

Original Paper

Peripheral Blood *MUC4* Gene Expression and Clinical Correlates in Iraqi Women with Recurrent Pregnancy Loss: A Case–Control Study

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ABSTRACT

Recurrent pregnancy loss (RPL), the loss of two or more pregnancies, affects 1–5% of couples and remains unexplained in about half of cases. Mucin 4 (*MUC4*) contributes to the anti-adhesive glycocalyx of the endometrial epithelium, but its expression has not been examined in Iraqi women with RPL. This study assessed *MUC4* mRNA expression in peripheral blood and its relationship with clinical and socio-demographic variables in women with RPL. In a case–control study conducted at Al-Yarmouk Hospital between January and June 2025, 50 women with RPL and 40 fertile controls were enrolled. Demographic, clinical and anthropometric data were collected with a structured questionnaire. *MUC4* mRNA was quantified in peripheral blood by quantitative reverse-transcription PCR (qRT-PCR), with *GAPDH* as the reference gene, and analysed by the $2^{-\Delta\Delta Ct}$ method. Women with RPL had a mean of 2.94 ± 1.12 pregnancy losses and a mean BMI of 26.7 ± 4.8 kg/m². Within the RPL group, the number of losses differed across BMI categories ($p = 0.034$) but not across age groups ($p = 0.078$), and economic status was not associated with the number of losses ($p = 0.164$). *MUC4* expression was markedly higher in women with RPL than in controls (fold change 195.4; 95% CI 164.2–266.5; $p < 0.0001$). Hypertension and diabetes were associated with a higher number of losses (OR = 1.85 and 1.73, respectively; $p < 0.05$). Higher BMI and increased peripheral-blood *MUC4* expression were therefore associated with RPL in this cohort. These findings are preliminary: the diagnostic value of *MUC4* and its relevance to endometrial receptivity require confirmation in larger studies with endometrial sampling and protein-level validation.

KEYWORDS: Recurrent pregnancy loss, *MUC4*, Gene expression, qRT-PCR, Body mass index, Iraq.

1-INTRODUCTION

Recurrent pregnancy loss (RPL), defined as the loss of two or more pregnancies before 24 weeks of gestation, affects 1–5% of couples attempting to conceive [1,2]. Its aetiology is multifactorial and involves genetic, immunological, endocrine, anatomical and environmental determinants [3]. Parental chromosomal rearrangements account for only a small proportion of cases, and in roughly half of couples no cause is identified despite full evaluation [4]. Successful implantation requires synchrony between a viable blastocyst and a receptive endometrium during the window of implantation (WOI). Ovarian steroids remodel the endometrial epithelium morphologically and molecularly, converting a non-receptive surface into one that permits blastocyst attachment [5,6].

Transmembrane mucins, including mucin 4 (*MUC4*, encoded by the *MUC4* gene), form the heavily glycosylated glycocalyx of the endometrial luminal epithelium. Outside the WOI this barrier protects the epithelium against microbial invasion, enzymatic degradation and mechanical stress and prevents premature cell-to-cell attachment; its down-regulation in the luminal epithelium is a prerequisite for embryo attachment [5]. In the human endometrium, *MUC1* and *MUC16* are the predominant transmembrane mucins, whereas *MUC4* is expressed at low and variable levels, and most evidence for a role of *MUC4* in implantation comes from animal models, so human

data remain limited [7]. Persistence of the mucin barrier during the WOI has been proposed as a mechanism of implantation failure and early pregnancy loss [5,7]. *MUC4* polymorphisms have been associated with endometriosis and endometriosis-related infertility, and mucin up-regulation is thought to favour adhesion and immune evasion of ectopic endometrial lesions [8,9].

To our knowledge, *MUC4* expression has not been examined in women with RPL, and no data are available for the Iraqi population. Because endometrial biopsy is invasive, peripheral blood offers an accessible sample, although *MUC4* transcripts in blood cells are normally scarce and their relationship to endometrial expression is unknown. The present study therefore evaluated *MUC4* mRNA expression in the peripheral blood of Iraqi women with RPL compared with fertile controls, and examined the association of age, BMI, socio-economic status and health conditions with the number of pregnancy losses.

2-MATERIALS AND METHODS

2.1. Study design and participants

A case–control study was conducted at Al-Yarmouk Teaching Hospital, Baghdad, between January and June 2025. Fifty women with RPL and 40 fertile women were enrolled. Eligible cases were aged 18–45 years and had experienced at least two pregnancy losses documented by ultrasound before 20 weeks of gestation. Women with known uterine anatomical abnormalities, endocrine disease (polycystic ovary syndrome, hyperprolactinaemia, thyroid dysfunction), autoimmune disease (antiphospholipid syndrome) or parental karyotype abnormalities were excluded. Controls were fertile women with at least one live birth and no history of pregnancy loss.

The study was approved by the Institutional Review Board of Al-Yarmouk Hospital (approval No. FM.SA.45, dated 22/12/2024) and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants before data and blood collection.

2.2. Data collection

Demographic (age, socio-economic status, education level, employment), clinical (number of pregnancy losses, number of live births, previous caesarean sections, hypertension, diabetes) and family-history data were recorded with a structured questionnaire; family history was coded as present or absent. Height and weight were measured at recruitment, and BMI (kg/m^2) was categorised according to WHO criteria (underweight < 18.5 ; normal 18.5–24.9; overweight 25.0–29.9; obese ≥ 30.0).

2.3. RNA extraction and qRT-PCR

Three millilitres of peripheral venous blood were collected into EDTA tubes from all participants. Controls were sampled in the early follicular phase (cycle days 2–5) and cases at least 6–8 weeks after the last miscarriage, to avoid acute post-abortion hormonal effects. Total RNA was extracted with TRIzol reagent (Thermo Fisher Scientific, USA). RNA concentration and purity were assessed with a NanoDrop spectrophotometer (A260/A280 1.8–2.0), and samples were stored at -80°C . cDNA was synthesised with PrimeScript RT Master Mix (Takara Bio Inc., Japan) according to the manufacturer's instructions.

qRT-PCR was performed with MonadAmp SYBR Green qPCR Mix (Monad Biotech Co., Ltd, China) on a real-time PCR system in a 20 μL reaction containing 10 μL master mix, 0.4 μM of each primer, 2 μL cDNA and nuclease-free water. Cycling conditions were 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 30 s, and a melting-curve step. *MUC4* primers (forward 5'-CGTTCTGGGACGATGCTGAC-3'; reverse 5'-GATGGCTTGCTAGGTGTTGCT-3') were taken from Li et al. [10], and *GAPDH* primers (forward 5'-GGAGCGAGATCCCTCCAAAAT-3'; reverse 5'-GGCTGTTGTCATACTTCTCATGG-3') from Strzalka-Mrozik et al. [11]. Relative expression was calculated by the $2^{-\Delta\Delta\text{Ct}}$ method with *GAPDH* as the reference gene.

2.4. Statistical analysis

Analyses were performed with IBM SPSS version 26.0. Normality was assessed with the Shapiro–Wilk test. Continuous variables are reported as mean $\pm$ SD or median (range) and categorical variables as n (%). Cases and

controls were compared with Student's t-test or the Mann–Whitney U test for continuous variables and the chi-square test for categorical variables. Within the RPL group, differences in the number of pregnancy losses across age and BMI categories were examined by one-way ANOVA with eta-squared (η^2) as the effect size. Associations of socio-demographic factors, family history and health conditions with the number of losses were examined by chi-square tests and multivariable logistic regression adjusted for age and BMI, reported as odds ratios (OR) with 95% confidence intervals (CI). ΔCt values were compared between groups with Student's t-test, and the fold change ($2^{\Delta\Delta\text{Ct}}$) is reported with its 95% CI. Statistical significance was set at $p < 0.05$.

3-RESULTS

3.1. Characteristics of the participants

Fifty women with RPL and 40 fertile controls were included. The characteristics of the RPL group are summarised in Table 1. Mean age was 30.45 ± 5.62 years (range 22–44 years). The women had experienced a mean of 2.94 ± 1.12 pregnancy losses (median 3) and had a mean of 1.76 ± 0.98 live births and 0.94 ± 1.12 previous caesarean sections. Mean BMI was 26.70 ± 4.80 kg/m², within the overweight range, and was significantly higher than that of the fertile controls (22.44 ± 1.02 kg/m²; $p < 0.001$). Thirty-two women (64.0%) were of middle or low socio-economic status, 21 (42.0%) were employed, 9 (18.0%) had hypertension and 7 (14.0%) had diabetes.

Table (1) Characteristics of women with recurrent pregnancy loss (n = 50)

Variable	n (%)	Mean $\pm$ SD	Median (range)
Age (years)	–	30.45 ± 5.62	29.00 (22–44)
BMI (kg/m ²)	–	26.70 ± 4.80	25.80 (18.9–34.2)
Number of pregnancy losses	–	2.94 ± 1.12	3.00
Number of live births	–	1.76 ± 0.98	2.00 (0–5)
Previous caesarean sections	–	0.94 ± 1.12	1.00 (0–3)
Economic status (middle/low)	32 (64.0%)	–	–
Hypertension	9 (18.0%)	–	–
Diabetes	7 (14.0%)	–	–

BMI: body mass index; SD: standard deviation. –: not applicable.

3.2. Age, BMI and the number of pregnancy losses

Within the RPL group, one-way ANOVA showed that the number of pregnancy losses differed across BMI categories ($p = 0.034$) but not across age groups ($p = 0.078$) (Table 2). Women in the higher BMI categories had a higher mean number of losses. Given the modest sample size and the absence of adjustment for confounders, this finding indicates an association rather than a causal effect.

Table (2) Association of age and BMI categories with the number of pregnancy losses in women with recurrent pregnancy loss (one-way ANOVA, n = 50)

Factor	df	P-value	Interpretation
Age group	4, 45	0.078	Not significant
BMI category	3, 46	0.034	Significant

BMI: body mass index; df: degrees of freedom (between groups, within groups).

3.3. Socio-demographic factors, family history and health conditions

Economic status (low vs. high/medium) was not significantly associated with the number of pregnancy losses (OR = 1.78, 95% CI 0.78–4.06; $p = 0.164$), whereas a positive family history (OR = 3.26, 95% CI 1.84–5.82; $p = 0.001$) and a lower education level (OR = 2.78, 95% CI 1.45–5.33; $p = 0.002$) were associated with a higher number of losses (Table 3). Hypertension (OR = 1.85, 95% CI 1.07–3.20; $p < 0.05$) and diabetes (OR = 1.73, 95% CI 1.02–2.94; $p < 0.05$) were also associated with a higher number of losses.

Table (3) Socio-demographic predictors of the number of pregnancy losses in women with recurrent pregnancy loss (multivariable logistic regression, n = 50)

Variable	Comparison	OR	95% CI	P-value
Family history	Present vs. absent	3.26	1.84–5.82	0.001
Education level	Low vs. high	2.78	1.45–5.33	0.002
Economic status	Low vs. high/medium	1.78	0.78–4.06	0.164

OR: odds ratio adjusted for age and BMI; CI: confidence interval.

3.4. *MUC4* gene expression in women with RPL

MUC4 amplification and melting curves are shown in Figure 1. The *MUC4* melting curve showed a predominant peak within the expected range (approximately 78–82 °C), consistent with amplification of the intended product. Relative to fertile controls, *MUC4* expression was 195.36-fold higher in women with RPL (95% CI 164.20–266.52; $p < 0.0001$) (Table 4).

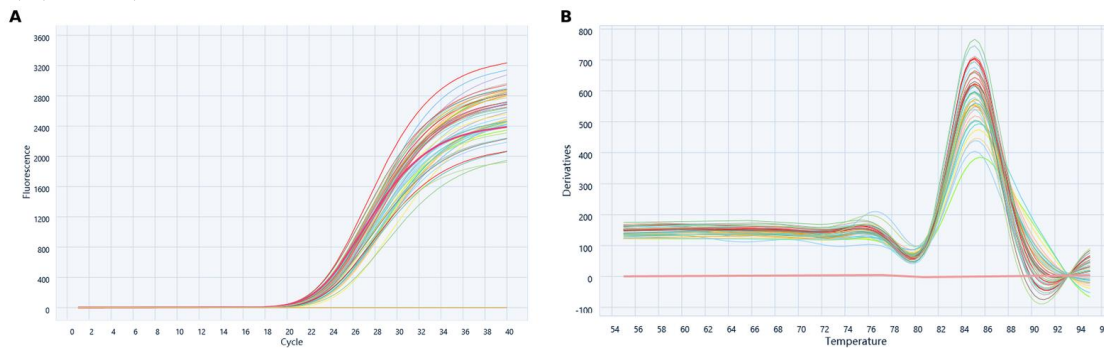


Figure (1): qRT-PCR profiles of *MUC4* in the control and RPL groups. (A) Amplification curves; (B) melting curve of the *MUC4* product.

Table (4) Relative expression of *MUC4* in women with recurrent pregnancy loss compared with fertile controls ($2^{-\Delta\Delta Ct}$ method)

Group (n)	ΔCt (mean $\pm$ SD)	$\Delta\Delta Ct$ (mean)	Relative expression ($2^{-\Delta\Delta Ct}$)	95% CI of fold change
Control (40)	19.09 $\pm$ 0.82	0.00	1.00	0.82–1.21
RPL (50)	11.48 $\pm$ 0.52	-7.61	195.36	164.20–266.52

ΔCt : $Ct(MUC4) - Ct(GAPDH)$; CI: confidence interval; RPL: recurrent pregnancy loss. ΔCt was compared between groups by Student's t-test; $p < 0.0001$, RPL vs. control.

4-DISCUSSION

In the present study, BMI was associated with the number of pregnancy losses within the RPL group ($p = 0.034$), and the mean BMI of women with RPL (26.70 ± 4.80 kg/m²) was higher than that of controls (22.44 ± 1.02 kg/m²). Elevated BMI is an established risk factor for miscarriage: a meta-analysis found the odds of miscarriage to be 1.67 times higher in obese women than in women of normal weight, with insulin resistance, altered progesterone action and impaired oocyte quality among the proposed mechanisms [12]. Low-grade systemic inflammation associated with adiposity is also thought to impair endometrial receptivity [6].

By contrast, no association was found between age group and the number of losses ($p = 0.078$). Maternal age is a major determinant of miscarriage risk through declining oocyte quality and increasing embryonic aneuploidy [13]; the absence of an effect here most likely reflects the narrow, young age range of the sample (30.45 ± 5.62 years), which limits contrasts with women over 40. Economic status was likewise not associated with the number of losses ($p = 0.164$), although lower socio-economic status has been linked to adverse pregnancy outcomes through limited access to antenatal care and higher physiological stress [14,15]. In this cohort, clinical and biological factors such

as BMI appear to matter more than socio-economic determinants, but the small sample and the within-case design limit this conclusion.

The principal molecular finding was a marked up-regulation of *MUC4* mRNA in the peripheral blood of women with RPL compared with fertile controls ($p < 0.0001$). In the endometrium, persistence of an anti-adhesive mucin barrier during the WOI would be expected to hinder blastocyst attachment, and altered patterns of adhesion-molecule expression have been described in the endometrium of women with recurrent miscarriage [16]. Multi-omics studies have also implicated dysregulated immune and adhesion pathways at the maternal–fetal interface in RPL [17]. However, *MUC4* was measured here in blood rather than in endometrial tissue, and the relationship between circulating leukocyte *MUC4* transcripts and endometrial *MUC4* expression is unknown. The present results therefore identify an association that requires confirmation at the tissue and protein level before any mechanistic interpretation can be made.

This study has several limitations. It was conducted at a single centre with a modest sample size and no *a priori* power calculation. The case–control design does not permit causal inference, and the analyses of age, BMI and socio-economic factors were performed within the RPL group without adjustment for confounders. *MUC4* expression was assessed in peripheral blood rather than in the endometrium, with a single reference gene (*GAPDH*) and without protein-level validation (for example, ELISA or western blotting); the very large fold change and the reference-gene Ct values require confirmation with efficiency-corrected, replicated assays. Multicentre studies with endometrial sampling timed to the window of implantation are recommended.

CONCLUSION

Within this cohort of Iraqi women, higher BMI and increased peripheral-blood *MUC4* expression were associated with recurrent pregnancy loss. These preliminary findings suggest that *MUC4* merits further investigation as a marker of altered endometrial receptivity in RPL, but its diagnostic value and biological relevance must be established in larger studies with endometrial and protein-level analyses.

DECLARATIONS

Ethical approval: The study was approved by the Institutional Review Board of Al-Yarmouk Hospital (approval No. FM.SA.45, dated 22/12/2024) and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

Conflicts of interest: The authors declare no conflict of interest.

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