



# Uterine and Serum Glycodelin Concentration in Recurrent Implantation Failure Versus Normal Fertile Women on Implantation Window

Robabeh Taheripناه<sup>1</sup>, Marzieh Zamaniyan<sup>2\*</sup>, Shahrzad Akhoondzadeh<sup>1</sup>, Anahita Taheripناه<sup>3</sup>, Narges Malih<sup>4</sup>

## Abstract

**Objectives:** Glycodelin is a factor which regulates immunological activity and is required in implantation window. The present study was conducted to compare glycodelin concentrations in blood and uterine flushing samples from women with in vitro fertilization (IVF) failure and fertile controls.

**Materials and Methods:** This was a prospective clinical trial including 20 women with IVF failure and 19 fertile participants. Both groups initially filled a special questionnaire including their demographic profile and prior failed IVF attempts. Then samples of uterine flushing and blood samples were taken to measure glycodelin concentrations.

**Results:** There were significant differences between mean glycodelin concentrations in the case and control groups, both in serum glycodelin ( $30.1 \pm 5.30$  vs.  $44.5 \pm 11.85$  ng/mL,  $P < 0.001$ ), and uterine flushing glycodelin concentrations ( $310.0 \pm 67.51$  vs.  $498.3 \pm 119.01$  ng/mL,  $P < 0.001$ ). The mean total glycodelin concentration in uterine flushing was higher than serum in both groups ( $399.4 \pm 133.95$  vs.  $36.9 \pm 1.52$  ng/mL,  $P < 0.001$ ).

**Conclusion:** We found a significant decrease in glycodelin concentrations among patients with IVF failure. Since glycodelin has a role in immune system during endometrial receptivity, therefore, it may be required for prevention of IVF failures.

**Keywords:** Embryo implantation, Endometrium, Fertilization in vitro, Glycodelin, Infertility

## Introduction

Glycodelin is a glycoprotein related to the lipocalin family. Other names for this glycoprotein are leukemia inhibitory factor, placenta protein 14 (leukemia inhibitory factor) and pregnancy-associated endometrial alpha 2-globulin (1). Based on three-dimensional molecule glycosylation properties of glycodelin, it has 3 different isoforms: type A, type F, and type S. Type A is secreted in uterine cavity by glandular cells and secretory-decidualized endometrium. Glycodelin A is a major glycoprotein involved in progesterone regulation. It has 2 different effects in fertile and implantation windows. In the fertile window period, glycodelin has inhibitory effects on the sperm-egg bindings; therefore, in fertile women there is a decrease in glycoprotein level to allow sperm-egg fertilization and ovum formation. In fact, at this time, glycodelin A inhibits sperm-egg binding in a dose-dependent form and has contraceptive effects. However, during the implantation window in the luteal phase on day 21 to 24 of reproductive cycles, glycodelin is an obligatory factor for fertility and acts as a marker of endometrial receptivity and implantation. The glycoprotein is required in implantation

window to inhibit the activity of natural killer (NK) cells and to increase the fetomaternal surface for prevention of fetal loss, as a semi allograft transplant (2).

In assisted reproduction, embryo settings and transfer methods have improved over the recent years, but the conception rates have not improved. It is suggested that about 10% of patients who undergo in vitro fertilization (IVF) cycles will experience some problems (3).

Due to the large number of IVF failures and high cost of IVF treatments and also the importance of glycodelin as an essential factor in endometrial receptivity, this study was conducted to compare the glycodelin in blood samples and also fluid from uterine flushing among patients with IVF failure versus fertile women. It should be noted that no such study has been performed in this field among Iranian women.

## Materials and Methods

This prospective clinical trial was performed from February 2012 till September 2012, in Imam Hussein Hospital, Tehran, Iran. A specialized questionnaire including demographic profile and prior failed IVF

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<sup>1</sup>Infertility and Reproductive Health Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. <sup>2</sup>Infertility center, Department of Obstetrics and Gynecology, Mazandaran University of Medical Sciences, Sari, Iran. <sup>3</sup>Department of Molecular and Cellular Sciences, Faculty of Advanced Sciences and Technology Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran. <sup>4</sup>Department of Health and Community Medicine, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

\*Corresponding Author: Marzieh Zamaniyan, Infertility center, Department of Obstetrics and Gynecology, Mazandaran University of Medical Sciences, Sari, Iran. Tel: +982122712234, Email: marziehzamaniyan@gmail.com



attempts was completed and informed consent from both case and control groups was obtained after explaining the safety of procedures for the participants. In total, 20 patients in the age range of 20 to 40 years old with IVF failure (at least 2 IVF failure cycles) were enrolled. The control group consisted of 19 fertile women including different hospital personnel who were in the same age range as the case group.

All participants were visited on days 21-22 of their menstrual cycles. After that a transvaginal ultrasound was requested to rule out the presence of polyps or uterine myoma. Participants, who had polyps, uterine myoma on ultrasound examination, history of hormone usage in the last 2 months, or active vaginal infection on speculum examination, were excluded from the study. A Pap smear sample was taken freely as a prize for all participants in the control group.

In both groups, a disposable speculum was entered into the vagina in lithotomy position and then 10 cc normal saline was injected in 2 stages into the uterine cavity using a uterine specific cannula (in each stage 5 cc was injected into uterine). The returned liquid from the uterus during the uterine flushing was collected. Also 5 cc blood clot samples were taken from both groups and centrifuged for 10 minutes. Then the serum was separated and the level of glycodeilin was determined using enzyme-linked immune sorbent assay (ELISA) with a Glycodeilin kit (Germany, BIOSERVE Company).

**Statistical Analysis**

The data analysis was done by SPSS version 21.0 (IBM Corp., Armonk, NY). *T* test was used to compare the results between the groups. The proportions were compared using chi-squared test. The data were reported as mean ± standard deviation (SD) and *P* values less than 0.05 were considered statistically significant.

**Results**

Demographic data for participants in the case and control groups are shown in Table 1. In the present study, 20 patients with IVF failure and age range of 23-40 years and 19 patients in the control group with age range of 22-40 years completed the study. There was no difference regarding the mean age in case and control group (33.5 ± 4.96 vs. 32.1 ± 5.88; Table 1).

Infertility factors in IVF failure group included tubal

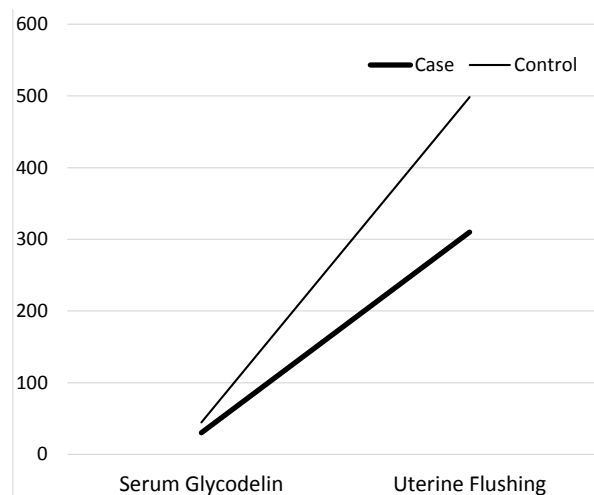
(n=4), male (n=8), unexplained (n=3) and polycystic ovary syndrome (PCOS) (n=5). The number of previous failed IVF cycles was 2 (n=5), 3 (n=12), 4 (n=2) and 5 (n=1) cycles.

There were significant differences between glycodeilin concentrations in the case and control group, both in serum (*P*<0.001) and uterine flushing (*P*<0.001). Total glycodeilin concentrations in uterine flushing was higher than in the serum in both groups (*P*<0.001; Table 1). Another finding was that serum glycodeilin levels increased in parallel with uterine flushing fluid glycodeilin levels (Figure 1).

**Discussion**

In this study, there was a significant difference in glycodeilin concentrations in serum samples and uterine flushing samples among IVF failure patients compared with the control group on days 21-23 of menstrual cycle. We found that, both serum and uterine flushing fluid glycodeilin concentrations were lower among patients with repeated IVF failures compared to the control group. We also found that, serum glycodeilin levels increased in parallel with uterine flushing fluid glycodeilin levels.

Salim et al measured the glycodeilin concentrations in uterine flushing fluid during the implantation window among 20 women with a history of recurrent pregnancy loss in the first half of their pregnancy versus 16 fertile



**Figure 1.** Correlation Between Uterine Flushing and Serum Glycodeilin Concentrations.

**Table 1.** Baseline Characteristics and Uterine Flushing and Blood Glycodeilin Concentrations Among Participants

Variables	Case group (n=20)	Control group (n=19)	Total Mean ± SD	P
	Mean ± SD	Mean ± SD		
Age (y)	33.5 ± 4.96	32.1 ± 5.88	32.9 ± 5.39	>0.05
Serum glycodeilin (ng/mL)	30.1 ± 5.30	44.5 ± 11.85	36.9 ± 11.52	<0.001
Uterine flushing glycodeilin (ng/mL)	310.0 ± 67.51	498.3 ± 119.01	399.4 ± 133.95	<0.001

women and 8 women with septate uteri (4). Similar to our findings, in their study the uterine flushing glycodelin concentrations among women at risk of early pregnancy failure was significantly lower than the other 2 groups. Underline immunological mechanisms for early pregnancy failure and repeated IVF failures were similar in our study and the study by Salim et al, but they did not measure the serum glycodelin concentrations. Glycodelin is an immunological factor regulating the implantation in endometrial cells and creating a satisfactory environment for growth of the pinopodes, which could explain the different concentrations between the case and the control groups.

We found a significant difference between glycodelin concentrations in uterine flushing and the serum in both the case and the control groups, which reinforces the theory that glycodelin is derived from the endometrial cells for the embryo implantation at the implantation window and acts as an endometrial receptivity factor for conception and prevents the rejection of the embryo (5).

Elbehery et al have reported that glycodelin levels are lower in patients with uterine polyps than controls, which improves postoperatively. This could explain the underlying mechanisms by which endometrial receptivity is disturbed in the presence of endometrial polyps (6).

Douglas et al have reported their assessment of serum glycodelin and Insulin-like growth factor binding protein 1 (IGFBP-1) in donor egg recipients undergoing IVF. They found that, the glycodelin/IGFBP-1 ratio on the day of embryo transfer was higher in egg recipients who had a successful pregnancy. At LH + 17, glycodelin was higher, and IGFBP-1 was lower among women who reached pregnancy compared to those who did not. These differences seem to be due to endometrial changes induced by effective implantation (7).

Bastu et al have evaluated the expression of Mucin 1 (MUC-1) and glycodelin A (GdA) in the endometrium and blood samples throughout the implantation window. Both blood and tissue measurements of MUC-1 and GdA were significantly lower in women with repeated implantation failures than controls during the implantation window. They also found a significant relationship between blood versus tissue levels of both MUC-1 and GdA. They suggested that receptivity could be evaluated with noninvasive blood test, rather than endometrial biopsy, as the serum and tissue levels of MUC-1 and GdA are much correlated (8). This was similar to our finding indicating that serum glycodelin levels increased in parallel with uterine flushing fluid glycodelin levels. The relatively small population of participants might be considered as a limitation of the present study which may have affected our outcomes.

## Conclusion

We found a significant decrease in glycodelin concentrations among patients with IVF failure compared

to fertile women. Since glycodelin acts as a protein regulator for immune system during endometrial receptivity, therefore, it may be required for prevention of the IVF failures.

## Ethical Issues

This study was approved by the Ethics Committee of Infertility and Reproductive Health Research Center (IRHRC), Shahid Beheshti University of Medical Sciences, Tehran, Iran (SBMU.RAM.REC.1386.16). Registration ID in IRCT was [IRCT2016082120408N5](https://www.irct.ir/trial/2016082120408N5). This was a prospective clinical trial study and written informed consent was taken from the women who underwent uterine or blood sampling according to mentioned protocols.

## Conflict of Interests

The authors have no conflict of interests with the subject matter of the manuscript.

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