Placenta 43 (2016) 77–80

Contents lists available at ScienceDirect

Placenta

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

Short communication

Placental development during early pregnancy: Effects of embryo origin on expression of chemokine ligand twelve (CXCL12)

K.E. Quinn [a], L.P. Reynolds [b], A.T. Grazul-Bilska [b], P.P. Borowicz [b, c], R.L. Ashley [a, *]

[a] Department of Animal and Range Sciences, New Mexico State University, 2980 South Espina Street, Las Cruces, NM, 88003, USA
[b] Center for Nutrition and Pregnancy and Department of Animal Sciences, North Dakota State University, Fargo, ND, 58108-6050, USA
[c] Advanced Imaging and Microscopy Core Laboratory, Dept. 7630, North Dakota State University, Fargo, ND, 58108-6050, USA

ARTICLE INFO

Article history:
Received 22 February 2016
Received in revised form
5 May 2016
Accepted 11 May 2016

Keywords:
CXCL12
Implantation
Placentation
Assisted reproductive technologies

ABSTRACT

The aim was to localize chemokine ligand twelve (CXCL12) in sheep placental tissues during early gestation and after assisted reproductive technologies (ART). Uteri were collected from naturally (NAT) mated ewes and ewes receiving embryo transfer (ET), in vitro fertilization (IVF) or in vitro activation (IVA). CXCL12 was immunolocalized to endometrial stroma, glands, and trophoblast. Greater CXCL12 immunoreactivity was present in trophoblast on day 22 and 24 and in NAT ewes compared to IVF and IVA. Increased CXCL12 expression suggests CXCL12 promotes implantation and placentation. Decreased CXCL12 in IVF and IVA embryos, may compromise pregnancy establishment when utilizing ART methods.

1. Introduction

CXCL12 binding to its receptor, CXCR4 influences pathways important for embryo attachment and placentation, leading to embryo survival [1–3]. To enhance our understanding of CXCL12 at the fetal-maternal interface in livestock, determining cell types that express CXCL12 is imperative. Our objectives were to localize CXCL12 within ovine utero-placenta from normal and assisted reproductive technologies (ART). We hypothesized CXCL12 would localize to trophoblast cells based on day of gestation and ART.

2. Materials and methods

2.1. Animals

All procedures were approved by North Dakota State University Institutional Animal Care and Use Committee as previously reported [4–7].

2.2. Experiment 1

Uteri were obtained from ewes (n = 3 per day) on days 18, 20, 22, 24, 26, and 28 after mating. Cross sections of gravid uterus were obtained followed by immersion in 10% neutral buffered formalin, and paraffin embedded according to standard histological procedures [8].

2.3. Experiment 2

Methods for experiment two are published [6,7,9]. Briefly, gravid uteri were collected on day 22 of gestation from control group (NAT), ewes in which pregnancy was achieved through embryo transfer after natural mating (NAT-ET), in vitro fertilization (IVF) or in vitro activation (IVA; parthenotes-production of embryo from female gamete only). Cross sections of gravid uterus were obtained followed by immersion in 10% neutral buffered formalin and paraffin embedded according to standard histological procedures [8].

2.4. Immunohistochemistry

Tissues sections (5 μm) were mounted onto glass slides, and deparaffinized (Histoclear; National Diagnostics, Atlanta, GA, USA) and rehydrated with a series of ethanol washes. Antigen retrieval was performed in 10 mM sodium citrate buffer, pH 6, with 0.05% Tween 20 in a 2100 retriever (Electron Microscopy Sciences, Hatfield, PA). Slides were rinsed twice in Tris-buffered saline with Triton X-100 (TBST; 0.05 M Tris, 0.15 M NaCl, 0.1% TritonX-100). To

* Corresponding author.
E-mail address: ryashley@nmsu.edu (R.L. Ashley).

http://dx.doi.org/10.1016/j.placenta.2016.05.008
0143-4004/

block nonspecific binding, slides were treated for 20 min with blocking buffer (10% normal goat serum). Tissue sections were incubated with anti CXCL12 antibody (1:50 dilution in 1X TBS, R&D Systems, Minneapolis, MN) followed by incubation with Alexa 647-labeled antibody (1:200 dilution, Invitrogen A21235, Grand Island, NY). Slides were mounted with Pro-Long Gold with 4,6-diamidino-2-phenylindole (DAPI; Life Technologies, Grand Island, NY) to counterstain nuclei. Control sections were incubated with normal goat serum in place of CXCL12 antibody.

2.5. Microscopy

Photomicrographs were taken at same exposure time with a Zeiss Imager. M2 epifluorescence microscope using a 10× objective and AxioCam HRm camera, as well as a Zeiss piezo automated stage controlled by MosaiX module of Zeiss AxioVision software (Carl Zeiss Microscopy, LLC; 1 Zeiss Dr., Thornwood, NY).

2.6. Image and statistical analyses

MosaiX images were analyzed using Fiji Is Just Image J software program [10]. Image background was subtracted and ten separate areas analyzed for CXCL12 intensity for each ewe. Significant differences ($P < 0.05$) in CXCL12 intensity were determined using one-way ANOVA analysis in Prism software (version 5, GraphPad Software, Inc.). Figures were designed using FigureJ [11].

3. Results

3.1. Experiment 1

CXCL12 was immunolocalized to endometrial stroma, glands, and trophoblast on each day of gestation (Fig. 1A–G). Intensity of CXCL12 staining in trophoblast was greater ($P < 0.05$) on day 22 compared to days 18 and 28, and day 24 was greater ($P < 0.05$) than days 18, 20 and 28 (Fig. 1H). The greatest intensity of CXCL12 immunofluorescence in trophoblast was on days 22 and 24, and the least on days 18 and 28, with staining intensity intermediate on days 20 and 26.

3.2. Experiment 2

Similar to experiment one, CXCL12 was localized to endometrial stroma, glands, and trophoblast in NAT and ART uterine cross sections (Fig. 2A–F). Intensity of CXCL12 immunofluorescence differed in ewes exposed to ART compared to NAT, with less trophoblast CXCL12 intensity in IVF and IVA ewes than NAT (Fig. 2G).

4. Discussion

Distinct CXCL12 localization in glandular endometrium implicates CXCL12 may be secreted by uterine glands and functions in a paracrine and/or autocrine fashion to support placental development and conceptus survival. Similar to CXCL12 staining in sheep, vascular endothelial growth factor (VEGF) localizes to uterine glandular endometrium during early gestation in cows [12]. Of note, the CXCL12-CXCR4 signaling axis stimulates VEGF synthesis [13] and in turn VEGF induces CXCR4 and CXCL12 production [14]. In support of CXCL12 and VEGF interplay, we demonstrated treatment of ovine trophoblast cells with CXCL12 results in greater *VEGF* and Fibroblast Growth Factor 2 (*FGF2*) expression [3]. Interestingly, expression of angiogenic factors in sheep utero-placental tissues is decreased after transfer of IVF and IVA embryos [9], which leads to poor placental development and embryo growth [9,15]. Poor growth of embryos created through IVF and IVA may be due to decreased CXCL12 and angiogenic factor synthesis, leading to impaired placental vascularization.

In sheep, a primary requirement for successful pregnancy is remodeling of luminal epithelium to support trophoblast attachment [16,17]. Staining of CXCL12 in apical regions of endometrial stroma follows similar patterns of integrin subunit localization, suggesting CXCL12 regulates endometrial stroma reorganization and embryo implantation [18]. The peak increase of CXCL12 in trophoblast on day 24 suggests CXCL12 signaling supports fetal-maternal communication and contributes to trophoblast attachment.

Results from this study established the expression pattern of CXCL12 in endometrial stroma, glands and trophoblast throughout early gestation and after application of ART. Based on localization of

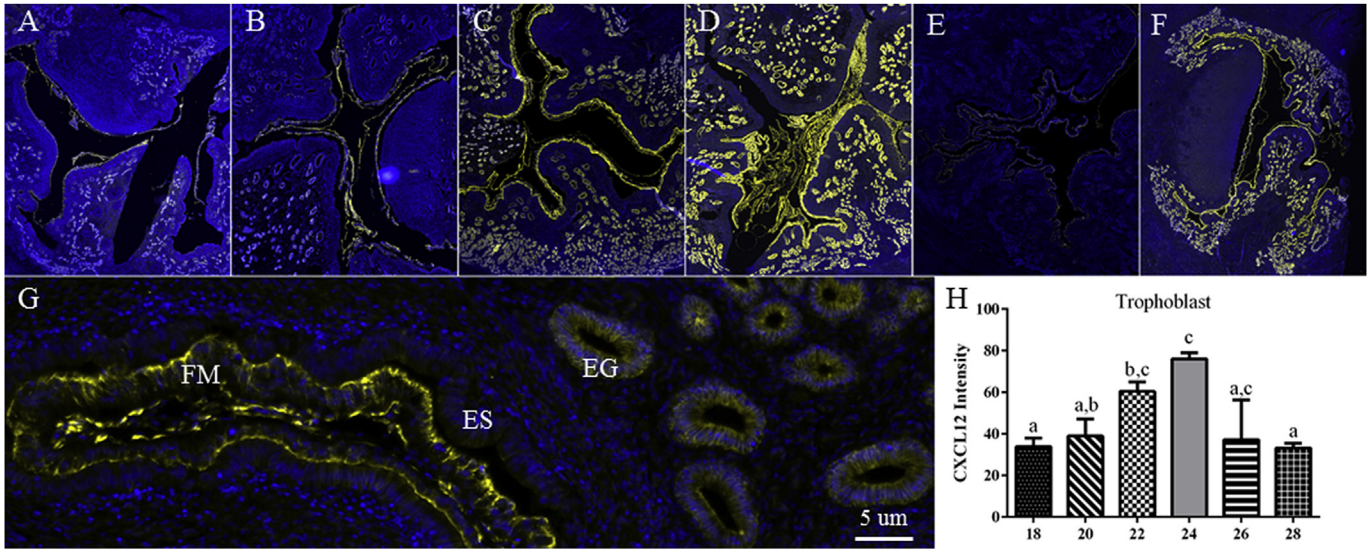

Fig. 1. CXCL12 intensity increases in trophoblast cells on day 22 and 24 of gestation in sheep. Representative MosaiX and 40× magnification (5 μm) images of CXCL12 in sheep uterine tissue on days 18, 20, 22, 24, 26 and 28 (A-F respectively). CXCL12 is localized in the endometrial stroma (ES), endometrial glands (EG), and trophoblast cells of fetal membrane (FM) (G). On day 22 and 24 CXCL12 intensity increased in FM compared to day 18 and 28 and day 18, 20 and 28 respectively (H). Different letters above each bar denote a significant ($P < 0.05$) difference. Yellow fluorescence signifies CXCL12 localization and blue represents 4,6-diamidino-2-phenylindole (DAPI) counterstaining of nuclei.