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Negative energy balance affects imprint stability in oocytes recovered from postpartum dairy cows

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ABSTRACT

Ovarian follicle development in post-partum, high-producing dairy cows, occurs in a compromised endogenous metabolic environment (referred to as negative energy balance, NEB). Key events that occur during oocyte/follicle growth, such as the vital process of genomic imprinting, may be detrimentally affected by this altered ovarian environment. Imprinting is crucial for placental function and regulation of fetal growth, therefore failure to establish and maintain imprints during oocyte growth may contribute to early embryonic loss. Using ovum pick-up (OPU), oocytes and follicular fluid samples were recovered from cows between days 20 and 115 post-calving, encompassing the NEB period. In a complimentary study, cumulus oocyte complexes were in vitro matured under high non-esterified fatty acid (NEFA) concentrations and in the presence of the methyl-donor S-adenosylmethionine (SAM). Pyrosequencing revealed the loss of methylation at several imprinted loci in the OPU derived oocytes. The loss of DNA methylation was observed at the *PLAGL1* locus in oocytes, following in vitro maturation (IVM) in the presence of elevated NEFAs and SAM. Finally, metabolomic analysis of postpartum follicular fluid samples revealed significant differences in several branched chain amino acids, with fatty acid profiles bearing similarities to those characteristic of lactating dairy cows. These results provide the first evidence that (1) the postpartum ovarian environment may affect maternal imprint acquisition and (2) elevated NEFAs during IVM can lead to the loss of imprinted gene methylation in bovine oocytes.

1. Introduction

The decline in the reproductive performance of high yielding Holstein dairy cows over the past five decades has been well documented [34,37,40]. Although these animals are positively characterized for their lactational performance and are capable of producing vast amounts of milk [14], this level of production comes at a cost. The mobilization of fat and muscle in support of milk production during lactation results in deteriorating body condition that is reflected in the cows' metabolic and hormone profiles, such as reduced glucose concentrations, increased levels of β-hydroxybutyrate & non-esterified fatty acids (NEFAs), lower IGF-1 and decreased insulin levels [34,43]. These metabolic and endocrinological fluctuations are associated with poor reproductive performance, attributed in part to a suboptimal follicular microenvironment and reduced developmental competence of the oocyte and subsequent embryo [35].

In order to present a viable gamete for fertilization, the oocyte must complete a series of critical processes during transition from the resting primordial stage to the preovulatory stage. These changes include proliferation and differentiation of cytoplasmic organelles, synthesis and storage of mRNA and proteins required to drive the initial cell cycles of embryogenesis, resumption of and completion of meiosis and attainment of epigenetic modifications (for review, see [16]). One such epigenetic process is the essential reprogramming of genomic imprints, which occurs during the mammalian oocyte growth stage [27,39,48]. This process involves reprogramming the maternal genome, through DNA methylation of differentially methylated regions (DMRs), to elicit parent-of-origin specific expression of a small group of genes, collectively known as imprinted genes, reviewed in [19]. Inappropriate methylation at imprinted loci can lead to ectopic expression of imprinted genes (e.g. both copies silenced or both copies expressed) and has been observed in a number of developmental and pathological conditions, such as Beckwith–Wiedemann syndrome [5], Prader–Willi syndrome and Angelman syndrome [8], spontaneous abortion [65], early embryonic lethality [50], and transient neonatal diabetes [41,58].

Recent studies have shown that the adverse metabolic environment induced by lactation alters the metabolomic, steroidogenic

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and transcriptomic profile of ovarian follicles during their development in postpartum lactating cows compared to non-lactating heifers [4,61,62]. These differences are characterized by higher concentrations of saturated fatty acids (SFAs), such as palmitic and stearic acids, lower concentrations of n−3 polyunsaturated fatty acids (PUFAs), higher levels of glycine and L-glutamine and lower levels of L-alanine and oxoproline in follicular fluid from lactating cows compared to those from dry heifers [4]. In lactating cows, this altered metabolic environment was associated with reduced dominant follicle estradiol and progesterone synthesis during differentiation and luteinization, respectively [62]. Analysis of the associated follicular cell transcriptome indicated altered expression profiles of transcripts associated with steroid biosynthesis [62], immune cell function and chemotaxis [61]. These findings suggest that the follicle microenvironment in lactating cows might have a detrimental impact on the oocytes within, placing them at a developmental disadvantage compared with heifer oocytes, and that this may contribute to well-characterized differences in fertility [63].

While the potential for an epigenetic effect of NEB on oocytes has been considered [21], to date there is little or no supporting data. Therefore, the objectives of the current study were to determine if the establishment of the maternal DNA methylation imprints is compromised in oocytes growing within the ovarian microenvironment of the postpartum dairy cow, to characterize the metabolome of the corresponding antral follicles and to investigate the epigenetic effect of high NEFA concentrations during in vitro maturation (IVM) on bovine oocytes.

2. Results

2.1. Imprinted gene DNA methylation during postpartum period

High grade oocytes isolated using transvaginal ultrasound guided ovum-pick-up (OPU) (Fig. 1) were separated into three groups according to the date of collection postpartum (dpp). The groups were as follows: <45 dpp (early), 46–85 dpp (mid) and >85 dpp (late). Average methylation values for the following maternally methylated imprinted gene DMRs, *SNRPN* (12 CpGs), *MEST* (15 CpGs), *IGF2R* (12 CpGs), *PLAGL1* (11 CpGs), and *PEG3* (7 CpGs) and a single paternally methylated imprinted gene, *H19* (8 CpGs), were determined using pyrosequencing

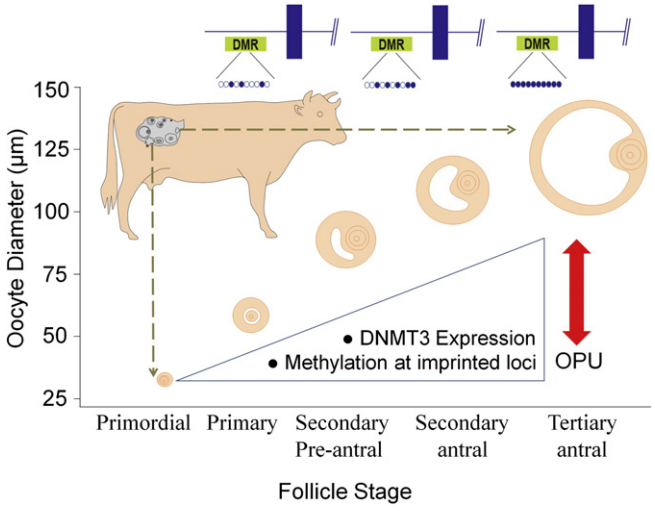

Fig. 1. Schematic diagram of bovine oocyte growth and DNA methylation imprint acquisition. The figure illustrates the dynamics of DNA methylation at the differentially methylated regions (DMRs) of maternally methylated imprinted genes. Circles are representative of individual CpG dinucleotides within the DMR and filled circles denote a methylated CpG. DNA methylation is acquired during the bovine oocyte growth phase, during a period of notable DNA methyltransferase expression (adapted from [48]). Fully grown oocytes used in this study were collected by ovum pick-up (OPU).

Table 1
Summary of DNA methylation data obtained from ovum pick-up derived bovine oocytes.

Gene	Animal ID	0–45 dpp	46–85 dpp	>85 dpp
PEG3	778	x	83.4% (6)	x
	813	1.4% (8.2)†	96.7% (7.6)	x
	779	x	96.3% (4.6)	x
	771	x	95.7% (3.3)	x
MEST	757	x	29.6% (5.8)	1.4% (9.6)†
IGF2R	458	x	87.4% (6.4)	x
	306	x	85.7% (8.5)	x
	701	x	83.2% (6.3)	x
PLAGL1	801	32% (4.5)†	x	x
	306	x	91.9% (8.5)	x
	448	x	x	1.5% (5.5)†
SNRPN	298	3% (4.1)†	x	x
	448	x	x	76.7% (5.5)

Values in brackets represent the level of methylation at the *H19* DMR. Values labeled † represent oocyte samples with a hypomethylated maternally methylated DMRs and a correspondingly low level of *H19* DMR methylation. x = no methylation result determined.

of these oocytes. Data from oocytes that had low level methylation at the *H19* DMR (<10%) are presented in Table 1 and summarized in Fig. 2 (all DMR pyrosequencing results are listed in Supplementary Table 1). For oocyte samples isolated up to 45 dpp, methylation values of 1.4% (*PEG3*), 32% (*PLAGL1*) and 3% (*SNRPN*) were recorded at maternal DMRs. In oocytes isolated from animals between 46 and 85 dpp, methylation values were as follows: 83.4–96.7% (*PEG3*); 29.6% (*MEST*); 83.2–87.4% (*IGF2R*) and 91.9% (*PLAGL1*). Methylation values of 1.4% & 11.6% (*MEST*), 1.5% (*PLAGL1*) and 76.7% (*SNRPN*) were recorded in fully-grown oocytes isolated between 86 and 115 dpp. Collectively, imprinted gene DMR methylation was highly variable in oocytes recovered within the first 45 days post-calving, or from 85 to 110 dpp.

2.2. Metabolomic analysis of follicular fluid

A total of 27 fatty acids were identified and quantified in the follicular fluid samples (Supplementary Table 2). Follicular fluid samples were divided according to days post-parturition as follows: <45 dpp (early $n = 12$), 46–80 dpp (mid $n = 11$) and >80 dpp (late, $n = 8$). In all three groups, the most abundant fatty acids were linoleic acid, oleic acid and stearic acid; erucic acid, heneicosanoic acid and arachidic acid were the least abundant. Of the 27 fatty acids identified, the abundance of six was significantly different ($P < 0.05$) between the three groups (Table 2 & Fig. 3); pentadecanoic acid and tricosanoic acid concentrations increased with increasing dpp, whereas concentrations of

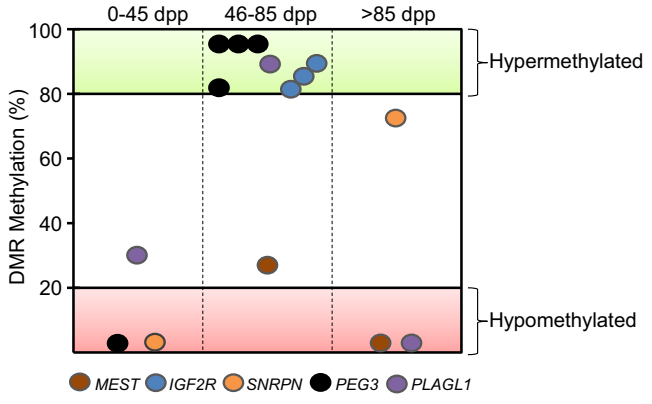

Fig. 2. DNA methylation status at maternally imprinted loci in oocytes relative to days postpartum. DNA methylation status at five maternally methylated DMRs (*MEST*, *PLAGL1*, *SNRPN*, *IGF2R* and *PEG3*) in postpartum derived oocytes was determined by pyrosequencing analysis. Only methylation values in pools of oocytes that had hypomethylation at the *H19* DMR are presented here.