Placenta 34 (2013) 1128–1135

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

Long-term type 1 diabetes impairs decidualization and extracellular matrix remodeling during early embryonic development in mice☆

R.R. Favaro [a,*], R.M. Salgado [a], A.C. Covarrubias [a], F. Bruni [b], C. Lima [b], Z.B. Fortes [c], T.M. Zorn [a]

[a] Laboratory of Reproductive and Extracellular Matrix Biology, Department of Cell and Developmental Biology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil
[b] Special Laboratory of Applied Toxinology (CAT/CEPID), Butantan Institute, São Paulo, Brazil
[c] Laboratory of Diabetes and Hypertension, Department of Pharmacology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil

ARTICLE INFO

Article history:
Accepted 18 September 2013

On occasion of the 30th anniversary of the Laboratory of Reproductive and Extracellular Matrix Biology we dedicate this article to its founder, Professor Paulo Abrahamsohn.

Keywords:
Decidua
Extracellular matrix
Maternal–fetal interface
Mouse
Early embryonic development
Pregnancy
Type 1 diabetes

ABSTRACT

Introduction: Endometrial decidualization and associated extracellular matrix (ECM) remodeling are critical events to the establishment of the maternal–fetal interface and successful pregnancy. Here, we investigated the impact of type 1 diabetes on these processes during early embryonic development, in order to contribute to the understanding of the maternal factors associated to diabetic embryopathies.
Methods: Alloxan-induced diabetic Swiss female mice were bred after different periods of time to determine the effects of diabetes progression on the development of gestational complications. Furthermore, the analyses focused on decidual development as well as mRNA expression, protein deposition and ultrastructural organization of decidual ECM.
Results: Decreased number of implantation sites and decidual dimensions were observed in the group mated 90–110 days after diabetes induction (D), but not in the 50–70D group. Picrosirius staining showed augmentation in the fibrillar collagen network in the 90–110D group and, following immunohistochemical examination, that this was associated with increase in types I and V collagens and decrease in type III collagen and collagen-associated proteoglycans biglycan and lumican. qPCR, however, demonstrated that only type I collagen mRNA levels were increased in the diabetic group. Alterations in the molecular ratio among distinct collagen types and proteoglycans were associated with abnormal collagen fibrillogenesis, analyzed by transmission electron microscopy.
Conclusions: Our results support the concept that the development of pregnancy complications is directly related with duration of diabetes (progression of the disease), and that this is a consequence of both systemic factors (i.e. disturbed maternal endocrine–metabolic profile) and uterine factors, including impaired decidualization and ECM remodeling.

1. Introduction

The outcome of pregnancies complicated by type 1 diabetes includes a higher incidence of malformations, intrauterine growth restriction and preterm labor [1]. Although evidence indicates that the maternal environment plays a key role in the development of diabetes-associated embryopathies [2], there is limited information about the impact of diabetes on the maternal–fetal interface.

In order to support embryo implantation and development, the human and rodent endometrium undergoes a series of biological events, collectively known as decidualization [3–5]. Upon decidualization, a remarkable remodeling of the extracellular matrix (ECM) occurs [3–5]. In mice, one of the most striking events is the increase in the diameter of collagen fibers [6,7]. Radioautographical

Abbreviations: D, days after diabetes induction; ECM, extracellular matrix; ELISA, enzyme-linked immunosorbent assay; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; hp, hours of pregnancy; IL11, interleukin 11; MMP, matrix metalloproteinase; PBS, phosphate buffered saline; qPCR, quantitative real time PCR; SLRPs, small leucine rich proteoglycans.

☆ This study was conducted as partial fulfillment of the requirements for a PhD degree by Rodolfo Favaro (advisor, Telma Zorn). Fellowship (04/14442-6) and grants were provided by FAPESP (07/55277-6) and CNPq (306336/2006-5).

* Corresponding author. Department of Cell and Developmental Biology, Institute of Biomedical Sciences, University of São Paulo, Av. Lineu Prestes 1524, CEP 05508-900 São Paulo, SP, Brazil. Tel./fax: +55 11 3091 7309.
E-mail address: rodolfofavaro@usp.br (R.R. Favaro).

0143-4004/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.placenta.2013.09.012

studies demonstrated the synthesis of collagen by decidual cells [8]. Immunohistochemical and biochemical studies showed that collagen types I, III and V are the major components of the thick collagen fibrils identified in the mouse decidua [9–11]. In addition, the expression and deposition of small leucine-rich proteoglycans (SLRPs) decorin, biglycan, fibromodulin and lumican are profoundly modulated by decidualization [12]. Biglycan co-localizes with thick collagen fibrils, whereas decorin is found interacting with the thinner ones in the non-decidualized endometrial stroma [13]. The uterus of decorin-deficient mice has collagen fibrils with larger diameter and irregular profile, indicating the participation of SLRPs in endometrial collagen fibrillogenesis [14]. Furthermore, interleukin 11 (IL11) receptor α-deficient mice are infertile due to impaired implantation and decidualization [15]. A further study revealed that impaired decidualization was associated with alterations in decidual ECM remodeling [16]. Taken together, these studies indicate that ECM remodeling is essential for successful decidualization, and that the decidua is decisive for the establishment of a functional maternal–fetal interface.

Previous studies indicate that diabetes impairs decidualization and creates a pro-inflammatory uterine environment by increasing reactive oxygen species, pro-inflammatory cytokines and prostaglandins, nitric oxide [17], as well as disturbed levels and activity of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) [18], indicating that the ECM may be affected by this condition. In order to verify this hypothesis, we investigated decidual development and ECM remodeling during early embryonic development in a mouse model of pregnancy complicated by long-term type 1 diabetes.

2. Material and methods

2.1. Induction of diabetes

All experiments were approved by the Animal Ethics Committee of the Institute of Biomedical Sciences of the University of São Paulo (144/2002).

Diabetes was induced in sixty day-old Swiss female mice by a single intravenous injection of alloxan, 40 mg/kg (Sigma, USA), at least 16 h after food deprivation. Control mice were injected with physiological saline alone. Females with non-fasting glycemia >400 mg/dl were selected for this study. In order to confirm the maintenance of the diabetic state, glycemia, glycosuria, ketonuria and body weight were evaluated every 25 or 30 days, and also at the moment of sacrifice. Insulinemia was measured by radioimmunoassay according to the instructions of the manufacturer (Linco Research Inc., USA). Food and water supply was constantly monitored as well as the animal housing was frequently cleaned due to excessive production of urine and excrements by diabetic females. No insulin was administered to the diabetic females.

Table 1
Pathophysiologic and reproductive parameters of control and diabetic groups.

	Control	50–70D	90–110D
Glycemia (mg/dl)	114.2 ± 10.25	498.2 ± 74.54*a*	513.7 ± 48.20*a*
Insulinemia (ng/ml)	1.9 ± 0.24	nd	nd
Glycosuria (mmol/l)	nd	≥278	≥278
Body weight (g)	31.3 ± 1.86	25.2 ± 2.79*a*	23.9 ± 0.85*a*
Decidua (mm^2)	5.45 ± 0.76	5.6 ± 0.81	3.84 ± 0.65*b,d*
Relation BW/DD	0.172 ± 0.021	0.222 ± 0.03*c,e*	0.161 ± 0.028
Implantation sites	14.5 ± 0.5	13.8 ± 2.9	10.2 ± 1.04*b,d*

Data are expressed as mean ± SEM.
a = $p < 0.001$ vs. control group.
b = $p < 0.01$ vs. control group.
c = $p < 0.05$ vs. control group.
d = $p < 0.01$ vs. 50–70D group.
e = $p < 0.01$ vs. 90–110D group.
nd = not detected.
BW/DD = body weight/decidual dimensions.
Modified from: Favaro et al. (2010).

2.2. Mating schedule

Females were divided into three groups according to the breeding attempt: (*i*) 50–70 days after diabetes induction (D), (*ii*) 90–110D and (*iii*) 120–140D. Control and diabetic females were bred with normoglycemic males. Control females were bred 90–110 days after saline injection, which was the longest duration of diabetes analyzed in pregnant animals. After 3-h mating the presence of a vaginal plug was considered zero hour of pregnancy. Uterine samples from control ($n = 5$), 50–70D ($n = 5$) and 90–110D ($n = 9$) groups were collected at 168 h of pregnancy (hp) (approximately day 7–8 of pregnancy when vaginal plug is considered day 1). Non-pregnant uteri from 120 to 140D group ($n = 3$) were collected, to confirm the anestrous state.

2.3. Tissue collection and processing for light and transmission electron microscopy

After anesthesia with Avertin® (0.025 ml/g body weight) (Sigma), a 2% (w/v) papaverin (Sigma) solution in distilled water was dripped onto the uterine horns prior to dissection to avoid undesired myometrial contractions. Three implantation sites from the uterus of each mouse were randomly selected and fixed by immersion in Methacarn solution (absolute methanol, chloroform and glacial acetic acid; 6:3:1) for 3 h at 4 °C, and processed for paraffin-embedding. These samples were submitted to Picrosirius staining for identification of fibrillar collagens and immunohistochemistry procedures for detection of specific collagens and proteoglycans.

For transmission electron microscopy (TEM) uterine fragments were fixed by immersion in Karnovsky's solution, post-fixed with 1% (w/v) osmium tetroxide and embedded in Spurr resin. Thin sections (50 nm thick) were stained with uranyl

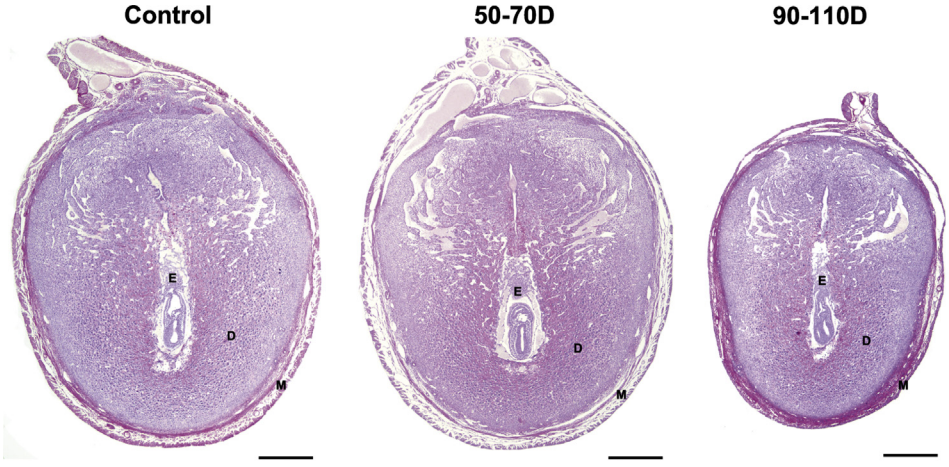

Fig. 1. Representative cross-sections of implantation sites from control ($n = 5$), 50–70D ($n = 5$) and 90–110D group ($n = 9$) showing that dimensions of the decidualized endometrium (D) are affected only in the latter. Blood vessels (asterisks), Embryo (E) and Myometrium (M). Picrosirius-hematoxylin. Scale bar = 200 μm.