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Effect of exosomes from plasma of dairy cows with or without an infected uterus on prostaglandin production by endometrial cell lines

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ABSTRACT

A contributing factor to declining fertility in dairy cows is an activated inflammatory system associated with uterine infection. Detecting uterine disease using biomarkers may allow earlier diagnosis and intervention with resultant improvements in fertility. Exosomes are known to participate in intercellular communication, paracrine, and endocrine signaling. Exosomes carry a cargo of proteins, lipids, and nucleic acids that represent specific cellular sources. Prostaglandins are lipids that are critical determinants of bovine fertility. In this study exosomes were isolated from the plasma of cows before (d 0) and during (d 10) the study in healthy animals or those with an induced uterine infection in a 2×2 factorial design. Exosomes were characterized for size and number (nanoparticle tracking analysis), exosomal marker expression (Western blot), and morphology (transmission electron microscopy). No significant differences were observed in exosome size or number. The abundance of exosome-enriched markers was confirmed in noninfected and infected animals. Transmission electron microscopy confirmed the morphology of the exosomes. These exosomes were co-incubated with bovine endometrial epithelial and stromal cells. Exosomes from d-10-infected animal plasma decreased $PGF_{2\alpha}$ production in endometrial epithelial but not stromal cells. For future research, the identification of effectors in the cargo may provide a useful basis for early diagnosis of uterine infection using an exosomal characterization approach.

Key words: dairy cow, uterine infection, exosome, prostaglandin

INTRODUCTION

Maintaining optimal reproductive performance is an important economic driver in dairy cattle production. Poor reproductive performance is associated with extended intervals between calvings, reduced milk production, and increased risk of cows being removed from the population, and thus significant economic losses (Inchaisri et al., 2010). Improved management has increased milk production, but fertility has declined due to increased metabolic pressure (Lucy, 2001; Garnsworthy et al., 2008; Roche et al., 2011).

Bacteria can be isolated from the majority of cows in the early postpartum period. However, many of these infections are self-limiting, but clinical disease is associated with the ongoing presence of *Escherichia coli*, *Trueperella pyogenes*, *Prevotella* spp., *Fusobacterium* spp., or *Bacteroides* spp. (Sheldon et al., 2002). Prevalence of clinical endometritis (presence of grossly evident purulent material in the vagina or uterus) is estimated to be 15 to 20% in dairy cows, and 30% are affected by subclinical endometritis (inflammation of the uterus as determined using cytology; Sheldon et al., 2009). Uterine inflammation, whether it is grossly evident or not, is associated with decreased conception rates and prolonged days to first service and days open, and it has been demonstrated that subclinical endometritis has an effect on survival and quality of the embryo (Kasimanickam et al., 2004; McDougall et al., 2011; Ribeiro et al., 2013).

Uterine disease is currently diagnosed using cow-side tests, such as gloved hand examination of the vaginal contents, vaginoscopy, use of an intravaginal probe to retrieve purulent material (Metricheck device, Simcro, Hamilton, New Zealand), or endometrial cytology among others (de Boer et al., 2014). Although endometrial cytology appears to have a higher sensitivity than other tests (McDougall et al., 2011), and is a better predictor of reproductive performance than other tests, it is an expensive and time-consuming test that re-

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quires laboratory infrastructure. Sensitive and specific, rapid, and cost-effective diagnostic tools for uterine disease need to be developed in dairy cattle, potentially through the use of biomarkers.

Exosomes are highly stable extracellular vesicles (**EV**) that bud from the cell membrane and are 30 to 120 nm in diameter (Raposo and Stoorvogel, 2013; Sohel et al., 2013). They are involved in cell-to-cell communication and may be useful in diagnosis of several biological conditions (Crookenden et al., 2016; Mitchell et al., 2016). Exosome number and content are altered under physiological pressures (e.g., cancer, pregnancy, and infection; Sabapatha et al., 2006; Silverman et al., 2010; King et al., 2012) and have a role in immunomodulation (Deng et al., 2013). Prostaglandins (**PG**), as well as being immunomodulatory agents, are key players in bovine fertility. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and prostaglandin E_2 (**PGE_2**) are the main PG associated with reproduction. The endometrium release of $PGF_{2\alpha}$ initiates luteolysis, and PGE_2 is a temporary luteotrophic signal, acting with interferon τ, for pregnancy maternal recognition (Arosh et al., 2002). During uterine infection in postpartum cows, plasma concentrations of $PGF_{2\alpha}$ metabolite are increased (Del Vecchio et al., 1994). In vitro studies of endometrial cells have shown PG are produced at differing rates. Stromal cells produce more PGE_2 than epithelial cells, with a reversal for $PGF_{2\alpha}$ (Fortier et al., 1988; Danet-Desnoyers et al., 1994; Asselin et al., 1996), whereas in another report secretion of PG was similar by epithelial and stromal cells (Betts and Hansen, 1992). Endometrium explants or cell lines challenged with LPS produced higher PGE_2 compared with $PGF_{2\alpha}$ (Herath et al., 2009).

This study aimed to assess whether the number or size of exosomes isolated from the plasma of cows with and without infection of the bovine uterus is altered, and furthermore, whether plasma exosomes from these cows could change PG production by endometrial cells.

MATERIALS AND METHODS

Animals

This negative controlled intervention study was conducted using 20 cows collected at d 0 and 10 sourced from a single research herd located in the north of New Zealand (Lye Farm, DairyNZ Vaile Rd., Newstead). Cows were selected based on calving date, freedom from grossly evident peripartum disease, and not having been treated with antimicrobials or nonsteroidal anti-inflammatories in the 14 d preceding the commencement of the study.

Cows were synchronized using the G-6-G/OvSynch protocol (that is a sequence of $PGF_{2\alpha}$, GnRH, GnRH, $PGF_{2\alpha}$, and GnRH at 2-, 6-, 7-, and 2-d intervals, respectively; Bello et al., 2006), and all cows were treated with an intrauterine antimicrobial (0.5 g of cephapirin, Metricure, MSD, Upper Hutt, New Zealand) at the time of the first GnRH injection.

At 96 h after the final GnRH of the synchrony protocol (i.e., approximately 3 d after ovulation), cows were blocked by age (2, 3, greater than 3 yr) and then randomly assigned to 2 groups (n = 18 per group). Half the cows were infused with saline, and the remaining half were further randomly (within age) assigned into 2 groups and infused into the uterus with either a dose of 10^7 cfu or 10^9 cfu of *T. pyogenes.* The strain of *T. pyogenes* and the technique of infusion was the same as that used by Amos et al. (2014).

Prior to infusion, and at d 10 after infusion, duplicate swabs were collected from the body of the uterus using triple guarded uterine catheters. One swab was used to prepare a glass slide for subsequent staining and cytological evaluation (Kasimanickam et al., 2004), and the second was placed in transport media for aerobic and capnophilic bacterial culture. The cows were observed daily for signs of clinical disease including increased rectal temperature, a drop in milk yield, and systemic signs of illness (dullness, dehydration, and so on), and daily transrectal ultrasonography to assess ovarian follicle development was undertaken. Blood samples (8 × 10 mL of EDTA, 2 × 10-mL plain vacutainers) were drawn on the days of uterine sampling from a jugular vein. Plasma progesterone was determined by a validated RIA (ImmunoChem Double Antibody Progesterone RIA Kit from MP Biomedical, Santa Ana, CA, catalog # 07–170105; https://www.mpbio.com/product.php?pid=07170105) in the laboratory of DairyNZ (Newstead, New Zealand). The minimum detectable concentration was 0.18 ng/mL. The within and between assays coefficients of variation were 2.3% and 5.2% for the high (2.1 ± 0.1 ng/mL; mean ± SD) and 3.9% and 10.0% for the low (0.25 ± 0.03 ng/mL) quality assurance standards, respectively.

Following culture, plasma from a subset of 10 cows from the control group with no evidence of infection and 10 cows from the *T. pyogenes*–treated group, in which heavy bacterial infection was confirmed by culture, were selected for this current study. These cows were all >12/16ths Holstein-Friesian, averaged 2.7 yr of age (SD = 0.9; range 2 to 4), and were an average of 48.2 DIM (SD = 4.1; range 43 to 58) at the time of intrauterine challenge. Cows had an average BW of 422 kg (SD = 47; range 360–526 kg) and a BCS of 4.1 (SD = 0.2; range 3.75–4.5 BCS; on a 1 to 10 scale, Roche et al., 2004) at 5 d before intrauterine challenge. Average daily milk yield over the 7 d preceding challenge was 21.2 L/cow per day (SD = 2.8; range 18.0–27.0