ORIGINAL ARTICLE: ASSISTED REPRODUCTION

Loss of placental thrombomodulin in oocyte donation pregnancies

Manon Bos, B.Sc.,[a] Hans J. Baelde, Ph.D.,[a] Jan A. Bruijn, M.D., Ph.D.,[a] Kitty W. M. Bloemenkamp, M.D., Ph.D.,[b] Marie-Louise P. van der Hoorn, M.D., Ph.D.,[c] and Rosanne J. Turner, B.Sc.[a]

[a] Department of Pathology, Leiden University Medical Centre, Leiden; [b] Department of Obstetrics, University Medical Center Utrecht, Wilhelmina Children's Hospital, Utrecht; and [c] Department of Obstetrics, Leiden University Medical Centre, Leiden, the Netherlands

Objective: To investigate whether thrombomodulin dysregulation is involved in the development of preeclampsia after oocyte donation (OD). Women who become pregnant after OD are prone to develop preeclampsia, a syndrome characterized by an aberrant immunologic response, hypercoagulability, and endothelial dysfunction. A mediator of inflammation and coagulation is thrombomodulin, which has a possible role to play in this syndrome.
Design: Case-control study.
Setting: Not applicable.
Patient(s): Placentas from 82 women with an uncomplicated pregnancy (48 naturally conceived, 21 IVF, and 33 OD pregnancies) and 9 women with an OD pregnancy complicated by preeclampsia have been studied.
Intervention(s): None.
Main Outcome Measure(s): Abundances of thrombomodulin protein and vitamin D receptor (VDR) were determined using immunohistochemistry; mRNA expression was determined using quantitative polymerase chain reaction.
Result(s): Placental thrombomodulin protein abundance was lower in OD pregnancies (diffuse pattern in 45%) than in controls (diffuse pattern in 96%). Placental thrombomodulin mRNA expression was lower in OD pregnancies complicated by preeclampsia (0.72 ± 0.47) compared with in uncomplicated OD pregnancies (0.43 ± 0.18). Thrombomodulin expression correlated with inflammation and coagulation. VDR expression was decreased in OD pregnancies complicated by preeclampsia and was correlated with thrombomodulin mRNA.
Conclusion(s): Pregnancies conceived through OD lose placental thrombomodulin expression. This loss is associated with an increased coagulation and inflammation and indicates that endothelial protection is diminished in OD pregnancies, which might be an explanation for the increased risk for preeclampsia. Vitamin D metabolism is dysregulated in OD pregnancies and might be a target for therapy. (Fertil Steril® 2016;■:■–■. ©2016 by American Society for Reproductive Medicine.)
Key Words: Preeclampsia, oocyte donation, placenta, thrombomodulin, vitamin D

Discuss: You can discuss this article with its authors and with other ASRM members at https://www.fertstertdialog.com/users/16110-fertility-and-sterility/posts/12484-22883

Oocyte donation (OD) is a technique that enables women with diminished ovarian reserve to conceive. Pregnancy is a challenging state for the mother's immune system, where a controlled environment with a regulated immune response to the semiallogeneic fetus has to be established. After OD, the fetus is completely allogeneic; this even more challenging environment for the immune system is presumed to contribute to the increased number of obstetrical complications observed after OD (1, 2). These complications can be explained, in large part, by the increased prevalence of pregnancy-induced hypertension and preeclampsia (3–8).

Preeclampsia, a hypertensive disorder during pregnancy, is a leading cause of maternal and neonatal morbidity and mortality worldwide (9). The pathophysiology of preeclampsia is not fully understood, but the syndrome is characterized by impaired placental development and subsequent shedding of syncytial trophoblast. This results in the release of antiangiogenic factors such as soluble Flt-1, which binds to vascular endothelial growth factor in the circulation (10). These factors contribute to a maternal intravascular systemic inflammatory response, leading to generalized endothelial dysfunction, enhanced leukocyte and complement activation, and coagulation (10).

The role of the placenta in the pathogenesis of preeclampsia in OD pregnancies is presumed to be different from that in preeclampsia in naturally conceived pregnancies (11–13). Although these patients are subject to an increased risk of preeclampsia because of older age (9), it is also

Received August 5, 2016; revised September 19, 2016; accepted October 2, 2016.
M.B. has nothing to disclose. H.J.B. has nothing to disclose. J.A.B. has nothing to disclose. K.W.M.B. has nothing to disclose. M.-L.P.v.d.H. has nothing to disclose. R.J.T. has nothing to disclose.
Reprint requests: Manon Bos, B.Sc., Leiden University Medical Centre, Department of Pathology, L1 Q, P.O. Box 9600, P0-107, 2300 RC Leiden, the Netherlands (E-mail: m.bos@lumc.nl).

Fertility and Sterility® Vol. ■, No. ■, ■ 2016 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2016.10.005

known that OD is an independent risk factor for hypertensive complications of pregnancy (14, 15). Furthermore, the clinical presentation of these patients is different from patients with preeclampsia after a naturally conceived pregnancy: growth restriction after OD pregnancies complicated by preeclampsia is less severe (6, 12, 16, 17). Moreover, the pathophysiology of preeclampsia after naturally conceived pregnancies seems to be of a more vascular origin (10), whereas preeclampsia after OD presumably has a more immunological origin (1, 11, 12, 18).

In women with preeclampsia in naturally conceived pregnancies, serum levels of the breakdown product of thrombomodulin are higher in comparison with uncomplicated naturally conceived pregnancies (19), and placental thrombomodulin protein and mRNA expression in naturally conceived pregnancies is decreased (20). Thrombomodulin is a protein essential for the maintenance of endothelium; it inhibits inflammatory pathways and apoptotic pathways in endothelial cells, and it inhibits coagulation (21). The pathways through which thrombomodulin is regulated in the placenta are currently not precisely known, but the angiogenic imbalance, as seen in preeclampsia, has been shown to decrease thrombomodulin expression (20). Another possible regulator of placental thrombomodulin is vitamin D; decreased vitamin D levels are associated with an increased incidence of preeclampsia (22), and vitamin D increases thrombomodulin expression in endothelial aorta cells (23).

Despite the fact that the placenta is presumed to have a different role in the pathophysiology of preeclampsia after OD pregnancies and naturally conceived pregnancies; the placental thrombomodulin expression might be alterd in OD pregnancies complicated by preeclampsia as well. In both naturally conceived and OD pregnancies, preeclampsia is characterized by endothelial dysfunction, inflammation, and hypercoagulability (12). Therefore, our objectives are to investigate placental thrombomodulin expression, downstream effects of thrombomodulin, and the regulation of thrombomodulin in women with preeclampsia after OD and in women with uncomplicated pregnancies that were either naturally conceived (20), induced by IVF, or induced by OD, as control subjects. We hypothesize that thrombomodulin expression is altered in OD pregnancies complicated by preeclampsia.

MATERIALS AND METHODS

Patients Who Underwent OD and Control Groups

A case-control study with 56 placentas from women pregnant after OD in the Leiden University Medical Centre (LUMC) and teaching hospitals in the region between 2004 and 2013 was performed; 40 placentas were from pregnancies without hypertensive complications, and 16 placentas were from women with preeclampsia according to International Society for the Study of Hypertension in Pregnancy (ISSHP) guidelines (24). Patients who had an OD with at least information on maternal age, gestational age, birth weight, highest diastolic blood pressure, and available paraffin-embedded placenta samples were included. Twenty-eight uncomplicated naturally conceived pregnancies and 21 IVF-induced pregnancies were selected from available patients as controls. Using IVF pregnancies and naturally conceived pregnancies as controls for OD pregnancies has been described before in comparable studies (12, 18). Controls were selected on the basis of mode of delivery, because mode of delivery has a broad impact on the placenta and influences gene expression (25). No other selection criteria were used.

Small for gestational age was defined as birth weight below the 10th percentile for gestational age according to the Dutch reference curves for birth weight by gestational age (26).

Patient characteristics were obtained from the medical records. From all placentas, paraffin-embedded samples were available for immunohistochemical staining. Frozen tissue, which was used for mRNA analysis, was available for 36 uncomplicated OD placentas, 16 placentas from an OD pregnancy complicated by preeclampsia, and 10 placentas from an uncomplicated naturally conceived pregnancy. As previously described, the placentas of twins and triplets were treated as individual samples, since placental pathology can be different in twins. Informed consent was obtained from all patients. This study was approved by the ethics committee of LUMC (P13.084).

Thrombomodulin and Maternal Age

Placentas from an additional group of older women ($n = 20$; maternal age >37) with an uncomplicated naturally conceived pregnancy were included to investigate the effect of maternal age on placental thrombomodulin protein expression.

Histochemical Staining

Histological phosphotungistic acid-hematoxylin (PTAH) staining was performed to investigate the presence of fibrin depositions. Sections were incubated in 0.25% potassium permanganate for 15 minutes followed by 5% oxalic acid for 5 minutes. Sections were then incubated in PTAH for 24 hours at room temperature.

Immunohistochemistry

Immunohistochemical staining was performed to investigate the placental protein abundance of thrombomodulin and the vitamin D receptor (VDR). We choose to determine placental VDR expression since decreased placental VDR expression has been described as a proper measure for disturbances in vitamin D signaling before (27, 28).

Sections were deparaffinized, and antigen retrieval was performed. Sections were incubated with anti-thrombomodulin mouse monoclonal antibody (1:200; Leica Biosystems) or an anti-VDR mouse monoclonal antibody (1:1,500) for 1 hour at room temperature. Binding of the primary antibody was visualized with a PO-labeled anti-mouse polymer (DAKO) and diaminobenzidine as a chromogen.

Scoring of Staining Patterns

Slides were scored by two observers blinded with respect to cases and control groups. Twenty percent of cases were scored differently between observers, and for those, consensus was