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The sheep conceptus modulates proteome profiles in caruncular endometrium during early pregnancy

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ABSTRACT

The stage-specific expression of functional proteins within the endometrium, and their regulation by conceptus-derived signals, are crucial for conceptus development and successful establishment of pregnancy. Accurate knowledge of endometrium-conceptus interactions is key for the development of effective strategies to improve conceptus implantation rates both following natural conception and/or assisted reproductive technologies. The unilateral pregnant ewe provides a powerful experimental model for the study of endometrial function in the presence or absence of conceptuses during the peri-implantation period. Two-dimensional gel electrophoresis and mass spectrometry-based proteomics were used to compare and identify differentially expressed proteins in caruncular endometrium collected from the gravid uterine horns and the non-gravid uterine horns at the time of conceptus attachment (day 16 of pregnancy) and early post-implantation period (day 20 of pregnancy). Fifty seven protein spots were up-regulated in the gravid horn at day 16 of pregnancy and twenty seven protein spots were up-regulated in the gravid horn at day 20 of pregnancy. Sixteen proteins with different functions such as protein metabolism, cholesterol and ion transport and cell adhesion were identified. In conclusion, the use of the unilaterally pregnant ewe model provides evidence that the early implantation and post-implanting conceptus-derived signals up-regulate caruncle endometrial proteins, including carbonic anhydrase 2 (CA-II) and apolipoprotein A-1 (APOA1) and down-regulate caruncle endometrial proteins, including adenosylhomocysteinase (AHCY) and heat shock 60 kDa protein 1 (HSP60). These regulated proteins are likely involved in providing a suitable intra-uterine environment required for conceptus attachment, implantation, early post-implantation development and the successful establishment of pregnancy in sheep.

1. Introduction

In sheep, goats and cattle, successful conceptus (embryo and associated extraembryonic membranes) implantation relies on elaborate cellular, biochemical and molecular cross-talk between the extraembryonic membranes and receptive uterine endometrial tissues that ensures corpus luteum (CL) progesterone production and optimal post-implantation conceptus development and survival (Paria et al., 2001; Imakawa et al., 2004). During early pregnancy, a high rate of embryonic morality occurs due to abnormal conceptus signalling (Goff, 2002; Dixon et al., 2007; Diskin and Morris, 2008). Although a multitude of molecular pathways involved in extraembryonic

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membrane-endometrium crosstalk during conceptus implantation and post-implantation periods have been identified through studies of gene expression, a comprehensive understanding of changes in many endometrium proteins expressed in the presence of conceptuses is currently lacking.

Our previous studies provided original evidence that several endometrial proteins with different functions, including protein synthesis and degradation, antioxidant defence, cell structural integrity, adhesion and signal transduction, play important roles in the establishment of early pregnancy in sheep (Al-Gubory et al., 2014). Of particular interest were proteins that were highly expressed in response to the presence of conceptuses at attachment and early post-implantation periods and, using our unilaterally pregnant ewe model, we demonstrated that the early implantation and post-implanting conceptus-derived signals up-regulate the expression of cytoplasmic tryptophanyl tRNA synthetase and the mitochondrial superoxide dismutase (Al-Gubory et al., 2014). The former is a key catalytic enzyme for the first step reaction in protein synthesis (Sallafranque et al., 1986) and the latter the first antioxidant defence enzyme against reactive oxygen species-induced mitochondrial oxidative damage (Orrenius et al., 2007), in sheep caruncular endometrium (Al-Gubory et al., 2015). In sheep, the endometrium caruncles (CAR) are highly vascularized stromal protuberances covered by a simple luminal epithelium. CAR areas are specialized sites of attachment of the outer covering extraembryonic membrane, the trophectoderm, and are privileged endometrial tissues for conceptus-uterine communication. Our hypothesis is that the early developing sheep conceptus modulates protein expression profiles in CAR endometrium during early pregnancy.

The unilateral pregnant sheep model enables changes in the expression of endometrial proteins in the presence or absence of conceptuses to be studied, providing a powerful model for the investigation of proteome changes during the peri-implantation period (Al-Gubory et al., 2015). The benefit of this model is that both uterine horns are exposed to similar concentrations of circulating hormones such as progesterone but only the gravid horn is under the direct action of local signalling molecules produced by the conceptus. In the present study, the unilateral pregnant ewes with functional ovaries were therefore employed to test our hypothesis. Two-dimensional gel electrophoresis (2DE) based proteomics (Fowler et al., 2007; Arianmanesh et al., 2011; Al-Gubory et al., 2014) was used to characterize specific alterations in the proteome of CAR endometrial tissues collected from the gravid uterine horns (GH) and the non-gravid uterine horns (NG) at the time of conceptus attachment (day 16 of pregnancy) and early post-implantation period (day 20 of pregnancy).

2. Materials and methods

2.1. Experimental animals

All procedures relating to care and use of animals were approved by the French Ministry of Agriculture according to the French regulation for animal experimentation (authorization no° 78-34). Ewes of the Préalpes-du-Sud breed (18 months of age) were used in this study. Unilaterally pregnant ewes were prepared surgically as described previously (Payne and Lamming, 1994; Lamming et al., 1995). Briefly, ewes were initially anesthetized with a mixture of pentobarbital (Sanofi, Paris, France) and thiopentone (Abbott, Aubervilliers, France). After endotracheal intubation, general anaesthesia was maintained by constant inhalation of a mixture of oxygen and halothane. Reproductive organs were exposed via midventral laparotomy and one ovary was removed. One uterine horn was ligated close to the uterine bifurcation so that after mating the conceptus is confined to the non-ligated pregnant horn. Three weeks after surgery, the ewes were treated for 14 days with intravaginal sponges containing 40 mg fluorogestone acetate (Intervet, Angers, France) to synchronize oestrous. Ewes were mated twice with fertile rams of the same breed, at an interval of 12 h during the synchronized oestrus. The ewes were housed under conditions of natural day-length and temperature and had free access to mineral licks and water.

2.2. Endometrial tissue collection

The ewes were slaughtered at a local abattoir in accordance with protocols approved by the local institutional animal use committee at the Institut National de la Recherche Agronomique (INRA, Jouy-en-Josas, France). Pregnant ewes were randomly allocated for slaughter at two specific stages of early pregnancy corresponding to the initial conceptus attachment to CAR areas (day 16, n = 4 ewes) and the early conceptus post-implantation period (day 20, n = 4 ewes). The stages of pregnancy were confirmed by the presence and the morphology of the conceptus in uterine flushings. Immediately after slaughter of the ewes, the reproductive tracts were collected, placed on crushed ice and transported to the laboratory. Endometrial CAR areas were collected from the entire GH (ipsilateral uterine horn) and NG (contralateral uterine horn) of each ewe, snap-frozen in liquid nitrogen and stored at −80 °C until processed for 2DE gel electrophoresis and Western blot.

2.3. Protein extraction and quantification for electrophoretic analysis

CAR from the gravid horns on days 16 (GH16) and 20 (GH20), non-gravid horns on days 16 (NG16) and 20 (NG20) were processed separately for 1DE and 2DE gel electrophoresis as described previously (Fowler et al., 2007). Briefly, tissues were combined with 5 ml lysis buffer/1 mg wet weight of tissue. The lysis buffer (0.01 M Tris–HCl, pH 7.4) contained 1 mM EDTA, 8 M urea, 0.05 M dithiothreitol, 10% (v/v) glycerol 5% (v/v), NP40, 6% (w/v), pH 3–10, resolyte (Merck Eurolab Ltd, Poole, Dorset, UK) and protease inhibitor cocktail (Roche Diagnostics). The tissues were disrupted using a Tissue Lyser (Qiagen Ltd) for 4 min at 30 Hz. Insoluble materials were removed from the lysates by centrifugation (50,000*g* at 4 °C) for 30 min. The protein content of the final supernatant had been determined by RC-DC assay (Bio-Rad Laboratories Ltd). The