J. Dairy Sci. 100:1–14
https://doi.org/10.3168/jds.2016-11880

The influence of postnatal nutrition on reproductive tract and endometrial gland development in dairy calves

Meghan L. Wilson,* Sarah R. McCoski,* Adam J. Geiger,† R. Michael Akers,† Sally E. Johnson,* and Alan D. Ealy*[1]
*Department of Animal and Poultry Sciences, and
†Department of Dairy Sciences, Virginia Tech, Blacksburg 24061

ABSTRACT

Uterine gland development occurs after birth in cattle and other mammals. The timeline of gland development has been described in various species, but little is known about how postnatal diet influences uterine gland development. This is especially concerning in dairy heifers, where a variety of milk replacer and whole milk nutrition options exist. Little work also exists in cattle to describe how early exposure to steroids influences reproductive tract and uterine gland development. The objective of this work was to determine the effects of early postnatal plane of nutrition and estrogen supplementation on uterine gland development in calves. In both studies, Holstein heifer calves were assigned to restricted milk replacer (R-MR) or enhanced milk replacer (EH-MR) diets. In study 1, calves (R-MR, n = 6; EH-MR, n = 5) were euthanized at 8 wk. In study 2, calves were weaned at 8 wk and administered estradiol (R-MR, n = 6; EH-MR, n = 6) or placebo (R-MR, n = 6; EH-MR, n = 5) for an additional 14 d before euthanasia. Average daily gain and final body weight was greater in both studies in heifers fed the enhanced diet. At 8 wk, EH-MR calves had a greater number of glands and a smaller average gland size, but total gland area was not different from the R-MR group. At 10 wk, uterine gland number and size were not affected by diet or estrogen. Expression profiles of several paracrine mediators of gland development were examined. Increases in transcript abundance for *IGF1* and *IGFBP3* and a decrease in abundance of *WNT7A* were detected in calves fed the enhanced diet at 8 wk of age. Plane of nutrition did not affect transcript profiles at 10 wk of age, but estradiol supplementation decreased *MET* and *WNT7A* transcript abundance. To conclude, heifer calves on a restricted diet exhibited a uterine morphology and transcript profile suggestive of delayed uterine gland development. These changes appear to be corrected by wk 10 of life. Also, this work provides evidence supporting the contention that early estradiol exposure has detrimental effects on uterine gene expression.
Key words: uterus, reproductive tract, growth factors, milk replacers

INTRODUCTION

Mammalian uteri are devoid of endometrial glands at birth. The postnatal development of glands, or adenogenesis, begins immediately after birth and is normally complete by 8 wk of age in gilts and ewe lambs (Bartol et al., 1988b, 1999). The timeline for uterine gland development in cattle has not been described. This process involves a coordinated series of events wherein luminal epithelium differentiate into glandular epithelium located in the glandular buds present throughout regions of the uterus that do not contain caruncles. These invaginations form tubules that eventually coil and branch throughout the stroma to form an extensive network of uterine glands (Gray et al., 2001a).

The primary function of uterine glands is to produce and secrete epithelial products and selectively transport serum compounds into the uterine luminal fluid (Spencer, 2014). This mix of ions, AA, carbohydrates, proteins, lipids, and biologically active factors (e.g., growth factors) are essential for the continuation of early pregnancy. Ewes lacking uterine glands because of neonatal steroid hormone exposure undergo normal estrous cyclicity and produce viable embryos, but the embryos die around the time of maternal recognition of pregnancy (Gray et al., 2001b).

Avoiding steroid-containing implants or legumes that contain large quantities of phytoestrogens is recommended for cattle and other ruminants before puberty to prevent diminished reproductive potential in adulthood (Adams, 1995a; Becker et al., 2011). Heifers implanted with Compudose estradiol implant (Elanco Animal Health, Greenfield, IN), a nonsteroidal

Received August 17, 2016.
Accepted December 19, 2016.
[1]Corresponding author: ealy@vt.edu

estrogen agonist, have increased incidence of ovarian cysts (Deutscher et al., 1986). Uterine gland development also is compromised with early postnatal steroid exposure. The most severe reductions in uterine gland number and size occur in sheep, pigs, and cattle when estradiol or progesterone exposure begins at birth. Females display a permanent reduction or a total absence of uterine glands that persists throughout life (Bartol et al., 1988a, 1995; Spencer et al., 1993; Gray et al., 2001a). Endometrial gland density and uterine fluid content also is reduced by estrogen administration in 3- and 6-wk-old heifers (Bartol et al., 1995).

It is unclear how early postnatal nutrition affects adenogenesis. This is especially concerning in dairy heifers, where a variety of milk replacer and whole milk nutrition options exist. A commonly used dairy calf milk replacer ration includes low amounts of protein and fat to promote early starter grain consumption, which hastens rumen development and, ultimately, reduces the overall feed costs (Brown et al., 2005; Hill et al., 2008). This scheme also eases the transition from a milk- to a grain-based diet at weaning. Other milk replacer rations contain greater protein and fat content, so they more closely approximate the composition and caloric density of normal cow milk. The high-protein, high-fat ration increases BW and ADG in the first several months of life (Hill et al., 2008; Daniels et al., 2009). Reductions in the age at puberty and first calving and increases in first-lactation milk production are reported in calves consuming high-protein, high-fat milk replacer rations when compared with calves consuming diets containing lower protein and fat (Davis Rincker et al., 2011; Soberon and Van Amburgh, 2013).

The current work was undertaken to obtain a more complete understanding of how early postnatal nutrition and estrogen supplementation at weaning influences reproductive tract morphology and uterine gland development in dairy heifers. Our hypothesis was that changes in preweaning nutrition (i.e., during the first 8 wk of life) influences reproductive tract development and uterine gland formation and that estradiol supplementation at weaning adversely affects these processes.

MATERIALS AND METHODS

Animal Handling and Experimental Design

Animal experiments were completed in accordance with and with the approval of the Virginia Tech Institutional Animal Care and Use Committee (#14–045-DASC). Two studies were initially conceived to examine how postnatal plane of nutrition and early estradiol exposure affects mammary gland development. An initial publication of these findings has been completed (Geiger et al., 2016). The feeding scheme, time intervals, and use of heifer calves (expensive to use for terminal studies) provided the opportunity for companion studies examining the influences of early plane of nutrition and estradiol exposure on reproductive tract development.

The 2 studies used the same early postnatal feeding scheme. In both studies, Holstein heifer calves (*Bos taurus taurus*) were purchased from a commercial producer and maintained at the Virginia Tech Dairy Center (Blacksburg, VA). Calves were fed similarly for the first 5 to 8 d of life (Geiger et al., 2016). At 6 ± 2 d of age, calves were assigned randomly to restricted (**R**) or enhanced (**EH**) milk replacer (**MR**). The R diet (20.9% CP, 19.8% fat, DM basis; Southern States Inc., Richmond, VA) was fed at 0.44 kg of MR powder/animal per day. The EH diet (28.9% CP, 26.2% fat, DM basis; Land O'Lakes Animal Milk Products Co., Shoreview, MN) was fed at 1.08 kg of MR powder/animal per day. Both MR were reconstituted to 15% (wt/vol) water and fed in 2 equal portions at 0600 and 1700 h daily. At the beginning of wk 8, the amount of MR offered was reduced by half to prepare calves for weaning. Heifers were weaned at the end of wk 8. Calves were offered water ad libitum throughout the study. At the end of wk 4, calves were pair-fed starter grain to keep starter intakes similar between groups (25.6% CP, 4.0% fat, 19.8% NDF, DM basis; Southern States Cooperative). This was achieved by offering R-fed calves the average amount of starter grain consumed by ad libitum, EH-fed calves on the preceding day. Milk replacer and feed refusals, weekly growth measurements (BW, hip and withers height, heart girth), and daily fecal and respiration scores are described elsewhere (Geiger et al., 2016). On only a few occasions did calves not consume their entire allotment of milk replacer, and these occasions usually occurred during the first week on their diet.

The 2 studies differed in the duration on diet. In the first study, R- (n = 6) and EH-fed (n = 5) calves were euthanized at the end of wk 8 (56 ± 2 d of age). In the second study, R- and EH-fed calves (R-MR, n = 12; EH-MR, n = 11) were weaned and then maintained exclusively on starter grain (pair-wise fed) until the end of wk 10. An estradiol implant (R-MR, n = 6; EH-MR, n = 6) or a blank silicone implant (R-MR n = 6; EH-MR n = 5) was inserted into the ear of calves at the time of weaning, as described previously (25.7 mg of estradiol-17β; Compudose Implant; Elanco Animal Health, Greenfield, IN; Lammers et al., 1999). Circulating estradiol concentrations were not determined, but, according to manufacturer specifications, calves were