Introduction

Besides its role in coagulation, von Willebrand factor (VWF) was shown to be involved in angiogenesis via multiple pathways including Integrin αVβ3 (ITG) and Angiopoietin (Ang)-2 [1]. Apart from symptoms seen in von Willebrand disease (VWD) patients relating to impaired coagulation, women affected by VWD may suffer from miscarriage [2]. Massive proliferation of vasculature is essential in uterus and placenta during pregnancy, indicating these processes might be insufficient in VWD patients, constituting the reason for the miscarriages. Therefore, the histological structure of blood vessels in the uterus was evaluated and VWF, ITG and Ang-2 were analyzed by immunohistochemistry (IHC) of uterus and oviduct samples in a pig model of VWD type 3 and 1 and controls.

Material and methods

Animals

Tissue samples of uterus and oviduct were collected from five mature pigs with different genotypes. One was affected by VWD type 3, two were heterozygous carriers (HC) showing symptoms consistent with VWD type 1, and two were wildtype individuals (WT).

Immunohistochemistry

After establishment of protocols ensuring specific staining, polyclonal antibodies against VWF, ITG and Ang-2 were used for IHC. As negative controls the actual primary antibody was replaced by pure dilution medium and isotype controls for both organs and all antibodies were performed.

Aim of our study

Characterization of porcine VWF and its influence on angiogenesis in the female reproductive tract as a model for the condition in humans. Therefore we analysed

• the conformation of blood vessels and
• the staining intensity and cellular distribution of VWF, ITG and Ang-2.

Hematoxylin-Eosin staining

Sections were stained with Hematoxylin-Eosin (HE) using standard protocols. The evaluation of blood vessel conformation comparing the different genotypes was conducted by light microscopy.

Results

Concerning the histological architecture, blood vessels in both the uterine and the oviduct lamina propria, are unremarkable.

Important staining of the apical EpC membrane.

Strong staining of EC and of the apical EpC membrane.

Strong staining of the apical cell membrane of EpC and GC.

Weak cytoplasmic staining of EpC and GC.

Strong cytoplasmic staining of EpC and of VWF.

Weak cytoplasmic staining of EpC and of VWF.

Strong cytoplasmic staining of EC and of VWF, indicating its binding to von Willebrand factor.

Fig. 1: Microscopic findings in uterine and oviduct tissue of wildtype, VWD type 1, and VWD type 3 pigs. For each genotype and analysis, uterus tissue is shown on the left and oviduct tissue on the right. The smaller pictures show exception sections containing blood vessels (scale bar = 100 µm). Asterisks = epithelial cells (EpC), arrows = glandular epithelial cells (GC), black arrowsheads = endothelial cells (EC), white arrowsheads = vascular smooth muscle cells (VSMC), BV = blood vessels.

Discussion

The alterations of the architecture of blood vessels in the uterus of the VWD-affected pig can be classified as angiodysplasia, already described for VWD patients, e.g. in the gastrointestinal tract [3] or naevus [4]. The staining pattern of VWF appears as expected concerning EC. However, the varying distribution regarding the EpC might influence the function of uterus and oviduct. For ITG, the staining pattern of the uterine EpC and GC seems to conform in vitro studies on human umbilical vein EC, postulating that ITG is unstable on the cell surface if VWF is missing [5]. This might explain the strong cytoplasmic staining of the VWD animal in contrast to the apical staining of WT and HC animals. The apical staining of the EpC can also be seen in the oviduct of WT pigs.

The reason for the clear differences among the genotypes regarding Ang-2 staining patterns seems to be the varying storage capacity of Ang-2. Since VWF is missing in the VWD pig, no Weibel-Palade-Bodies (WPB) can be formed. As Ang-2 is commonly stored within these organelles, their absence might lead to uncontrolled release and distribution of Ang-2.

Conclusion

To the best of our knowledge, this is the first study showing an influence of VWF on blood vessel conformation as well as on the expression and distribution of ITG and Ang-2 in the female reproductive tract of pigs. Our results strongly indicate a connection of VWD with miscarriage, since the detected alterations might affect the function of oviduct and uterus. In future, supplementary investigations will be conducted in both non-pregnant and pregnant pigs examining the described as well as further angiogenetic factors by IHC and on a molecular genetic level, respectively, to elucidate the exact mechanisms connecting VWF and female fertility.

References


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