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Plasma exosome profiles from dairy cows with divergent fertility phenotypes

M. D. Mitchell,*[1] K. Scholz-Romero,* S. Reed,* H. N. Peiris,* Y. Q. Koh,* S. Meier,† C. G. Walker,‡[2] C. R. Burke,† J. R. Roche,† G. Rice,* and C. Salomon*
*Centre for Clinical Diagnostics, University of Queensland Centre for Clinical Research, Royal Brisbane and Women's Hospital, Queensland, Australia
†DairyNZ Ltd., Private Bag 3221, Hamilton 3240, New Zealand
‡DairyNZ Ltd., 3A Symonds Street, Auckland 1010, New Zealand

ABSTRACT

Cell-to-cell communication in physiological and pathological conditions may be influenced by neighboring cells, distant tissues, or local environmental factors. Exosomes are specific subsets of extracellular vesicles that internalize and deliver their content to near and distant sites. Exosomes may play a role in the maternal-embryo crosstalk vital for the recognition and maintenance of a pregnancy; however, their role in dairy cow reproduction has not been established. This study aimed to characterize the exosome profile in the plasma of 2 strains of dairy cow with divergent fertility phenotypes. Plasma was obtained and characterized on the basis of genetic ancestry as fertile (FERT; <23% North American genetics, New Zealand Holstein-Friesian strain, n = 8) or subfertile (SUBFERT; >92% North American genetics, North American Holstein-Friesian strain, n = 8). Exosomes were isolated by differential and buoyant density centrifugation and characterized by size distribution (nanoparticle tracking analysis, NanoSight NS500, NanoSight Ltd., Amesbury, UK), the presence of CD63 (Western blot), and their morphology (electron microscopy). The total number of exosomes was determined by quantifying the immunoreactive CD63 (ExoELISA kit, System Biosciences), and the protein content established by mass spectrometry. Enriched exosome fractions were identified as cup-shape vesicles with diameters around 100 nm and positive for the CD63 marker. The concentration of exosomes was 50% greater in FERT cows. Mass spectrometry identified 104 and 117 proteins in FERT and SUBFERT cows, of which 23 and 36 were unique, respectively. Gene ontology analysis revealed enrichment for proteins involved in immunomodulatory processes and cell-to-cell communication. Although the role of exosomes in dairy cow reproduction remains to be elucidated, their quantification and content in models with divergent fertility phenotypes could provide novel information to support both physiological and genetic approaches to improving dairy cow fertility.
Key words: exosomes, fertility, dairy cow

INTRODUCTION

Until recently, genetic selection in dairy cows has focused primarily on milk production traits, with very few countries including functional traits such as fertility indices (Miglior et al., 2005). As a result, milk production capacity of the modern dairy cow has increased dramatically, but fertility has declined steadily (Garnsworthy et al., 2008). This decline is attributed to a reduction in pregnancy rate in the modern cow, chiefly associated with an increase in metabolic pressure due to increases in milk production (Butler, 2000; Roche et al., 2011). However, another critical detrimental factor to cow fertility is the presence of an activated inflammatory system through infectious or immune challenge (Formigoni and Trevisi, 2003; Piccinini et al., 2004; McDougall et al., 2007).

The need for, and utility of, markers of early disease onset (or vulnerability to diseases), which often can lead to early intervention and greater survival rates, has accelerated the development of methods for biomarker discovery in humans (Pan et al., 2005; Peddinti et al., 2008). The same methods could be used to predict desirable traits in animals, such as likelihood of pregnancy success. The use of epigenetic biomarkers is still in its infancy but already some positive results have been obtained (Magee et al., 2010; Berkowicz et al., 2011). We believe that pregnancy rates can be improved by use of protein and epigenetic biomarkers that can be delineated and then used prognostically for positive reproductive performance and, potentially,

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[1]Corresponding author: murray.mitchell@uq.edu.au
[2]Current address: Growing Up in New Zealand, Centre of Longitudinal Research, University of Auckland, Auckland, New Zealand 1010.

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to monitor the effectiveness of intervention strategies, such as nutritional or anti-inflammatory approaches.

Recently, the utility of circulating nanovesicles, such as exosomes, as biomarkers of disease has been reported (Alvarez et al., 2012; Bala et al., 2012). Exosomes are small (40–100 nm) membrane vesicles that are secreted from all cell types through the inward budding of multivesicular bodies with the plasma membrane. They contain protein, lipid, and nucleic acids and are hypothesized to function as messengers for cellular communication between tissues (De Toro et al., 2015). The number of exosomes found in bodily fluids such as blood is increased in pathological conditions and the protein cargo of exosomes can be used to diagnose diseases such as cancer with high specificity and sensitivity (Melo et al., 2015). Furthermore, exosomes have recently been used as a marker for pregnancy and pregnancy-related pathologies in humans (Tsochandaridis et al., 2015). In sheep, exosomes derived from uterine flushings stimulate trophoectoderm cell lines to proliferate and secrete interferon tau, the pregnancy recognition signal (Ruiz-Gonzalez et al., 2015). The miRNA content of exosomes has also been characterized in bovine follicular fluid, with content differing depending on the oocytes stage of development (Sohel et al., 2013).

Our hypothesis was that exosomes containing specific effector molecules (e.g., proteins and miRNA) will be detectable in bovine plasma and that the number of exosomes and the exosomal cargo will differ in cows of divergent fertility phenotype.

MATERIALS AND METHODS

Animals and Management

All procedures were undertaken with the approval of the Ruakura Animal Ethics Committee (Hamilton, New Zealand). The study was conducted at DairyNZ Limited (No 5. Dairy, Hamilton, New Zealand). The original study was a terminal study, ending approximately 86 d in milk (DIM; SD 8 d; n = 27) (Meier et al., 2009, 2014). For the purposes of the current study, a selective set of 40 samples suitable for exosome analyses across a range of DIM were evaluated. These samples represented 16 from 27 lactating Holstein-Friesian cows. Sample description are provided in Figure 1. Cows were representative of 2 genetic strains [<23% North American genetics (fertile, **FERT**; **NZ**, n = 8) or >92% North American genetics (subfertile, **SUBFERT**; **NA**, n = 8)]. This designation (FERT and SUBFERT) is consistent with the poorer oocyte and embryo quality, lower conception rate to first and second services, and lower 6-wk pregnancy rate and overall lower pregnancy rate for the NA compared with NZ strain Holstein-Friesian dairy cows (Horan et al., 2005; de Feu et al., 2008; Macdonald et al., 2008). Table 1 provides a summary of the phenotypic descriptions of the dairy cows used, including ancestry, age, BW, BCS, DIM, milk yield, milk composition, and pedigree information of this subset of animals.

All cows were managed as a single herd, with fresh pasture grazed in an intensive rotational manner similar

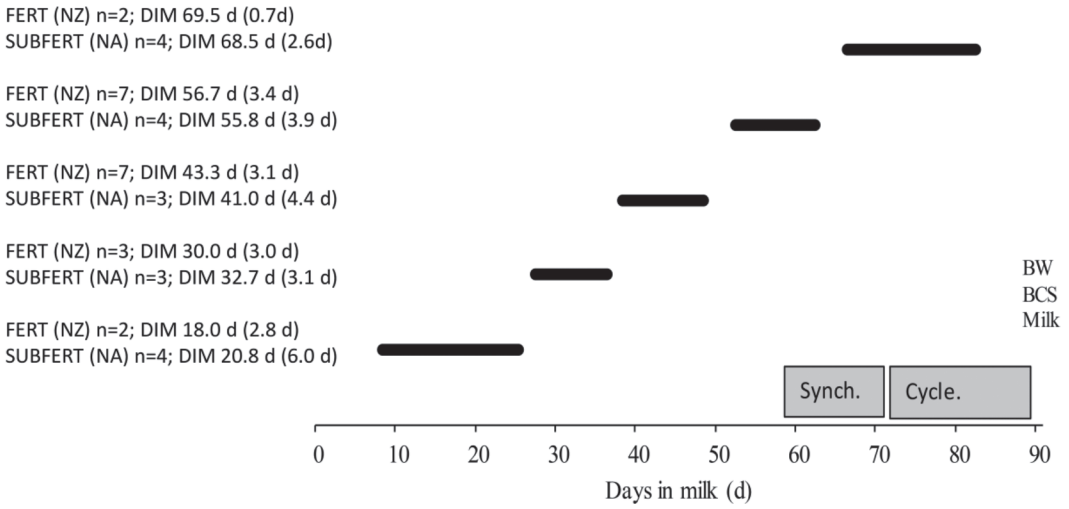

Figure 1. Schematic diagram of the samples used for exosome analyses for each group including DIM and SD (in parentheses). A synchrony program (Synch) was initiated at approximately 58 to 60 DIM, followed by a reproductive cycle (Cycle). Eight of the 16 animals underwent embryo transfer 7 d after the synchrony [fertile (FERT), n = 4; subfertile (SUBFERT), n = 4], whereas the remaining 8 animals did not undergo embryo transfer (FERT, n = 4; SUBFERT, n = 4; Meier et al., 2009, 2014). NZ = New Zealand Holstein-Friesian strain; NA = North American Holstein-Friesian strain.

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