Available online at www.sciencedirect.com
ScienceDirect
European Journal of Obstetrics & Gynecology and Reproductive Biology 133 (2007) 76–80
ejog European Journal of Obstetrics & Gynecology and Reproductive Biology
www.elsevier.com/locate/ejogrb

A comparative study of glycodelin concentrations in uterine flushings in women with subseptate uteri, history of unexplained recurrent miscarriage and healthy controls

R. Salim [a,*], J. Miel [b], M. Savvas [a], C. Lee [a], D. Jurkovic [a]

[a] *The Early Pregnancy & Gynaecology Assessment Unit, King's College Hospital, London, UK*
[b] *Department of Medicine, GKT Medical School, King's College, London, UK*

Received 17 November 2005; received in revised form 7 September 2006; accepted 8 September 2006

Abstract

Objective: To compare the concentration of glycodelin in uterine flushing at the implantation window obtained from women with subseptate uteri, women with a history of recurrent first trimester miscarriage and fertile controls.
Study design: Glycodelin concentration was assessed using Enzyme Linked Immunohistochemistry (ELISA) at The Early Pregnancy & Gynaecology Assessment Unit, King's College Hospital, London, England. Eight women with a subseptate uterus, 20 women with a history of unexplained recurrent first trimester miscarriage and 16 fertile controls had uterine cavity flushing, for glycodelin concentration, done 7 days after the luteinising hormone surge.
Results: Glycodelin concentrations in uterine flushing obtained from women with subseptate uteri (n =8) (median 32.9 ng/ml, range 17.1–52.4 ng/ml) and recurrent miscarriage (n =20) (median 26.8 ng/ml, range 9.7–78.5 ng/ml) were significantly lower than in the control group (n = 16) (median 67.7 ng/ml, range 59.0–77.6 ng/ml) ($\chi^2 = 19.565$, $p < 0.001$).
Conclusion: Peri-implantation levels of glycodelin are lower in women at high risk of early pregnancy failure.

Keywords: Subseptate uterus; Recurrent miscarriage; Glycodelin

1. Introduction

Glycodelin, previously known as placental protein 14, is one of the most abundantly secreted endometrial glycoproteins [1]. However, its expression is limited from the mid to late luteal phase of the menstrual cycle, which indicates that glycodelin may play a physiological role in facilitating implantation and early pregnancy development [2]. Previous studies have shown that glycodelin has potent immunosuppressive properties and has been shown to suppress both lymphocyte function and cytokine production[3,4]. In early pregnancy development, the role of glycodelin is thought to be to provide a microenvironment that protects the conceptus from the maternal immune system [5]. In women at high risk of miscarriage, such as those with a history of recurrent first trimester miscarriages, the expression of glycodelin is significantly lower in the mid to late luteal phase of the menstrual cycle, supporting the hypothesis for its role in early pregnancy development [6].

The subseptate uterus is a congenital uterine anomaly associated specifically with a high risk of first trimester miscarriage [7]. Recent studies have shown that women with an incidental finding of subseptate uterus on ultrasound scan are three times more likely to experience early pregnancy loss compared to women with normal uteri [8]. In women with a history of recurrent miscarriages, the prevalence of subseptate uterus is three times higher compared to general population of women [9]. In addition, the distortion of uterine cavity is more severe in women with recurrent

* Corresponding author at: The Early Pregnancy & Gynaecology Assessment Unit, Suite 8 Golden Jubilee Wing, King's College Hospital, Denmark Hill, London SE5 9PJ, UK. Tel.: +44 207 346 3168; fax: +44 207 346 3471.
E-mail address: rehan.salim@talk21.com (R. Salim).

0301-2115/$ – see front matter
doi:10.1016/j.ejogrb.2006.09.005

pregnancy loss in comparison to those with an incidental diagnosis of subseptate uterus [9]. Although there is a substantial body of evidence showing that subseptate uterus is associated with adverse pregnancy outcomes, no study has been performed so far to assess the physiological function of endometrium in women with subseptate uteri. The aim of this study was to investigate the expression of glycodelin in women with subseptate uteri compared to women with recurrent first trimester miscarriages and fertile controls, in order to obtain indirect information about possible differences in uterine environment and conditions for early pregnancy development.

2. Materials and methods

Eight women with an incidental diagnosis of subseptate uterus on ultrasound scan were included in the study. Twenty women with a history of unexplained recurrent first trimester miscarriages were also included in the study. Recurrent miscarriage was defined as three consecutive pregnancy losses which occurred before 12 complete weeks' gestation. Women in this subgroup had no evidence of thrombophilia, had normal karyotype and had no anatomical uterine abnormality found on ultrasound examination. A control group of 16 parous women who were awaiting sterilization were also recruited into the study. These women had no history of recurrent first trimester miscarriage or infertility and had no anatomical pelvic abnormality found on ultrasound examination.

All women included into the study had regular ovulatory cycles (range 28–34 days) and none of the women were taking any hormonal contraception for the duration of the investigative cycle. They all had transvaginal ultrasound examinations performed in the dorsal lithotomy position. A two-dimensional B mode ultrasound scan was used first to screen for congenital uterine anomalies. Uterine anomalies were suspected when there was evidence of duplication of uterine cavity or when the interstitial portion of either Fallopian tube could not be seen. All women with suspected anomalies were then examined by three-dimensional ultrasound (Voluson 730, Kretztechnik, Zipf, Austria). In order to obtain three-dimensional ultrasound images, the uterus was visualized in the longitudinal plane. A three-dimensional ultrasound volume was then generated by the automatic sweep of the mechanical transducer. The acquired volumes were in the shape of a truncated cone with the depth of 4.3–8.6 cm and a vertical angle $\alpha = 90^{\circ}$. The volumes were immediately stored on removable hard disc cartridges (Magneto-Optic 3.0′, 640 MB: Olympus Optical Co., Hamburg, Germany). The analysis of uterine morphology was then performed on-line using the technique of planar reformatted sections with the interstitial portions of the Fallopian tubes as reference points. Congenital uterine anomalies were classified according to the modified American Fertility Society Classification of Congenital Uterine Anomalies [10].

Uterine flushing was performed exactly 7 days after the luteinising hormone (LH) surge. LH surge was confirmed by sequential assay of early morning urine sample analysis using a commercially available kit (ClearPlan, Unipath Ltd., Bedford, England). Ovulation was confirmed, on the day of flushing, by the presence of a corpus luteum on transvaginal colour Doppler ultrasound scan. The uterine flushing was obtained in the dorsal lithotomy position with a Cusco's bivalve speculum to expose the cervix, which was thoroughly cleaned with sterile saline solution to remove all visible potential contaminants. An 8 F paediatric Foley balloon catheter (Schering AG, Berlin, Germany) was then passed into the uterine cavity through the cervix and the balloon then inflated with 2 ml of air. Under simultaneous transvaginal ultrasound guidance, the balloon was withdrawn to lie above the level of the internal cervical is identified at the reflection of the urinary bladder. Five aliquots of 2 ml sterile 0.154 M sodium chloride solution were then sequentially injected and aspirated from the uterine cavity over approximately 10 s. Ultrasound guidance was used to confirm that the fluid did not enter the Fallopian tubes or cervical canal, with re-aspiration being done when the fluid was seen distending the upper uterine cavity, at the level of the interstitial portions of the Fallopian tubes. The five 2 ml aliquots that were obtained were then pooled and immediately frozen and stored at −20 °C. Each aliquot was thawed for analysis on a single occasion only and any remaining uterine flushing fluid was discarded. After thawing and prior to commencement of any assay, all flushings were centrifuged at 2500 rpm at 18 °C for 10 min. Glycodelin assay was performed using a commercially available solid phase enzyme linked immunoassay (ELISA) based on the sandwich principle (Bioserve Diagnostics, Rostock, Germany). All assays were performed in duplicate and any samples where either of the duplicate values did not fall into within 10% of the other were reassayed. When a quality control value differed by greater than 10% from previous assay means, the assay was repeated. The assay was tested for specificity, by the manufacturer, against human chorionic gonadotrophin (2000 iU/l), alpha feto-protein (300 kU/l), human placental lactogen (20 mg/l) and prolactin (200 μg/l) with glycodelin being undetectable in all cases. The sensitivity of the assay was 6 ng/ml. The intra assay and inter assay precision were 6.2 and 1.2%, respectively.

2.1. Statistical analysis

A database file was set up using Microsoft Excel (Redmond, WA, USA) for Windows to facilitate data entry and retrieval. Statistical analysis was then performed using SPSS (Version 12.0, SPSS Inc., Chicago, IL, USA) for Windows. The median age, number of miscarriages, live births and glycodelin levels were compared using the Kurskal–Wallis analysis of variance in the three groups of women. Comparisons of the concentration of glycodelin