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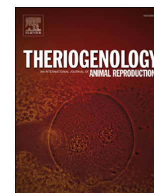

Timing of transcriptomic and proteomic changes in the bovine placentome after parturition☆

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ABSTRACT

Proper post-partum reproductive performance is important for reproductive efficiency in beef cows, and dystocia decreases post-partum fertility. Crossbred beef cows (n = 1676) were evaluated for lifetime performance based on degree of dystocia at presentation of the first calf. Cows that experienced moderate or severe dystocia produced fewer calves during their productive life ($P < 0.01$). The exact mechanism is unclear, but may be due to the contributions of dystocia to abnormal placental separation. Proteolytic activity is hypothesized to contribute to placental separation in ruminants; however, when ovine placentomes were collected following caesarian section, no proteolytic activity was detected. We hypothesized that stage 2 of parturition was necessary to stimulate proteolysis and initiate placental separation. Serial placentome collections were performed on mature cows (n = 21 initiated; 7 with complete sampling) at hourly intervals for the first 2 h after expulsion of the calf. An intact piece of each placentome was fixed for histological evaluation, and a separate piece of caruncular and cotyledonary tissue from each placentome was frozen for transcriptomic and proteolytic analysis. A full set of placentomes was collected from only 7 of 21 cows at 0, 1, and 2 h, and all cows had expelled fetal membranes by 6 h. Histological, transcriptomic and proteolytic analysis was performed on placentomes from cows from which three placentomes were collected (n = 7). The microscopic distance between maternal and fetal tissues increased at 1 h ($P = 0.01$). Relative transcript abundance of matrix metalloprotease 14 (*MMP14*) tended to increase with time ($P = 0.06$). The relative transcript abundance of plasminogen activator urokinase-type (*PLAU*) was greater in caruncles than cotyledons ($P = 0.01$), and tended ($P = 0.10$) to increase in the caruncle between 0 and 2 h while remaining unchanged in the cotyledon over the same span of time. Greater PLAU and plasminogen activator tissue-type (PLAT) proteolytic activity was detected by zymography in the caruncle than the cotyledon immediately post-partum ($P < 0.01$). From these findings we conclude that 1) dystocia during the first parity decreases lifetime productivity in beef cattle, 2) the PA system is present at both the transcript and protein level in the bovine plactentome during parturition and 3) proteolytic activity is localized to the caruncular aspect of the placentome.

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1. Introduction

Repeat breeder beef cows were more likely to have experienced calving difficulty at some point in their life [1], and calving difficulty is a major contributor to retained fetal membranes (RFM) in cows [2]. In beef cows, induction of parturition (e.g., with dexamethasone) or caesarian section also increase the risk of RFM [2,3], and impair subsequent reproductive performance [4]. Thus, a better

☆ Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of names by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

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understanding of the mechanisms controlling placental separation could explain why cows with dystocia and without RFM suffer suppressed reproductive performance which could lead to improved reproductive management of post-partum beef cows.

Compared to placental development, surprisingly little is known about the molecular mechanisms meditating placental separation during normal placental expulsion. Placental development in ruminants is mediated by trophoblast adhesion mainly within the placentome. Adhesion of trophoblasts to endometrial cells is mediated by the actions of integrins and their interaction with extracellular matrix proteins, most notably osteopontin [5]. During parturition, this integrin-mediated adhesion must be disrupted in order to facilitate proper separation and expulsion of the fetal membranes, an important step for proper involution of the uterus following pregnancy [6]. Studies that have investigated the molecular mechanism of placentome separation have focused primarily on the collagenolytic family of proteases, and results of several studies have produced conflicting results in terms of whether or not collagenolytic activity is reduced in cases of RFM [7–9].

Collagenases, also known as matrix-metalloproteases (MMPs), are a family of 26 Zn^{2+} dependent endoproteases that are synthesized and secreted as inactive zymogens. Four inhibitors (tissue inhibitor of matrix-metalloproteases: TIMPs) of these proteases are known to play roles in modulating the activities of the MMP and their inhibition is not covalently mediated (i.e., not SDS stable). Each MMP possesses a spectrum of specificities for different extracellular matrix (ECM) proteins with some functional overlap, the most well studied being collagen [10]. Activation of MMP involves proteolytic removal of the N-terminus to expose the catalytic zinc ion, referred to as the cysteine switch [11]. Once pro-MMPs are secreted, proteases within the plasminogen activator (PA) family are responsible for MMP activation [12,13]. The PA family consists of the enzymes plasmin/plasminogen (PLG), plasminogen activator tissue-type (PLAT), plasminogen activator urokinase-type (PLAU) and its receptor (PLAUR). Inhibitors of the PA family also exist including α_2-antiplasmin, plasminogen activator inhibitor-1 (SERPINE1), plasminogen activator inhibitor-2 (SERPINB2) and protein C inhibitor (SERPINA5). Additionally, PA activation of MMP can result in the activation of pro-PA and other MMP, further propagating the proteolytic cascade [12,14,15]. The known role of PA in activation of MMP, combined with reports that placentomes from retained fetal membranes possess reduced MMP activity, suggests PA activity may play a role in placental separation and the pathogenesis of RFM of cattle.

When placentomes were collected from sheep at stage 2 of parturition following delivery of the lambs by caesarian section, we were unable to detect PA activity, indicating that there may be stimulatory signals that occur during parturition that are necessary to activate the PA system [16]. This could explain why RFM occurs at a greater frequency after caesarian sections are performed [3]. Surprisingly little is known, however, about placental morphology during parturition and how MMP activation *in vivo* is mediated. To investigate this process we refined a technique to perform the serial collection of placentomes in post-partum cows [17], and in the present study, we compared PA transcript abundance and protease activity in placentomes collected at one h intervals after calf expulsion from spontaneously calving cows without any difficulties. Given the lack of basic information regarding the physiologic mechanisms responsible for normal placental separation, we chose to investigate the basic mechanisms placental separation in lieu of contrasts affected and unaffected animals (eg dystocia, RFM). The objective of this study was to a) quantify the effects of dystocia on lifetime productivity in beef cattle and 2) determine if the PA system is present in the bovine placentome during parturition. We hypothesized that: 1) experiencing calving difficulty during the first calving season would decrease lifetime productivity in beef cows, and 2) in cases of normal parturition placental morphology would change as a function of time after parturition, and would be associated with increases in PA system transcript abundance and proteolytic activity after expulsion of the calf.

2. Materials and methods

2.1. Influence of calving difficulty on reproductive longevity of beef cows

All procedures were approved by the U.S. Meat Animal Research Center (USMARC) Animal Care and Use Committee and were in accordance with the FASS guidelines for the care and use of agricultural animals in research. Standard management practices at the U.S. Meat Animal Research Center include recording calf birth dates, calf sex, calving difficulty score [18,19], calf birth weight, and calf weaning weights each year. In addition, each calf is provided with an individual identification and all data are stored in the USMARC database. Data were extracted for 2-yr-old crossbred cows (n = 1676) giving birth for the first time in the spring of 2000–2002. These years were chosen because they did not overlap with our previous published reports of calving day and calving difficulty with heifers giving birth for the first time before those years [18], and did not impinge on new experiments started in 2003 that examined the influence of nutritional treatments on many of these endpoints in heifers giving birth for the first time after those years [20–22].

At parturition, cows were assigned a calving difficulty score based on a standard that has been previously reported for USMARC [18,19]. Briefly, calving was subjectively evaluated on the following 6-point scale: 1 = no assistance; 2 = little difficulty, assistance given by hand; 3 = little difficulty, mechanical pull; 4 = slight difficulty, mechanical pull; 5 = moderate mechanical pull; 6 = hard mechanical pull. Cows with a calving score of 1 received no post calving care after delivery. If a cow required some assistance (score of 2, 3, 4) they were treated with oxytocin (5 ml, i.v.), and penicillin G benzathine (3.5 ml/100 lbs, s.q). Cows with more difficult deliveries (score of 5, 6) were treated with oxytocin (5 ml, i.v.), penicillin G aqueous (3.5 ml/100 lbs, i.m) for three days, and given two oxytetracycline intrauterine boluses. Any cows with additional medical needs were treated following USMARC protocols.

2.2. Placentomectomies

Placentomes were collected from 3-yr-old MARCII (25% Angus, 25% Hereford, 25% Simmental and 25% Gelbvieh) beef cows (n = 21), using the transvaginal collection technique that we described previously [17]. The experiment was designed to collect placentomes at 0, 1, and 2 h after expulsion of the calf without any difficulties to determine if normal progression through stage 2 of parturition stimulated proteolytic activity or altered transcript abundance within the bovine placentome. A single intact representative placentome was excised at each of the three times, excess fetal membranes were trimmed and the placentomes separated into cotyledonary and caruncular components. Samples for gene expression and proteolytic analysis were placed into 2.0 ml cryovials and snap frozen in liquid nitrogen within 5 min of collection. Samples were transported back to the laboratory in liquid nitrogen and stored at −80 °C until total cellular RNA and protein were extracted. Only seven cows maintained intact placentomes through the 2 h collection and all cows completely expelled the fetal membranes within 6 h of parturition. Therefore, further analysis was only performed using tissues from the seven cows that

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