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Comparison of oocyte developmental competence and follicular steroid content of nulliparous heifers and cows at different stages of lactation

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Abstract

Reduced reproductive performance and lower conception rates of lactating cows are closely associated with genetic progress for high milk production. In contrast, the fertility of nulliparous Holstein heifers has remained fairly stable over the years and appears to be markedly higher than that of mature lactating cows. Possible differences in oocyte quality and follicular steroid levels, which could be associated with the low fertility of high-lactating cows, were examined in 13-month-old heifers, cows around the time of first AI (60–95 d post-partum, yielding 49 ± 2.4 kg/d) and cows at mid-lactation (120–225 d post-partum, yielding 37 ± 2.1 kg/d). Estrus was synchronized by two doses of $PGF_{2\alpha}$ and follicles (5–8 mm) were aspirated on days 4, 8, 11 and 15 of the cycle by an ultrasound-guided procedure. Oocytes were morphologically examined, matured *in vitro,* chemically activated and cultured for 8 d. Cleavage rate and the proportion of developing parthenogenetic blastocysts were determined on days 3 and 8 post-activation, respectively. On day 17, heifers and cows received additional $PGF_{2\alpha}$ and follicular fluids from preovulatory follicles were collected on day 19 perior to the expected estrus. Follicular-fluid volumes were similar in cows and heifers, as were estradiol, progesterone and androstenedione concentrations in the follicular fluid. Percentages of high-grade oocytes, proportions of cleaved oocytes and developed blastocysts did not differ between the groups. Results suggest that the fertility gap between nulliparous heifers and high-lactating cows is not directly related to steroid content in the preovulatory follicular fluid or oocyte developmental competence.

Keywords: Oocyte competence; Lactating cows; Heifers

1. Introduction

Reduced reproductive performance of lactating cows over the last decades is a well-studied phenomenon which is mostly associated with intensive genetic selection for increased milk production [1,2]. Based on the Israeli data herd book (more than 100,000 first AIs per year), the conception rate of nulliparous Holstein heifers has remained stable at about 65%, whereas that of lactating cows has decreased over the years to below 40%, suggesting that the phenotype of high genetic-merit cows, rather than their genotype, underlies the above convincing evidence. While it is not yet entirely clear, the reduced reproductive performance in high-lactating cows seems to be multifactorial in nature with

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an association to physiological and/or endocrine alterations throughout the lactation period.

Several studies pointed that animals exposed to stress present inferior fertility. Low fertility of high-lactating cows during the summer is associated with their difficulties to maintain normothermia. The classic work of Berman et al. [3] showed that the thermoregulatory competence of lactating cows decreases due to increased metabolic heat production upon milk production. In this respect, Sartori et al. [4] recorded a low fertilization rate and low proportion of high-quality embryos in lactating cows relative to heifers during the hot summer.

As a consequence of increased blood flow and hepatic metabolism, high-producing cows are characterized by elevated clearance of steroid hormones from the circulation, with lower progesterone and estradiol levels compared to nulliparous heifers [4,5]. This hormonal pattern has been found to be associated with various alterations in reproductive features, such as follicular dynamics, cycle length, gonadotropin concentration and estrous behavior, and to be correlated with an increased rate of multiple ovulations [5–7]. In a study in which follicular dynamics was examined, the duration of dominance of the large follicle in cows was found to be 4 d longer than in heifers [5]. Prolonged dominance is associated with a decreased concentration of progesterone, increased LH-pulse frequency and prolonged high concentrations of estradiol [8,9], which may lead to premature activation of oocytes, reduced oocyte quality and poor embryonic development [10,11].

The intensive selection for high milk production in recent decades has also increased the gap between energy intake and output (i.e. milk production), resulting in increased negative energy balance (NEB). The energy status of the high-lactating cows appears to be a main factor in determining successful conception when cows are inseminated around 70–100 d post-calving [12]. A severe post-partum NEB is often associated with ovarian dysfunction [13], cystic ovarian follicle [14] and reduced estrous behavior [6]. Given the importance of the quality of the follicle-enclosed oocyte in determining the final fertility outcome, it has been suggested that metabolic alterations due to unavoidable post-partum NEB impair the follicular fluid content, which in turn disrupts oocyte developmental competence. For example, some studies have indicated that increased concentrations of non-esterified fatty acids (NEFA) and β-hydroxybutyrate in the follicular fluid, which are associated with post-partum NEB, adversely affect oocyte developmental competence [15–17]. In addition, NEFA have been shown to reduce steroidogenesis and proliferation in the follicular thecal cells [18], which in turn may induce alterations in the oocytes' microenvironment within the follicle and disrupt its developmental competence.

The current study compared oocyte quality and follicular-fluid steroid content in non-lactating heifers with that of cows at two different stages of lactation to associate potential differences with fertility failure. Given the potential deleterious effect of NEB in the early post-partum period and that of environmental thermal stress, the experiment was performed during the spring season on high-lactating cows that were at least 60 d post-partum, i.e. around the time of the first AI or later, at mid-lactation, when most of the cows are no longer under NEB.

2. Materials and methods

2.1. Animals

The study involved nulliparous cyclic, non-lactating Holstein heifers ($n = 7$), aged 13 months, and multiparous cyclic Holstein cows in their second to fifth lactation. Cows were assigned to two experimental groups ($n = 5$ cows/group) according to days in lactation and level of milk production. The AI group included cows at an earlier stage of lactation, around the first AI or breeding period (at 60–95 d post-partum), yielding 49 ± 2.4 kg milk/d. The mid-lactation group included cows at 120–225 d post-partum, yielding 36.5 ± 2.1 kg milk/d. The latter groups comprised of cows that did not conceive after two or three inseminations ($n = 3$), or those whose first insemination was delayed as per the farmer's decision (i.e. high milk production, body condition score, lactation curve, $n = 2$). In fact, the experimental groups in this study closely reflect a contemporary reproductive management in several dairy farms world-wide. All cows were examined by the same veterinarian, were defined as healthy and cyclic, and exhibited normal pattern of ovarian structures, as determined by ultrasonographic scanning. Pedometric data (AfiFarm, Afikim, Israel) prior to the experiment indicated normal intervals of estrous cycles.

Cows and heifers were managed in the same farm and kept in an open shed with access to an adjacent yard. The cows were milked three times a day and fed a complete mixed ration containing 17.1% protein and 1.81 Mcal/kg of dry-matter intake. The heifers were fed a complete mixed ration containing 13.1% protein and 1.33 Mcal/kg of dry-matter intake. Body condition (on a