Domestic Animal Endocrinology 46 (2014) 49–57

Contents lists available at ScienceDirect

Domestic Animal Endocrinology

journal homepage: www.domesticanimalendo.com

Global proteomic characterization of uterine histotroph recovered from beef heifers yielding good quality and degenerate day 7 embryos

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ARTICLE INFO

Article history:
Received 3 September 2013
Received in revised form 2 October 2013
Accepted 8 October 2013

Keywords:
Proteomics
Histotroph
Embryo development

ABSTRACT

The objective was to analyze the proteomic composition of uterine flushes collected from beef heifers on day 7 after insemination. Estrus was synchronized in crossbred beef heifers by using a protocol with a controlled intravaginal drug releasing device. Heifers detected in standing estrus (within 24–48 h after removal of controlled intravaginal drug releasing device) were inseminated (estrus = day 0) with frozen-thawed semen from a single ejaculate of a bull with proven fertility. Heifers from which an embryo was recovered (after slaughter on day 7) were classified as either having a viable embryo (morula/blastocyst stage) or a degenerate embryo (arrested at the 2- to 16-cell stage). The overall recovery rate (viable and degenerate combined) was 64%. Global liquid chromatography coupled to tandem mass spectrometry proteomic analysis of the histotroph collected identified 40 high-confidence proteins present on day 7; 26 proteins in the viable group, 10 in the degenerate group, and 4 shared between both groups. Five proteins (platelet-activating factor acetylhydrolase IB subunit γ [PAFAH1B3], tubulin α-1D chain, tubulin β-4A chain, cytochrome C, and dihydropyrimidinase-related protein-2) were unique or more abundant in the histotroph collected from animals with a viable embryo, and 1 protein (S100-A4) was more abundant in the histotroph collected from animals with a degenerate embryo. Of interest, PAFAH1B3, detected only in histotroph from the group yielding viable embryos, belongs to the group of platelet-activating factors that are known to be important for the development of the pre-implantation embryo in other species. To our knowledge this is the first report of PAFAH1B3 in relation to bovine early embryonic development.

1. Introduction

Embryo mortality in cattle, reflected in reduced conception rate/calving rate per service, is a major cause of economic loss for the farming industry. In heifers, only 60% of single inseminations lead to a successful full-term pregnancy despite a fertilization rate of 90% to 95% [1]. Because the period of greatest reproductive wastage in cattle occurs before day 16 [1,2], the underlying molecular events that regulate early conceptus development up to the time of maternal recognition of pregnancy in cattle have not been clearly elucidated. It is clear, however, that the uterine endometrium plays a central role in early conceptus–maternal communication for establishment and maintenance of pregnancy. This involves dynamic changes in the

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http://dx.doi.org/10.1016/j.domaniend.2013.10.003

uterine epithelium that are tightly regulated by changes in steroid hormones. The embryo leaves the oviduct and enters the uterus between day 4 and 5 after fertilization at the 8- to 16-cell stage [3]. From this point onward until the start of implantation, which occurs around day 19, the embryo is not attached in the uterus and completely depends on the uterine secretions for its further development [4,5]. As such it is of vital importance that the composition of the histotroph meets the requirements of a developing embryo. Indeed, changes in endometrial gene expression around this time, under the influence of progesterone, can lead to changes in the composition of the histotroph to which the developing conceptus is exposed [6,7]. The importance of histotroph for conceptus development has been demonstrated in the uterine gland knockout model in sheep in which embryos developed to day 9 of gestation but then failed to develop beyond the blastocyst stage, that is, day 14, in adult uterine gland knockout ewes [8,9]. The process of conceptus elongation after hatching is regulated mainly by histotroph-derived factors as evidenced by the fact that, despite attempts to artificially induce this process, hatched bovine blastocysts fail to elongate in vitro but will do so if transferred to the uterus of a recipient female [10,11].

The histotroph is composed of proteins, carbohydrates, sugars, lipids, and ions produced by the endometrial glands that are necessary to sustain the conceptus. The protein components of the histotroph are important for conceptus–maternal interaction, specifically the processes of elongation of the trophoblast, recognition of pregnancy, implantation, and placentation [12,13]. Many components of the histotroph are secreted under the influence of progesterone and in the early luteal phase [14,15] also by estradiol [16], but the optimum biochemical composition of the histotroph that supports the development of a healthy embryo/conceptus is not yet known. Recent studies have described the proteomic composition of uterine histotroph during the estrous cycle and in comparison with plasma [17–19].

The objective of this study was to analyze the proteomic composition of uterine flushes of inseminated beef heifers with normal and degenerate embryos on day 7 after insemination to elucidate what potential proteins are present in the uterus to support the embryo until blastocyst development. Our hypothesis is that there will be differences in composition between the animals with different types of embryos.

2. Materials and methods

All experimental procedures that involved animals were approved by the Animal Research Ethics Committee of University College Dublin and were licensed by the Department of Health and Children, Ireland, in accordance with the Cruelty to Animals Act (Ireland 1876) and European Community Directive 86/609/EC.

2.1. Animal management and treatments

The experimental design used for this study has been described previously [20]. Crossbred beef heifers, approximately 2 y old and weighing 524 ± 5.5 kg housed in a slatted floor facility in a commercial feedlot were used. All heifers were housed under the same management conditions with ad libitum access to a total mixed ration designed to achieve an average live-weight gain of 1.3 kg/heifer/d. Estrus (day 0) was synchronized by insertion of a controlled internal drug release (1.36 g of progesterone; Pfizer, Sandwich, UK) device placed per vagina for 8 d with a 2-mL injection of placental growth factor 2α analogue (Prosolvin; Intervet Ireland Ltd, Dublin, Ireland) given on day 7. Heifers were checked for signs of estrus 4 times per day, commencing 36 h after removal of the controlled internal drug release device. Twelve to 18 h after onset of estrus (day 0) only those heifers recorded in standing estrus within a narrow window were inseminated with frozen-thawed semen from a single ejaculate of a bull with proven fertility. Jugular blood samples were collected on days 4, 6, and 7 after estrus from all heifers. Blood samples were stored at room temperature for 1 h and at 4°C for a further 16 h. Serum was decanted after centrifugation for 20 min at 1,600 × *g* and stored at −20°C until subsequent analyses. All heifers were slaughtered on day 7 of pregnancy.

2.2. Progesterone assay

Serum progesterone concentrations were measured in all heifers on days 4, 6, and 7 after estrus with the use of a time-resolved fluorescence immunoassay with an Auto-DELFIA Progesterone kit (PerkinElmer, Wallac Oy, Turku, Finland), as previously described. All samples were assayed within a single assay with a sensitivity of 0.01 ng/mL for the progesterone assay. The intra-assay CVs were 4.6%, 5.5%, and 4.6% for high, medium, and low progesterone quality control sera, respectively.

2.3. Flush collection

Heifers from which an embryo was recovered were assigned to either a) the viable group when the embryo was at the correct developmental stage for age (ie, morula/early blastocyst) or b) the degenerate group when the embryo was arrested at the 2- to 16-cell stage. Heifers from which an unfertilized oocyte was recovered or from which no structure was recovered were omitted from the study. Within 30 min of slaughter the reproductive tract of all heifers was flushed with 20 mL of 10 m*M* Tris (pH 7.2; Sigma, Dublin, Ireland) by injecting this volume into the tip of the uterine horn and collecting it at the caudal end of the uterine body. All flushes were subsequently transported on ice to the laboratory, and flushes were centrifuged at 4,000 × *g* for 30 min at 4°C before snap-freezing in liquid nitrogen and storage at −80°C until further analysis.

2.4. Protein extraction from uterine flushes

Proteins were extracted from 6 samples (3 heifers per group) that were visibly free from blood (no red color) with the use of acetone precipitation as described previously [14]. Each sample was thawed on ice and split into 2 aliquots of 10 mL. Four volumes of ice-cold acetone were added, and samples were stored at −80°C overnight. Samples were then thawed, centrifuged at 4,000 × *g* for 30 min at 4°C, and the supernatant were removed. The pellets were resuspended in