Relationships between the Number of Small Follicles Prior to Superovulatory Treatment and Superovulatory Response in Holstein Cows

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ABSTRACT. In order to determine relationships between the number of small follicles prior to superovulatory treatment and superovulatory response, a total of 55 superovulations were induced in Holstein cows. The ovaries were examined ultrasonographically once 0–1.5 days before the initiation of superovulatory treatment. The number of small follicles 3–6 mm in diameter on both ovaries before superovulatory treatment was found to be significantly correlated with the numbers of corpora lutea after superovulation (r=0.440, P<0.001), total ova recovered (r=0.503, P<0.001) and transferable embryos recovered (r=0.482, P<0.001). These results indicate that a single ultrasonographic examination of follicles 3–6 mm in diameter prior to superovulatory treatment can be utilized to predict superovulatory response. — KEY WORDS: small follicle, superovulation, ultrasonography.

With the advent of ultrasonography, it is now possible to obtain a more precise description of the ovaries during the normal estrous cycle [2, 3, 11]. Great variations between individual cows in their response to superovulatory treatment has stimulated various studies that investigate whether ultrasonographic examinations of ovaries can be utilized to predict superovulatory response.

The presence of a dominant follicle before superovulatory treatment is known to decrease superovulatory response [5–7]. But Guilbault et al. [6] defined that dominant follicles had a diameter of >9 mm and were in a growing phase or stable for <4 days. This means that in order to predict superovulatory response by means of this relation, it is necessary to first monitor the ovaries of embryo donor cows for at least 4 consecutive days before the initiation of superovulatory treatment, and confirm whether a follicle is functionally dominant or not. This is impractical under field conditions.

On the other hand, a few investigators [4, 10, 12] have used ultrasonography, and found a positive correlation between the number of small follicles prior to superovulatory treatment and superovulatory response. But these experiments involved mostly heifers from which only a limited number of ova was recovered. The purpose of this study was to confirm this correlation in cows, and to see whether single ultrasonographical examinations of small follicles prior to superovulatory treatment could be applied in commercial embryo transfer programs for the prediction of superovulatory response.

Twenty-one Holstein cows (2.3–10.8 years of age) were superovulated a total of 55 times, from October 1990 to October 1992. The cows were housed at the Tokachi Livestock Management and other dairy farms in Tokachi, Hokkaido. The ovaries and uterus of each cow were examined ultrasonographically once 0–1.5 days before initiation of the superovulatory treatment, with a real-time linear scanning ultrasound diagnostic system (SSD-210 DXII, 5 MHz, Aloka Co., Ltd., Tokyo). The number and size of follicles were determined, as described by Pierson and Ginther [9]. Since follicles as small as 2–3 mm are within the resolving power of an ultrasound unit with a 5 MHz transducer, all follicles ≥3 mm in diameter on both ovaries were measured. Follicles 3–6 mm in diameter were categorized as being small follicles, as described by Romero et al. [10]. Superovulatory treatment was initiated between Days 9 and 13 of the estrous cycle (Day 0 = day of estrus). Superovulation was induced with 12 hr interval injections of decreasing doses of FSH (Antrin 10, Denka Co., Ltd., Kanagawa) for 4 days for a total dose of 40–52 mg. On the fourth day, 50–60 mg of PGF2α (Veterinary Pronalgon F Injection, Upjohn Company, MI, U.S.A.) was administered in two doses given 12 hr apart. The cows were artificially inseminated with semen at 12 and 24 hr after the onset of estrus. Embryos were recovered nonsurgically on Day 7 post estrus. The number of corpora lutea (CL) was evaluated by rectal palpation immediately before the embryo collection. Correlations between the number of pretreatment follicles 3–6 mm in diameter and the numbers of CL after superovulation, total ova recovered and transferable embryos recovered, were examined by linear regression analysis.

As shown in Table 1, the presence of a follicle ≥10 mm in diameter prior to superovulatory treatment did not relate to any significant differences in the mean numbers of CL after superovulation, total ova recovered and transferable embryos recovered. These results coincide with those of Wilson et al. [14], who found that the presence of a morphologically dominant follicle at the initiation of superovulatory treatment had no effect on the superovulatory response. On the other hand, several studies [5–7] have reported that functionally dominant follicles prior to superovulatory treatment decreased superovulatory response. Furthermore, Takagi et al. [13] reported that in the middle of the estrous cycle follicles larger than 10 mm in diameter were mostly morphologically degenerated and functionally non-dominant. Therefore it was suggested that the morphologically largest follicles in this study were not necessarily functionally dominant. Even though functionally dominant follicles prior to superovulatory treatment are known to decrease superovulatory response, this relation cannot be used to predict superovulatory response utilizing single ultrasonographic examinations, as this study shows that several consecutive days of ultrasonographic examinations are required to evaluate whether a large follicle is functionally dominant or not.
As shown in Fig. 1, the number of small follicles 3–6 mm in diameter, 0–1.5 days prior to the initiation of superovulatory treatment, was significantly correlated with the numbers of CL after superovulation (r=0.440, P<0.001), total ova recovered (r=0.503, P<0.001) and transferable embryos recovered (r=0.482, P<0.001). These results are similar to those of Romero et al. [10], who did ultrasonographic evaluations on cows and heifers, and indicated that the number of small follicles 3–6 mm at the start of superovulatory treatment was correlated with...
superoxovulatory response \((r=0.31, P<0.02)\). Grasso et al. [4] ultrasonographically monitored daily follicular development in heifers, and reported that follicles 4–6 mm on the day of initiation of superovulatory treatment, subsequently grew into a pool of larger follicles during treatment, and further grew to be >10 mm at estrus. Furthermore, they demonstrated that the number of follicles 4–6 mm at the time of initiation of treatment was positively correlated with the number of ovulations, and of embryos and unfertilized ova recovered \((r=0.57, P<0.05)\).

Antral follicles within the ovary in cattle are considered to fall into three size categories: a large pool of small (<3 mm) follicles, a few selected follicles of medium size (3–7 mm) that have been recruited from the large pool, and one or two large (≥8 mm) follicles that are dominant [1]. Gonadotropins are of utmost importance in the control of recruitment, selection and dominance [2]. Monniaux et al. [8] hypothesized that normal follicles ≥1.7 mm in diameter are the follicles that are the most involved in the ovulatory response to exogenous gonadotropin. It has been suggested that some of these follicles that are 3–6 mm in diameter before superovulatory treatment continue growing in reaction to exogenous gonadotropin, and eventually ovulate. Therefore, it is postulated that the larger the number of small follicles at the time of initiation of superovulatory treatment, the larger the number of follicles that respond to exogenous gonadotropin and better the resulting superovulatory response.

In summary, it was suggested that a single evaluation of the number of follicles 3–6 mm in diameter prior to the initiation of superovulatory treatment, can be used to predict superovulatory response. This method can be applied in field work; to decide whether to conduct gonadotropin treatments on a cow or not, to have an idea of how many recipients to prepare, and to assist in the selection of the breeding sire, etc.

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REFERENCES