J. Dairy Sci. 98:2322–2336
http://dx.doi.org/10.3168/jds.2014-7970

Free fatty acid levels in fluid of dominant follicles at the preferred insemination time in dairy cows are not affected by early postpartum fatty acid stress

Hilde Aardema,*[1] Bart M. Gadella,*† Chris H. A. van de Lest,† Jos F. H. M. Brouwers,† Tom A. E. Stout,‡ Bernard A. J. Roelen,*‡ and Peter L. A. M. Vos*

*Department of Farm Animal Health,
†Department of Biochemistry and Cell Biology, and
‡Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 7, 3584 CL Utrecht, the Netherlands

ABSTRACT

The fertility of high-yielding dairy cows has declined during the last 3 decades, in association with a more profound negative energy balance (NEB) during the early weeks postpartum. One feature of this NEB is a marked elevation in circulating free fatty acid (FFA) concentrations. During the early postpartum period (≤d 42), circulatory FFA levels were measured weekly, and progesterone concentrations and the diameter of the dominant follicles were determined thrice weekly. Retrospectively, cows that ovulated within 35 d postpartum were grouped as "normal ovulating" cows (n = 5), and the others were grouped as "delayed ovulating" cows (n = 5). In both groups, high total FFA levels (>500 μ*M*) were evident immediately postpartum. Interestingly, cows with delayed ovulation had higher plasma FFA concentrations in the first weeks postpartum compared with normal ovulating cows. In both cow groups, FFA decreased to control levels of non-NEB cows within 3 wk postpartum. The FFA compositions and concentrations in fluids from the dominant follicles of postpartum cows were not different between the normal and delayed ovulating cows when measured at potential insemination points: d 55, 80, and 105 postpartum. Interestingly, the concentration of monounsaturated oleic acid was higher and that of saturated stearic acid lower in follicular fluids of both groups compared with that in blood. The level of FFA in follicular fluid was correlated with the ratio of 17β-estradiol (E_2) to progesterone (P_4) in follicular fluid, with a relatively high level of unsaturated FFA in follicles with a low E_2:P_4 ratio. Taken together, these results indicate that a more severe NEB early postpartum is related to a delay in the first postpartum ovulation and does not affect FFA composition in follicular fluid at the preferred insemination time. The high FFA level in dominant follicles with a low E_2:P_4 ratio may be due to a different FFA metabolism in these follicles. The diagnostic value of this observation for selective screening of dominant follicles needs further investigation.

Key words: free fatty acid, dairy cow, follicular fluid, postpartum

INTRODUCTION

The reproductive performance of high-yielding dairy cows has declined markedly during the last 4 decades, from a calving rate of around 55% per insemination in the 1980s to 40% today (Britt, 1992; Sartori et al., 2002; Walters et al., 2002; Butler, 2003; van Knegsel et al., 2005; Diskin et al., 2006). In contrast, the fertility of nonlactating heifers has remained undiminished throughout this period (Sartori et al., 2002). During this period of declining fertility, the milk production of cows has increased appreciably, with the consequence that cows experience a more profound period of negative energy balance (**NEB**) in the early postpartum period. This NEB is considered to be, at least in part, responsible for the decrease in fertility (Britt, 1992; Walters et al., 2002; Butler, 2003; van Knegsel et al., 2005; Kawashima et al., 2012). Cows with a more severe NEB, and consequent more dramatic loss of body condition during the peripartal period, show compromised reproductive performance in terms of delayed resumption in ovarian activity and reduced ovulation rate from the first follicular wave postpartum, compared with cows with less severe body condition loss (Butler and Smith, 1989; Beam and Butler, 1998). A major metabolic characteristic of NEB is the elevation in circulating FFA concentrations. The FFA are released into the circulation from adipose tissue during NEB and periods of glucose deprivation and are transported into albumin complexes to enable uptake by peripheral tissues, where they can be used as an alternative energy source for aerobic functional cells

Received January 21, 2014.
Accepted December 16, 2014.
[1]Corresponding author: H.Aardema@uu.nl

(Contreras and Sordillo, 2011). Beyond their role as an alternative energy source, however, elevated levels of FFA (especially saturated FFA) may induce lipotoxic stress in several somatic cell types (Cnop et al., 2001; Listenberger et al., 2001; Maedler et al., 2001; Listenberger et al., 2003; Mishra and Simonson, 2005; Coll et al., 2008; Henique et al., 2010). Granulosa and theca cells may also be adversely affected by elevated FFA levels of, in particular, saturated FFA: in vitro exposure results in reduced cell proliferation and increased apoptosis in both cell types (Mu et al., 2001; Vanholder et al., 2005, 2006). Moreover, exposure of the cumulus–oocyte complex to elevated levels of saturated FFA during final maturation can result in an oocyte with a diminished mitochondrial membrane potential and impaired capacity to develop into a blastocyst (Leroy et al., 2005; Aardema et al., 2011; Wu et al., 2012). These observations indicate that the increase in FFA levels that occurs during NEB may well represent a threat for the maturing follicles and internal structures such as the oocyte.

The main focus of in vivo research has been on the acute effect of elevated FFA levels in blood during NEB on the dominant follicle (Leroy et al., 2005; Aardema et al., 2013a). Information on potential dominant follicles selected around the preferred insemination period is lacking. In general, dairy cows are inseminated from around 60 d postpartum onward to achieve a desired calving interval of 12 to 13 mo (Stevenson, 2007). The sequential growth stages of a presumptive dominant follicle, from the primordial stage until the preovulatory stage, take around 80 d in the cow (Britt, 1992). Initially, during early preantral follicular growth, the follicle contains a single layer of granulosa cells surrounding the oocyte but, during subsequent growth phases, the follicle develops into a structure with several cell layers (Fair et al., 1997). A major function of the follicle is to provide a "blood–follicle barrier" to create a favorable environment for the growing oocyte, which is also demonstrated by the distinct FFA composition between blood and follicular fluid (Leroy et al., 2005; Aardema et al., 2013a). The length of the period of follicular growth and development implies that follicles, which gain dominance at 60 to 80 d postpartum, have been recruited and have started their development in the peripartum period during the NEB. Consequently, these follicles have been fully exposed to the NEB-induced metabolic status and hence elevated FFA levels during the early (preantral) follicular growth stages. It has been hypothesized that exposure of follicles to the unfavorable metabolic conditions of NEB during early follicular growth may have a latent effect on the function of the follicle and hence the quality of its contained oocyte (Spicer and Echternkamp, 1986; Britt, 1992). The NEB condition may consequently contribute to the reduced fertility in high-yielding dairy cows for several weeks to months after the period of the NEB.

In this study, we investigated whether NEB affects FFA composition in follicular fluid of the dominant follicle near the recommended time of insemination. To this end, we determined the concentration and composition of FFA in blood and follicular fluid collected from dominant follicles during the period of postpartum insemination; namely, d 55, 80, and 105 postpartum. Furthermore, the potential effect of elevated FFA levels in the early postpartum period on reproductive activity was investigated by monitoring follicular growth and development and the timing of the first postpartum ovulation. To investigate whether the levels of FFA in follicular fluid were associated with follicular function, we performed a correlation analysis between FFA concentrations and the calculated 17β-estradiol ($\mathbf{E_2}$):progesterone ($\mathbf{P_4}$) ratio from E_2 (parameter for follicular function) and P_4 levels in follicular fluid.

MATERIALS AND METHODS

Chemicals

Unless stated otherwise, all chemicals were obtained from Sigma Chemical Co. (St. Louis, MO) and were of the highest available purity. Solvents (acetone, acetonitrile, chloroform, methanol, and hexane) were of HPLC grade (Labscan, Dublin, Ireland).

Experimental Procedures on Animals

All animal experiments were approved by Utrecht University's Animal Experimental Procedures Committee. Clinically healthy, pregnant Holstein-Friesian heifers (n = 10) were included in the experiment from the time of calving until d 105 postpartum. The heifers were fed 10 kg of maize, 0.5 kg of soybean hulls, ad libitum grass silage daily, supplemented with 2 kg of concentrate feed (Synchro-optimaal, De Heus Voeders BV, Ede, the Netherlands) on the day of calving, increasing to 8 kg of concentrate on d 14 postcalving until the end of the experiment. The heifers had unlimited access to water and, once a week, their BCS (1–5 scale) was determined. From d 14 postpartum, per rectum ultrasonography was performed 3 times a week using a 240 Parus scanner (Pie Medical, Maastricht, the Netherlands) equipped with a 7.5-MHz linear array transducer, and the diameters of ovarian follicles (>5mm) were recorded. Cows with a first ovulation before d 35 postpartum were grouped as "normally ovulating," and