J. Dairy Sci. 101:1–15
https://doi.org/10.3168/jds.2017-13221

Identification of potential embryokines in the bovine reproductive tract

P. Tríbulo,*† L. G. B. Siqueira,*†‡ L. J. Oliveira,†§ T. Scheffler,* and P. J. Hansen*†#[1]
*Department of Animal Sciences, and
†D.H. Barron Reproductive and Perinatal Biology Research Program, University of Florida, Gainesville 32611
‡Embrapa Gado de Leite, Juiz de Fora, MG, Brazil 36038-330
§Department of Infectious Disease and Pathology, College of Veterinary Medicine, University of Florida, Gainesville 32611-0910
#Genetics Institute, University of Florida, Gainesville 32611

ABSTRACT

Knowledge of the molecules used by the maternal reproductive tract to regulate development of the preimplantation embryo is largely incomplete. The goal of the present experiment was to identify candidates for this function. The approach was to assess expression patterns in the endometrium and oviduct of 93 genes encoding for hormones, growth factors, chemokines, cytokines, and WNT-related molecules. Results show that all of the genes were expressed in the reproductive tract. Expression in oviduct was affected by day of the estrous cycle for 21 genes with 11 genes having highest expression at estrus (*CCL21*, *CTGF*, *CXCL10*, *CXCL16*, *DKK3*, *FGF10*, *IL18*, *IL33*, *IL34*, *PGF*, and *SFRP2*), one gene at d 3 (*WNT4*), 8 at d 5 (*BMP7*, *HGF*, *IL6*, *SFRP1*, *TGFB1*, *WIF1*, *WNT2*, and *WNT5A*), and one at d 7 (*IK*). For endometrium, expression of 34 genes was affected by day of the estrous cycle with 11 having highest expression at d 0 (*BMP7*, *CCL14*, *CCL21*, *CCL26*, *CTGF*, *CXCL12*, *IGF2*, *IL16*, *IL33*, *SFRP2*, and *WIF1*), 2 at d 3 (*HDGF*, *IL15*), 14 at d 5 (*CSF2*, *CX3CL1*, *CXCL3*, *FGF1*, *FGF2*, *GRO1*, *HGF*, *IGF1*, *IL1B*, *IL8*, *SFRP1*, *SFRP4*, *WNT5A*, and *WNT16*), and 7 at d 7 (*CXCL16*, *FGF13*, *HDGFRP2*, *TDGF1*, *VEGFB*, *WNT7A*, and *WNT11*). Results are consistent with a set of genes regulated by estradiol early in the estrous cycle and another set regulated by progesterone later in the cycle. The cell-signaling genes identified here as being expressed in the oviduct and endometrium could serve to regulate early embryonic development in a stage-of-pregnancy-specific manner.
Key words: embryokine, histotroph, endometrium, oviduct, maternal-embryo crosstalk

INTRODUCTION

The environment established by the mother for the preimplantation embryo plays a key role in ensuring that development proceeds in a manner that optimizes pregnancy success and postnatal development. Disruption of maternal physiology during the preimplantation period can compromise embryonic survival. Examples include the effect of establishing an abnormal ratio of estradiol to progesterone in mice (Yoshinaga and Adams, 1966) and humans (Simón et al., 1995) and exposure of female embryo transfer recipients to bisphenol A in mice (Xiao et al., 2011). Other conditions can enhance maternal capacity for supporting development, as shown in cattle for treatment with somatotropin (Moreira et al., 2002). The maternal environment during the preimplantation period can also alter the developmental program of the embryo in a manner that alters postnatal phenotype [see review by Hansen et al. (2016)]. For example, feeding a diet low in protein during the preimplantation period modified postnatal growth and accumulation of body fat in rodents (Fleming et al., 2015).

The importance of the maternal environment for embryonic development is illustrated by the consequences of embryo production in vitro. In cattle, embryos produced in vitro experience altered gene expression (Corcoran et al., 2006; McHughes et al., 2009; Gad et al., 2012), metabolism (Khurana and Niemann, 2000), lipid content (Crosier et al., 2000; Sudano et al., 2012), ultrastructure (Boni et al., 1999; Rizos et al., 2002), DNA methylation (Niemann et al., 2010), and competence to establish pregnancy (Lonergan et al., 2007; Pontes et al., 2009). Moreover, properties of the resultant offspring can be disrupted in cattle (Siqueira et al., 2017) and other species (Fernandez-Gonzalez et al., 2004; Farin et al., 2006; Ceelen et al., 2008). Transfer of embryos produced in vitro to the oviduct can mitigate at least some of these abnormalities (Enright et al., 2000; Lazzari et al., 2010; Gad et al., 2012), indicating the importance of maternal signals for optimal development.

Received August 15, 2017.
Accepted September 19, 2017.
[1]Corresponding author: pjhansen@ufl.edu

An important mechanism by which the maternal oviduct and endometrium direct embryonic development is through secretion of regulatory molecules called embryokines. Several growth factors can affect embryonic development in various species. Among the most studied embryokines are colony-stimulating factor 2 (**CSF2**; Sjoblom et al., 1999; Loureiro et al., 2009, 2011; Denicol et al., 2014), IGF1 (Lin et al., 2003; Jousan and Hansen, 2007; Bonilla et al., 2011), and leukemia inhibitor factor (**LIF**; Kauma and Matt, 1995; Mohamed et al., 2004; Neira et al., 2010).

In many cases, it is unknown whether molecules that affect embryonic development in vitro are present in the reproductive tract at times coincident with development of the preimplantation embryo. In addition, other regulatory molecules are likely produced by the reproductive tract that can act on the preimplantation embryo. Indeed, the embryo is poised to respond to a plethora of maternal regulatory molecules because of the wide range of growth factor and hormone receptor genes that it expresses (Graf et al., 2014; Zuo et al., 2016).

The objective of the present study was to identify potential embryokines during the first 7 d after ovulation in the cow. It is during this period that the bovine embryo develops from the zygote to the blastocyst stage, where it spends the first 4 to 5 d in the oviduct, and then moves into the uterine lumen (Betteridge and Fléchon, 1988). The approach was to collect oviductal and endometrial tissue and determine the relative amounts of expression of genes for 93 hormones, growth factors, cytokines, chemokines, and WNT-related molecules that could potentially function as embryokines.

MATERIALS AND METHODS

Synchronization of the Estrous Cycle

The reproductive status of nonlactating Holstein cows was assessed by transrectal ultrasonography and 20 cows with a detectable corpus luteum were subjected to a hormonal protocol to synchronize ovulation. On d −18 (day of expected ovulation = d 0), cows were injected i.m. with 25 mg of $PGF_{2\alpha}$ (Lutalyse, Zoetis, Florham Park, NJ) followed by 100 μg of gonadorelin (GnRH; Cystorelin, Merial Inc., Duluth, GA) on d −16. A second, identical injection of GnRH was given on d −9 and a progesterone-containing controlled internal drug release device (CIDR, Zoetis) was inserted intravaginally. At d −4, each cow was administered 25 mg of $PGF_{2\alpha}$ i.m. and the intravaginal device was removed. Another 25 mg of $PGF_{2\alpha}$ was injected at d −3 and 100 μg of GnRH was injected i.m. at d −2 (i.e., 24 h after $PGF_{2\alpha}$). Transrectal ultrasonography of ovaries was performed on d −4, −1, and 0 to confirm ovulation. A total of 15 cows were successfully synchronized and slaughtered at either d 0 (n = 4), 3 (n = 4), 5 (n = 3), or 7 (n = 4) relative to the day of ovulation. Cows within group were split into 2 groups that were slaughtered on 2 different days. Slaughter was by captive-bolt stunning and exsanguination at a commercial abattoir.

Collection of Oviductal and Endometrial Tissues and Flushings

Reproductive tracts were obtained immediately after slaughter and placed on ice. Processing of all organs was completed within a maximum of 4 h from slaughter of the first cow. Side of the reproductive tract was identified as being ipsilateral or contralateral to the side of ovulation. Ovulation of cows slaughtered at d 0 was confirmed by absence of a preovulatory follicle in 3 of 4 cows. For the remaining cow, ovaries were lost during processing and ovulation could not be confirmed.

After dissecting the oviduct free from the mesosalpinx, the caudal third of the oviduct, corresponding to the isthmus, was used to cut transversal 1-mm sections while the oviduct was gently stretched. Samples were snap frozen in liquid N_2 for evaluation of gene expression. Samples were stored in liquid N_2 until transport to the laboratory and storage at −80°C.

Uterine flushings and samples of endometrium were collected separately from both uterine horns. The mesometrium was removed and each uterine horn was clamped near the uterine body. The end near the uterotubal junction was opened with a 0.5-cm incision and 30 mL of Dulbeccos's PBS at room temperature were flushed into the uterine horn from the opposite end using an 18-gauge needle. The fluid was propelled by massage along the uterine horn through the incision. Recovered fluid was kept on ice; after centrifugation at 3,000 × *g* for 15 min at 4°C, the supernatant fraction was obtained and stored at −20°C.

After flushing, each uterine horn was opened with a longitudinal incision along the major curvature. Intercaruncular regions of endometrial tissue were harvested from the middle section of uterine horns using a scalpel and tweezers. Three samples per cow were snap frozen, and 2 other samples were frozen in optimal cutting temperature medium (O.C.T., Sakura Finetek USA Inc., Torrance, CA) on dry ice covered with 2 methylbutyrate for immunofluorescence analyses. All samples were stored in liquid N_2 until transport to the laboratory where they were stored at −80°C.

Note that limited resources prevented collection of caruncular endometrium. Intercaruncular endometrium