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Association between antral follicle count and reproductive measures in New Zealand lactating dairy cows maintained in a pasture-based production system

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ABSTRACT

The antral follicle count (AFC) in cattle is consistent throughout the estrous cycle of individual cows, and cows with a lower AFC have lower fertility. We assessed the AFC at random stages of the estrous cycle, examined the correlation between AFC classifications, and determined the relationship between the most rapid and practical laboratory-based AFC classification (AFC of follicles of ≥2 mm in diameter) and fertility measures in New Zealand lactating dairy cows. Cows detected in estrus (n = 202) or not (n = 239) during the first 4 weeks of the breeding season were subjected to ultrasonography and classified as having a high, medium, or low AFC at the time of scanning (on-site classification). Images from ultrasound scanning were recorded onto video for accurate follicle counting in an imaging laboratory. A strong association ($P < 0.05$) between the AFC of follicles with a diameter of 2 mm or greater and fertility was observed. Cows with a high AFC had a shorter ($P < 0.05$) interval from calving to conception by artificial insemination (AI; 82.4 ± 1.6 vs. 87.3 ± 1.2 days) and greater pregnancy rates (PRs; i.e., PR to the first AI [68.1% vs. 45.3%], 6-week PR [81.9% vs. 67.3%], and overall PR [91.3% vs. 79.7%]) than cows with a low AFC. The AFC was positively associated ($P < 0.0001$) with age. Progesterone concentrations during diestrus were greater ($P < 0.05$) in high-AFC cows (7.6 ± 0.3 ng/mL) than in low-AFC cows (6.5 ± 0.3 ng/mL), whether these were pregnant (7.7 ± 0.3 ng/mL) or not (6.3 ± 0.2 ng/mL). A rapid on-site scoring system determined that cows classified as having a high AFC had a shorter ($P < 0.05$) interval from calving to the first AI (76.5 ± 1.7 vs. 82.3 ± 1.9 days) and were more likely to show estrus ($P < 0.01$; 56.8% vs. 36.4%) and have a CL at the beginning of the breeding season ($P < 0.01$; 93.4% vs. 79.6%) than cows with a low on-site AFC. Collectively, we have confirmed an association between AFC2 and fertility, and these results support the hypothesis that cows with a greater number of antral follicles are more fertile than cows with a lesser number of follicles. Although the on-site classification was related to resumption of estrous cycles after calving, associations with other fertility measurements could not be observed, highlighting a need for further refinement of the on-site classification system for rapid phenotyping of the AFC.

1. Introduction

Variability in the number of ovarian follicles has been reported, and a putative relationship between the follicle number, as a predictor of the ovarian reserve, and fertility

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has been proposed [1]. As part of the ovarian reserve, germ cells are contained in quiescent (primordial) follicles or growing (primary, secondary, and antral) follicles. These follicle populations form the preestablished or dynamic ovarian reserve, respectively [2]. During development, germ cells are surrounded by somatic (granulosa) cells to form primordial follicles. As the follicles begin to grow, the morphology of the granulosa cells change, becoming cuboidal in nature and they begin to proliferate, forming primary follicles. The granulosa cells continue to proliferate forming secondary follicles, which develop a cavity within the follicle becoming antral follicles. Because of this developmental path, there is a positive correlation between primordial and other more-developed follicle classes including antral follicles [1]. Therefore, the number of antral follicles is commonly referred to as the antral follicle count (AFC) and is currently used to represent the ovarian reserve in an individual. Antral follicles can be readily identified by ultrasonography in cattle [3,4] and can be used to predict the ovarian reserve *in vivo*. This is important because a heifer calf is born with a larger ovarian reserve than can be accurately assessed early in life [5].

As a result of the initial findings on the individual variability of the follicle count [1], it was envisaged that a relationship may exist between the ovarian reserve (germ cell numbers) and the reproductive performance. In a later study, Erickson et al. [6] showed that fertile cows had a greater number of growing follicles than infertile cows. In cattle, the AFC is highly variable between animals [1] but highly repeatable within an individual [7]. Real-time ultrasonography has been used to monitor follicles with a diameter of 3 mm or greater across the stages of the estrous cycle of the same individual [7] and to predict ovarian response to superovulatory treatment in cattle [8]. The repeatability of AFC is advantageous as one ultrasound assessment is sufficient to predict ovarian response to hormone treatments [9]. Cattle with a different AFC also have different reproductive characteristics including ovarian response to superovulatory treatments [8,10,11], blood hormone concentrations [7,11–17], ovary weight [3,4], oocyte yield after ovum pickup, embryo production [4], endometrial thickness, and CL function [17]. For instance, in low-AFC cattle, endometrial thickness was reduced during the first days of the estrous cycle and circulating progesterone was lower during diestrus when compared to high-AFC cattle [17]. Collectively, these findings suggested a putative association between the AFC and the reproductive performance [1,6], and more recent studies have confirmed an association between the AFC and fertility in beef [3] and dairy [18] cattle. Cushman et al. [3] observed that low-AFC beef heifers had small ovaries and reduced pregnancy rate in comparison with high-AFC heifers. Beef heifers that calved during the first 21 days of their first calving season had a greater number of follicles than those calving later in the calving season [19]. In a study carried out on Irish dairy cows, Mossa et al. [18] concluded that cows with an AFC of less than 15 follicles with a diameter of 3 mm or greater (low AFC) had lower reproductive performance than cows with a higher number of follicles [6].

The genetic composition of the New Zealand (NZ) dairy herd is particularly different [20,21], while the environment and management of NZ dairy farms also differ from those of other countries [22]. For example, NZ Holstein cows may be better adapted to pastoral systems because they have been selected to perform in this environment. In a pastoral system, their milk yield was similar to that of North American (NA) Holstein cows, while losing less body condition than their NA counterparts [23,24]. However, milk production in response to energy supplementation was found to be better in the NA Holsteins under the same conditions [23,25,26]. In fertility traits, AFC heritability estimates were 0.31 ± 0.14 and 0.25 ± 0.13 for Irish dairy cows and NA heifers, respectively. The AFC of offspring from cows that were lactating during pregnancy was greater than the AFC of daughters from dams that were not lactating (heifers) during gestation [27]. Daughters from cows under nutritional restriction during pregnancy had a lower AFC [28], indicating an interaction between the AFC and the environment. Because NZ dairy cattle have a different genetic composition and environment and pastoral management are different from other parts of the world [25,26], we expected that NZ dairy cows will have different characteristics of the AFC and its relationship with fertility. In a study carried out in Ireland, some differences in conception rates between strains of NZ and NA cows were observed [29]. The high fertility of NZ cows may also be due to the seasonal nature of the New Zealand dairy industry, which involves an intensive selection pressure, with an increased necessity for a large number of pregnant cows very early in the breeding season and a strict culling of nonpregnant cows at the end of the season.

We hypothesized that the AFC assessed at random stages of follicle development in lactating cattle is associated with reproductive hormone concentrations and fertility parameters. The objective was to determine whether there is an association between the AFC, assessed at random stages of their reproductive cycle, and the fertility parameters in NZ lactating dairy cows. In addition, a scoring system was used to estimate the AFC, at the time of ovarian ultrasonography, in a more rapid fashion than the actual follicle counts performed at the imaging laboratory. Score values were analyzed for their association with fertility variables.

2. Materials and methods

This study was carried out under the Animal Welfare Act Regulations of New Zealand guidelines with approval granted by the Animal Ethics Committee of the Invermay Agricultural Centre (Application No. 12207).

2.1. Animals

Postpartum dairy cows (aged 2–10 years, 80.6 ± 19.1 days from calving to ultrasound scanning) from four herds (two with predominantly Holstein genetics and two with predominantly Jersey genetics) in the South Island of New Zealand were used in the trial.

The breeding management of these farms was a representative of the New Zealand standard breeding systems. Briefly, the breeding season started on the day of the planned start of mating (PSM) with estrus detection and artificial insemination (AI) for a period of approximately