Endogenous retroviruses and placental syncytium formation during early gestation and establishment of pregnancy

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Animal agriculture faces immense challenges in the near future. The rapidly growing world population increases the need to develop efficient and sustainable livestock production practices, which begin with a female's ability to conceive and maintain a pregnancy. The objectives of this project were to elucidate the possible role of endogenous retroviral genes in placental formation, placental function and establishment of pregnancy. Syncytin-Rum1 and BERV-K1 were differentially expressed during the early gestation and may be important to the establishment of pregnancy.

Summary

Sustainable and efficient production is necessary if animal agriculture is going to meet the ever-growing demand for animal products. Animal efficiency begins with the mother's ability to become and remain pregnant. Endogenous retroviruses (ERV) may play a vital role in the establishment of pregnancy in cattle, as in other placental mammals such as mice and humans. Therefore, the objectives of this study were to examine the changes in mRNA expression of ERV in the placenta during the establishment of pregnancy and initial placentation. Crossbred Angus heifers (n = 46, ~15 months of age; body weight [BW] = 362 ± 34.7 kilograms [kg]) were estrus synchronized and artificially inseminated. Heifers then were ovariohysterectomized on various days of early gestation (day 16, 22, 28, 34, 40 or 50 after breeding) or served as nonpregnant controls to provide insight into placental development and changes in ERV expression. Fetal placental tissue expressed high levels of interferon-τ mRNA, the maternal recognition signal in cattle, at day 22 of gestation; however mRNA expression was close to undetectable at any later time point. Placental mRNA expression of pregnancy-specific protein-B increased dramatically as pregnancy progressed, with maternal mRNA expression at day 50 being 129,508-fold greater ($P < 0.001$) than in nonpregnant controls. Both s*yncytin-Rum1* and *BERV-K1*, which are thought to be major ERV in cattle, had a slight numerical increase in mRNA expression on day 16, compared with nonpregnant, and day 22, which may indicate some immune suppressant function around the time of maternal recognition of pregnancy. However, the mRNA expression of s*yncytin-Rum1* and *BERV-K1* increased dramatically on day 50 in caruncular (maternal placental) tissue (81.5 ± 14.4- and 202.7 ± 43.7-fold, respectively; $P < 0.001$). These data confirmed that placental mRNA expression of interferon-τ and pregnancy-specific protein-B change dramatically during early gestation. They also suggest that ERV may be performing vital functions of immune suppression and cell-to-cell fusion during placental development, which may dictate the viability of the developing embryo and success of the pregnancy.

Introduction

The mammalian genome contains thousands of endogenous retroviruses (ERV), most of which are nonfunctional; however, those that are functional promote the fusion of stem cells. Endogenous envelope genes of retroviral origin (Gifford and Tristem, 2003) make up a significant portion of the genome: 8 percent in humans (Kurth and Bannert, 2010), 10 percent in mice (Jern and Coffin, 2008) and 18 percent in cattle (Adelson et al., 2008).

Endogenous retroviruses have fusogenic and immunosuppressive functions during the development of fetal membranes, attachment and invasion into the uterine endometrium and subsequent viability of the pregnancy (Sharif et al., 2013). A class of these known as syncytins contribute to the formation of the syncytiotrophoblast in humans (Blond et al., 2000), mice (Dupressoir et al., 2005; 2007), rabbits (Heidmann et al., 2009) and carnivores (Cornelis et al., 2012), and syncytial plaques in ruminants (Cornelis et al., 2013). Syncytin is an ERV thought to be involved in cell-cell fusion (syncytium formation) and immunosuppression within the mammalian placenta and, therefore, may be critical for pregnancy establishment (Dunlap et al., 2005; Cornelis et al., 2013).

Placental syncytium formation in ruminants is unique among mammals because syncytial plaques take the place of the syncytiotrophoblast

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found in other species. Trophoblast stem cells fuse during early gestation to form syncytial plaques, which are multinucleated cells within the placentomes of ruminants (Gifford and Tristem, 2003). They can contain up to 25 nuclei in sheep (Wooding, 1984) and eight nuclei in cattle (Wooding and Wathes, 1980). The syncytiotrophoblast of other mammals forms the feto-maternal interface and is involved in the exchange of nutrients and gases between maternal and fetal circulation, hormone production and conceptus protection from a maternal immune response (Moffett and Loke, 2006).

Formation of the feto-maternal interface and secretion of interferon-τ (INFτ; Spencer and Bazer, 2004) is essential for implantation and normal fetal development. The objectives of the current study were to establish the baseline mRNA expression patterns for *syncytin-Rum1*, *BERV-K1*, INFτ and pregnancy-specific protein B, which we hypothesized would be expressed differentially during critical time points during the establishment of gestation.

Experimental Procedures

All protocols were approved by the North Dakota State University Animal Care and Use Committee. Crossbred Angus heifers (n = 46, ~15 months of age; BW = 362 ± 34.7 kg) underwent a five-day CO-Synch + CIDR estrus synchronization protocol. Seven heifers were not inseminated after synchronization protocol to serve as nonbred, nonpregnant (NP) controls. The NP controls were ovariohysterectomized on day 16 of the subsequent estrous cycle. The remaining heifers (n = five to nine per time point) were bred via artificial insemination 12 h after observed estrus and ovariohysterectomized at day 16, 22, 28, 34, 40 or 50 of gestation.

Immediately following ovariohysterectomy, utero-placental tissues (caruncle, CAR; intercaruncular endometrium, ICAR; and fetal membranes, FM) were obtained from the gravid uterus. Fetal membranes were collected only on day 22 and later days due to inadequate quantities of FM on day 16 and the absence of FM in NP controls. Once collected, all tissues were snap frozen in liquid nitrogen-cooled isopentane and stored at minus 80 C.

The RNA was extracted and purified using RNeasy Mini Kit (Qiagen, Valencia, Calif.), and cDNA was synthesized utilizing QuantiTect Reverse Transcription Kit (Qiagen, Valencia, Calif.). Total quantity of RNA was determined using the Take3 module of a Synergy H1 Microplate Reader (BioTek, Winooski, Vt.). After primer validation, dilutions of 1:100 were utilized for *syncytin-Rum1, BERV-K1*, pregnancy-specific protein B (PSP-B) and INFτ RTqPCR. mRNA levels were quantified using a 7500 Fast Real-Time PCR System (Applied Biosystems, Grand Island, N.Y.) with SYBR Green Master Mix (Bio-Rad Laboratories, Hercules, Calif.). The ΔΔCT method with genes of interest normalized to β-actin and NP CT values was used to analyze data for *syncytin-Rum1, BERV-K1* and PSP-B. Interferon-τ was analyzed via the ΔΔCT method, with INFτ normalized to β-actin and day 22 CT values.

All statistical analyses were conducted with the GLM procedure of SAS (SAS Inst. Inc., Cary, N.C.), with individual heifer serving as the experimental unit. Means were separated using the LSMEANS procedure of SAS and *P*-values ≤ 0.05 were considered different.

Results and Discussion

In the current study, mRNA expression of INFτ was greatest ($P < 0.001$) in day 22 FM and then decreased to negligible levels. These results agree with previous research (Bazer, 1992; Spencer and Bazer, 2004) that reported maternal recognition in cattle occurring between days 16 and 18 of gestation due to secretion of INFτ from fetal trophoblast cells. Pregnancy-specific protein-B mRNA expression increased exponentially during the first 50 days of gestation with a 129,509-fold increase ($P < 0.001$) over NP mRNA expression of PSP-B (Fig. 1). Austen et al. (1999) suggest that PSP-B may synergize with INFτ to maintain pregnancy and stimulate the secretion of uterine proteins necessary for normal embryonic development. However, our data indicates PSP-B increases at similar times to the major cattle ERV, after INFτ has decreased to minimal levels.

The role of syncytial plaques in ruminants has yet to be determined; however, these data may suggest similar functions to the syncytiotrophoblast in mice and humans during early pregnancy. *Syncytin-Rum1* (Fig. 2) had a slight numeric increase (16.3 ± 9.6-fold; $P < 0.36$) on day 16 in CAR of pregnancy uteri; however, CAR increased dramatically on day 50 (81.5 ± 14.4-fold; $P < 0.001$), compared with NP controls. The increase at days 16 and 50 suggests that *syncytin-Rum1* could be responsible for characteristic functions of all syncytins, immune suppression and cell-to-cell fusion, which may aide in placental formation and the establishment of pregnancy.

In addition to *syncytin-Rum1*, four retroviral genes, *BERVE-A*, *BERVE-B, BERV-K1* and *BERV-K2*, are expressed in the bovine trophoblast (Koshi et al., 2012). The envelope proteins of *BERVE-A* and *BERV-K1* may be involved with

increased binucleation and fusion between maternal and fetal placental cells that occurs during early gestation. Similar to *syncytin-Rum1*, we observed an increase on day 16 (20.8-fold) over NP but due to large variation was not statistically significant ($P = 0.52$).

These data suggest that cattle may have two ERV involved in the establishment of pregnancy, which is similar to mice (Dupressior et al., 2005) and humans (Blond et al., 2000), both of which have at least two ERV involved in pregnancy. Greater fusogenic capabilities of *BERV-K1* has been reported, compared with those of either *BERVE-A* or *syncytin-Rum1* (Nakaya et al., 2013). This simply may be due to higher expression of *BERV-K1* (Fig. 2). *BERV-K1* mRNA expression increased 202.7 ± 43.7-fold ($P < 0.001$) on day 50, compared with NP.

Syncytin-Rum1 and *BERV-K1* may be involved in the establishment of pregnancy and normal placental development. Abnormal placental growth has been reported to cause impaired pregnancy and fetal growth because placental growth is closely correlated to fetal growth (Caton and Hess, 2010). Impaired pregnancies have long-term effects on the offspring by decreasing their health and productivity throughout their lifetime, such as decreasing muscle mass and adipocyte diameter and increasing yield grade at harvest (Long et al., 2012).

Metabolically and otherwise compromised animals are major deterrents to efficient and sustainable livestock production systems. Therefore, improvements in fetal and placental development leading to better pregnancy outcomes would have a significant impact on animal agriculture and beef cattle production.

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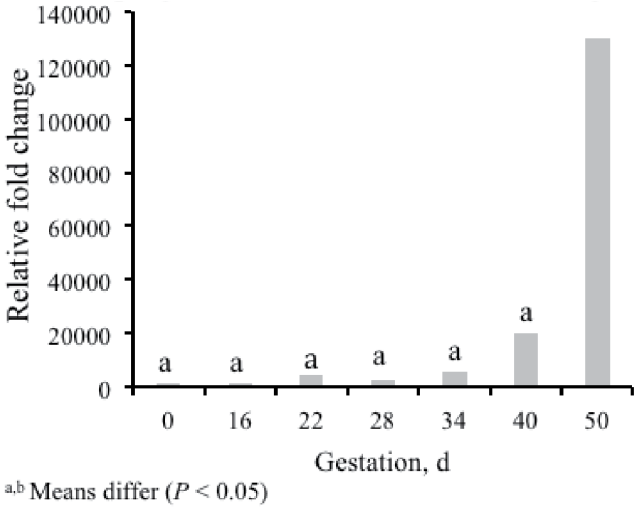

Figure 1. Basal mRNA expression of pregnancy-specific protein-B during early gestation.

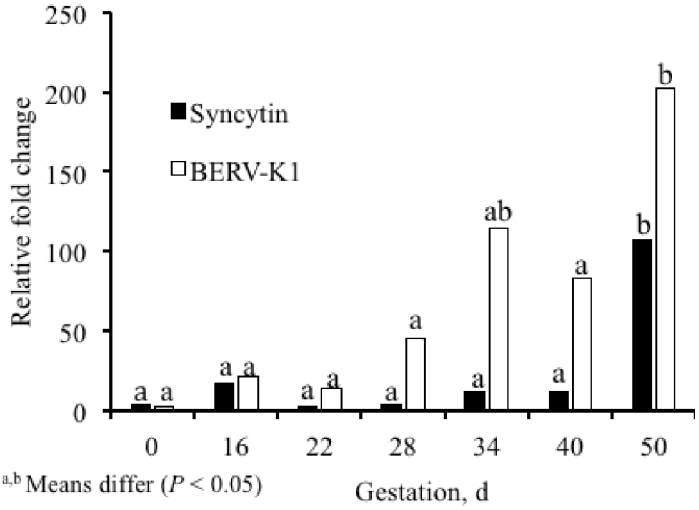

Figure 2. Basal mRNA expression of endogenous retroviral elements during early gestation.

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