulin resistance was defined as HOMA-IR ≥ 2.1, FPG ≥ 6.1 mmol/L, 2hPG ≥ 7.8 mmol/L, and/or 2hINS > 1hINS.

	Control (N = 123)	Simple RPL (N = 212)	Complicated (N = 67)	Simple RIF (N = 123)	p-value
[a] compared with the Control group, $P < 0.05$;					
[b] compared with the clean RPL group, $p < 0.05$;					
[c] compared with RPL with RIF group, $p < 0.05$;					
[d] compared with the RIF group, $p < 0.05$.					

3.2 Glucose and insulin features of subjects

No significant differences in FPG were observed among the four groups, while the simple RPL group and the simple RIF group had significantly higher 2hPG than the control group ($P < 0.01$). Compared with the control group, both the simple RPL group and the complicated group had significantly higher FINS, HOMA-IR and HOMA-β, while the simple RIF group did not. The FINS, AUCI, HOMA-IR and HOMA-β of the simple RIF group were the lowest, with significantly lower levels than the simple RPL group and the complicated group. Meanwhile, the medians of those parameters of the simple RIF group were all lower than the control group though there was no statistical difference. The incidences of insulin resistance were significantly higher in both the simple RPL group and the complicated group than in the other two groups (Table 1).

After adjusted for age and WHR, compared with the control group, the other three groups had higher FINS and HOMA-IR. The simple RIF group had the highest FPG [adjusted-mean (95%CI), 5.20 (5.09–5.33) mmol/L] and lower FINS [adjusted-mean (95%CI), 10.77 (9.25–12.29) mU/L] and HOMA-β [adjusted-mean (95%CI), 127.76 (83.56-171.97)]. On the contrary, the clean RPL group had the highest FINS [adjusted-mean (95%CI), 12.09 (11.21–12.98) mU/L] and HOMA-β [adjusted-mean (95%CI), 189.74 (164.29-215.18)] and lower FPG [adjusted-mean (95%CI), 5.03 (4.97–5.10) mmol/L, Fig. 1].

3.3 Separate analysis among subjects without pregnancy loss history

The FPG, FINS, HOMA-IR and HOMA-β of study subjects in terms of times of pregnancy loss and implantation failure were shown in Fig. 2. None of these measures increased linearly with times of pregnancy loss or implantation failure.

Further separate analyses were conducted among subjects without any pregnancy loss history. The change of FPG did not show any significant trend with the times of implantation failure ($P = 0.379$, Fig. 2A and 2E). The FINS showed a significant increase with times of implantation failure among those patients with six and fewer times implantation failure history. However, the trend turned over as the FIN decreased dramatically ($P < 0.05$) in patients with more than six times implantation failure history (Fig. 2B

and 2F). Similar tendencies were observed in HOMA-IR and HOMA-β, although the result has no statistical significance.

4 Discussion

In this retrospective study, we investigated multiple glucose metabolism parameters in patients undergoing ART with RPL and/or RIF. We found the simple RPL group and the simple RIF group had significantly higher 2hPG than the control group. Patients with RPL history, no matter with or without RIF history, had significantly higher FINS, HOMA-IR and HOMA-β, while the FINS, AUCI, HOMA-IR and HOMA-β of the simple RIF group were at the lowest level. The incidences of insulin resistance were significantly higher in the simple RPL group and complicated group. After adjusted for age and WHR, FINS and HOMA-β were of the lowest level in the simple RIF group were the highest level in the simple RPL group. In the simple RIF group, the FINS tended to increase with times of implantation failure among those patients with six or fewer times implantation failure history, while those with more than six times implantation failure history have a significantly lower level of FINS, and similar tendencies were observed in HOMA-IR and HOMA-β but without significance.

The relationship between IR and idiopathic RPL is controversial. Studies revealed that patients with RPL have a significantly increased prevalence of IR than the fertile controls[13, 14, 21]. Other clinical investigations showed that IR may be the risk factor for early-stage embryonic miscarriage of PCOS patients[22–24]. Metformin, a sensitizer of insulin, has been reported to be contributive to the reduction of IR and reduction of miscarriage rate[22, 24]. However, Diejomaoh *et al* demonstrated that insulin resistance was not significantly associated with the recurrent spontaneous abortion of unknown aetiology[25]. Abnormal glucose levels may be also involved in the etiology of RPL. Diabetic women with good metabolic control were less likely than nondiabetic women to occur the loss of pregnancy, but diabetic women with elevated blood glucose and glycosylated hemoglobin levels in the first trimester have a significantly increased risk of having a spontaneous abortion[26]. Zolghadri *et al* found that patients with RSA were found to have an abnormal glucose tolerance test result compared with patients in the normal pregnancy group[27]. Romero *et al* demonstrated a glycemic control marker serum fructosamine was increased in women with RPL[28]. The results of our study was consistent with the above mentioned reports that IR was related to RPL.

Few studies involve the relation between IR and RIF directly. Studies found that ART outcomes such as implantation rate, pregnancy rate, and live birth rate were impaired in IR patients with PCOS[16–18], or even in IR patients without PCOS[29]. Chang *et al* found that the effects of hyperinsulinemia on endometrial function and implantation process maybe underlie the significant decrease in ART outcomes in IR patients with PCOS[16].

The hypothesized reasons of IR on ART failure in both RIF and RPL were that IR may be related to the decreased oocyte quality[30, 31], poor embryonic development[32, 33] and decreased endometrial receptivity[34–36].

As an important part of endometrial receptivity, the endometrial decidual process is fundamental for functional fetomaternal interface formation. It maintains tissue homeostasis in the process of endovascular trophoblast invasion and provides tissue resistance to stress signals, including protection against oxidative cell death[37]. Abnormal endometrial decidualization leads to abnormal implantation/placentation and ultimately to clinical disease such as abortion[38]. Frolova *et al* demonstrated lower glucose availability leads to lower decidualization marker expression[39], abnormal glucose metabolism leads to inefficient decidualization both *in vitro* and *in vivo.* That may be an important cause of incomplete embryo implantation and miscarriages[40]. IR and hyperinsulinemia may influence decidualization by multiple factors like prokineticin 1, forkhead-O1[41], insulin-like growth factor binding protein-1[42], hox gene A10[34] and endometrial protein PP14[43]. A study conducted by Hu *et al* on pregnant rats demonstrated that insulin resistance induced damage mediated by mitochondria as well as the subsequent imbalance of oxidative stress responses in the gravid uterus[44]. Oocyte quality is another key factor of female fertility, studies have suggested that a changed maternal metabolic environment leads to an abnormal follicular fluid microenvironment, and afterwards poor oocyte and embryo quality. Another study found ascending level of insulin coexisted with ascending C-reactive protein level in follicular fluid implying the status of inflammation and increased oxidative stress, which was associated with declined developmental potential in the oocyte[31]. Ou et al studied an insulin-resistant mouse model, finding that IR contributed to oxidative stress and damaged mitochondrial function in mouse oocytes, which may disturb the accurate transmission of mtDNA in intergeneration. Therefore, it resulted in poor oocyte quality and detrimental embryonic development of insulin-resistant mice[33, 45].

In the present study, the FINS levels of the simple RIF group had a significant decline with more than six times implantation failure history, and similar tendencies were observed in HOMA-IR and HOMA-β. This finding may indicate a dysfunction of the pancreatic β cells, which has been less studied. Only a few studies have reported the loss function of the pancreatic β cells were due to abnormal DNA methylation of CD4 T cells, T-reg cells, or NK cells in latent autoimmune diabetes in adults, and this abnormal glucose metabolism finally has adverse effects on pregnancy[46]. The autoimmune thyroid disease and autoimmune diabetes usually exist in the same individual due to a strongly shared genetic susceptibility[47]. Alecsandru *et al* studied patients with RIF or RPL in ART. All the patients had a normal metabolic state before ART under the indices of HbA1c, fasting glucose and insulin level. Eighty-five percent of these patients had thyroid autoimmune disease and a family history of diabetes mellitus (100%), and 70% had a low level of 2 hours' insulin secretion after OGTT. They deduced patients with agnogenic RPL or RIF with thyroid autoimmune disorders, family history of diabetes mellitus and abnormal insulin response after glucose load could be taken as the same subset of patients[48]. Thus, we can also suppose that insulin secretion impairment may be related to RIF.

Being a retrospective study, a well-designed prospective study would be necessary to clarify the role of IR in RPL and RIF.

5 Conclusions

In patients undergoing ART, insulin resistance may be a common etiopathogenesis of RPL and RIF and insulin secretion impairment may be related to RIF.

Abbreviations

IR: insulin resistance

RPL: recurrent pregnancy loss

RIF: recurrent implantation failure

ART: assisted reproductive technology

OGTT: the oral glucose tolerance test

AUGG: the area under the curve of glucose

AUGI: the area under the curve of insulin

HOMA-IR: the homeostasis model assessment for insulin resistance

HOMA-β: the homeostasis model assessment for β cell function

FINS: fasting insulin

WHR: waist-hip ratio

FPG: fasting plasma glucose

IVF: in vitro fertilization

ICSI: intracytoplasmic sperm injection

PCOS: polycystic ovary syndrome

Declarations

Ethics approval and consent to participate

The study was approved by the institutional review board of Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University (SYSEC-KY-KS-2020-030).

Consent for publication

Written informed consent for publication was obtained from all participants.

Availability of data and materials

The data sets supporting the results of this article are included within the article and its additional files.

Competing interests

The authors declare that they have no competing interests.

FUNDING

This study was supported by National Natural Science Foundation of China (81771545), the Guangdong Basic and Applied Basic Research Foundation (2020B1515020001), the Special Fund for Clinical Research of Chinese Medical Association (18010180747), and the 5010 grants of Sun Yat-sen University (SYSU2019003).

Authors' contributions

Miao Ding, Fengyi He, Xiaojia Li, Sushi Jiang collected and analyzed the data, wrote the manuscript; Yacong Cao and Yanting Zou helped perform the analysis with constructive discussions; Dongzi Yang and Xiaomiao Zhoa designed the study and revised the manuscript. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

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Figures

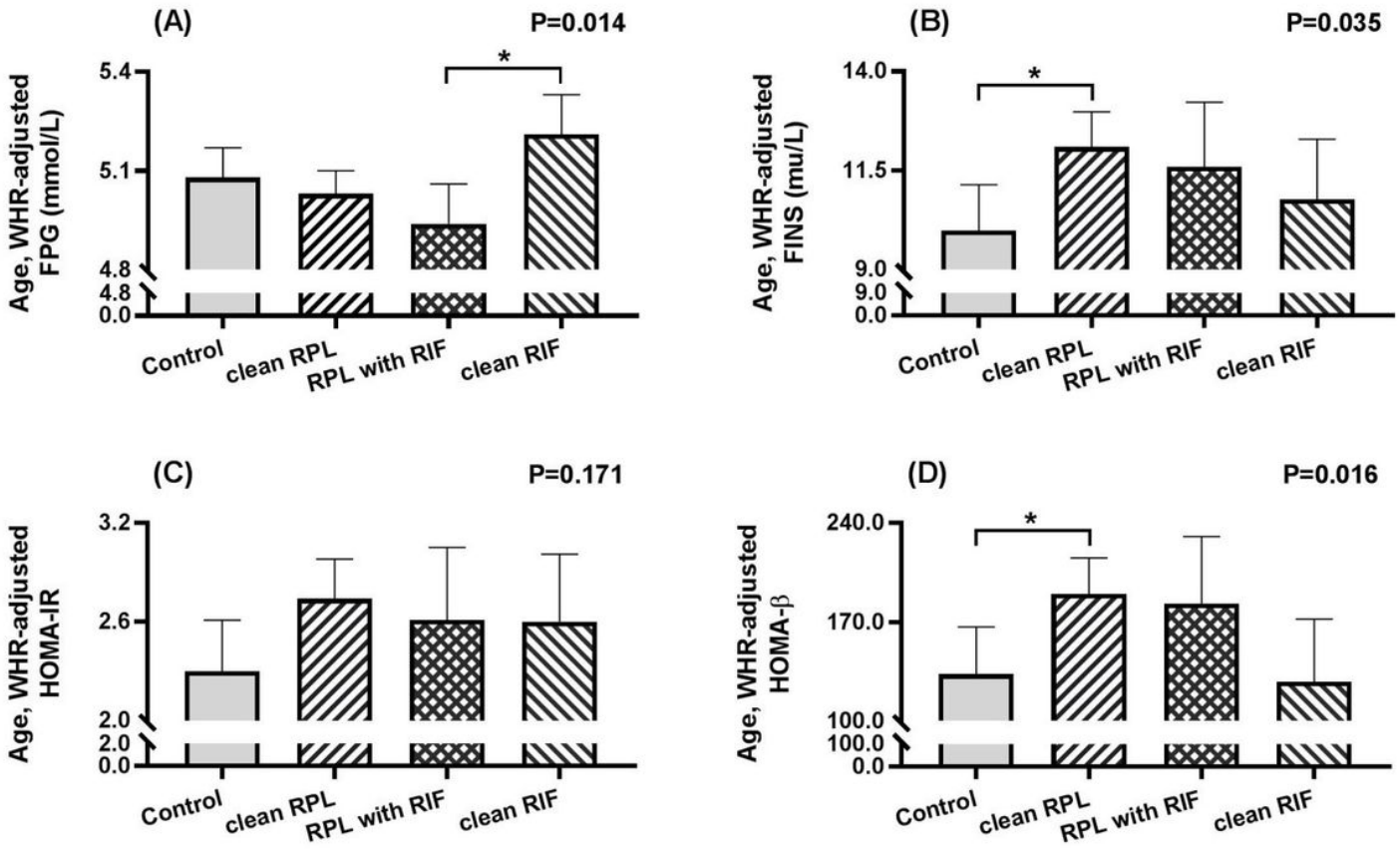

Figure 1

The comparisons of adjusted-glucose and insulin measures of the study subjects The bars (errors) indicate the means (95%CI). The comparison analysis was performed by ANCOVA test, adjusting for age and WHR. Non-normally distributed variables were log-transformed before statistical disposal. * Bonferroni corrected p-value<0.05. RPL, recurrent pregnancy loss; RIF, repeated implantation failure; WHR, waist-to-hip ratio; FPG, fasting plasma glucose; FINS, fasting insulin; HOMA-IR, homeostasis model assessment for insulin resistance; HOMA-β, homeostasis model assessment for β-cell function.

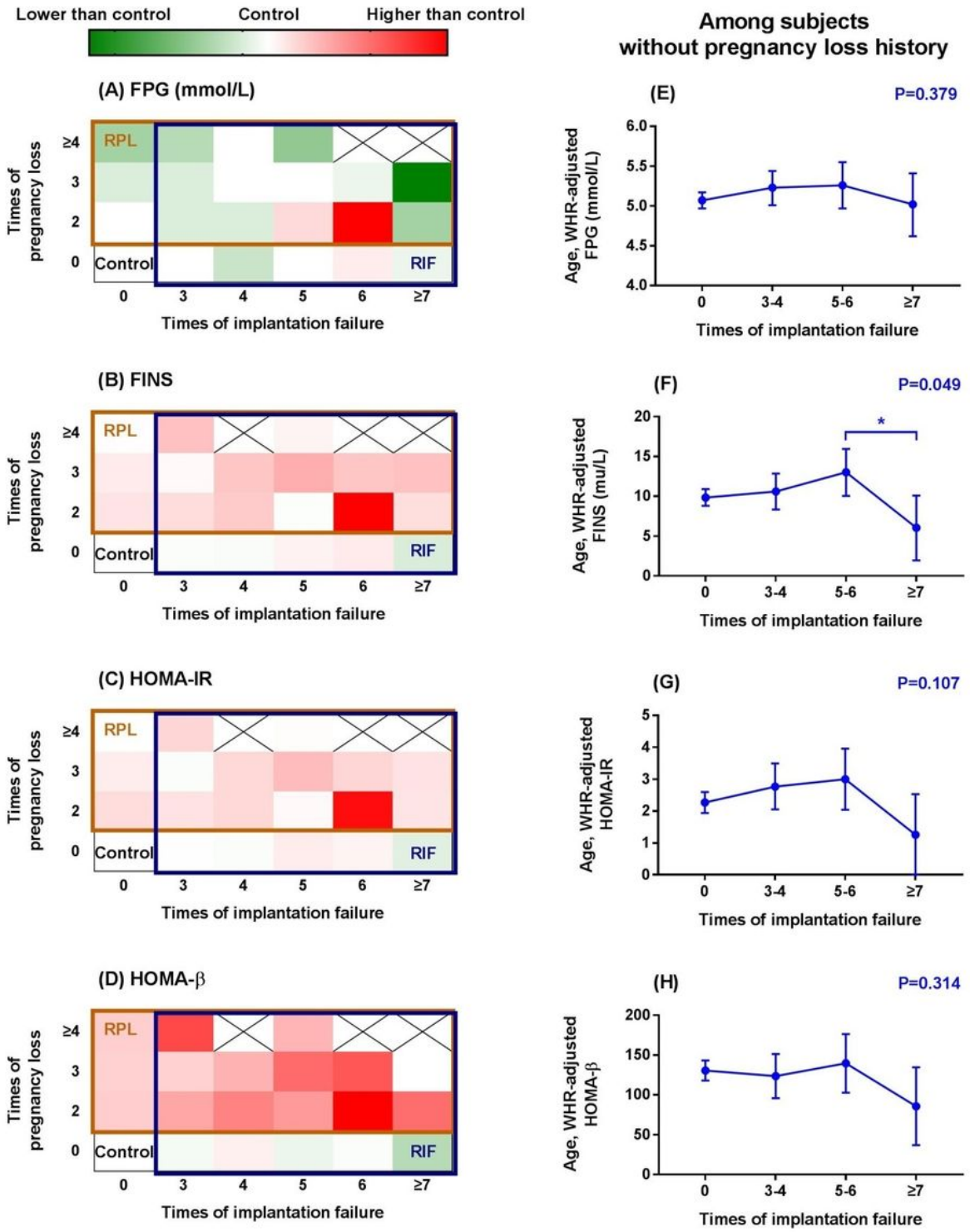

Figure 2

Glucose and insulin measures of study subjects according to times of pregnancy loss and implantation failure The heat maps (A-D) indicate the crude medians of glucose and insulin measures of all subjects. The line charts (E-H) indicate the multiple-adjusted means (95%CI) of measures of subjects without pregnancy loss history. The comparison analysis was performed by ANCOVA test, adjusting for age and WHR. Non-normally distributed variables were log-transformed before statistical disposal. * P<0.05. RPL,

recurrent pregnancy loss; RIF, repeated implantation failure; FPG, fasting plasma glucose; FINS, fasting insulin; HOMA-IR, homeostasis model assessment for insulin resistance; HOMA-β, homeostasis model assessment for β-cell function.