The importance of follicular waves in cattle has been discussed in two previous Michigan Dairy Review articles (What Do Dairy Cows Have in Common with Chickens and Lake Michigan? May 1999 and Tick-tock? July 2002). Based on our understanding of growth and function of dominant follicles during follicular waves, reproductive physiologists have learned a great deal about how to manipulate the estrous cycle to improve reproductive management. For example, Richard Pursley in our Department of Animal Science at Michigan State University collaborated with Milo Wiltbank at the University of Wisconsin to develop Ovsynch, an efficient method which has been adopted widely by the dairy industry to regulate dominant follicle growth and ovulation during the follicular waves occurring in estrous cycles of cattle (Figure 1).

Moreover, we now understand that some of the variation associated with responses of cattle to superovulation procedures is attributable to the presence of dominant follicles on ovaries, which diminishes growth of new follicles during treatments. So, improvements in superovulation will require better control over dominant follicle growth during follicular waves. Most importantly, future advances in management of reproductive efficiency of cattle should be based on the understanding that groups of ovarian follicles grow in recurrent waves as depicted in Figure 1 and follicular waves are required for the normal development, function, and ovulation of dominant ovulatory follicles.

Pursley worked with graduate student Nora Bello to take advantage of this new information to generate preliminary data indicating that shortening the lifespan of the dominant follicle during follicular waves by only a couple of days may improve fertility in dairy cows. The information in Figure 1 also has been used in my laboratory to design studies that take advantage of the variation in number of follicles growing during follicular waves to develop new ways to predict fertility in cattle.

Follicular Waves and Fertility

That’s all very interesting stuff, you might say, but what do follicular waves have to do with discovery of a new measure for fertility? During discussions with Department of Animal Science colleagues, it dawned on me that the variation in numbers of follicles and oocytes (eggs) in ovaries (also called...
The ovarian reserve) may have an important but overlooked role in fertility of cattle.

We all agreed that little was known about the degree of variation in follicle numbers growing during follicular waves and even less about the importance of the variation in follicle numbers to fertility. A search turned up no information on the importance of the variation of follicle numbers during waves to fertility, but did reveal numerous research articles reporting that high compared with relatively low numbers of follicles in ovaries of a variety of species, including cattle, are positively associated with numerous measures of fertility including responses to superovulation, in vitro fertilization rate, embryo recovery, return to estrus postpartum, and the number of calves born.

This begged the question: Why hadn’t anyone examined the association of numbers of follicles growing during follicular waves with fertility, especially considering the relatively easy use of ultrasound to count follicles?

I suspect the association of numbers of follicles with fertility hasn’t been examined in cattle for four reasons.

- The understanding that follicles grow in two or three different waves during a 21-day estrous cycle (Figure 1) is a relatively recent discovery.
- The long hours and money required to hire and train someone to use ultrasound to measure accurately alterations in numbers of follicles on ovaries during all the different waves of an estrous cycle in a relatively large group of cattle.
- Although dozens of follicles may grow during waves, cattle ovulate only a single dominant follicle (Figure 1). So, why would variation in numbers of follicles growing during a wave affect fertility when only one dominant follicle ovulates?
- Because follicular waves develop in quite different hormonal environments during the follicular and luteal phases of an estrous cycle, it is likely that numbers of follicles growing during the different waves of an estrous cycle are random rather than similar. If follicular growth is random during different waves, then it is unlikely to be very highly repeatable within individuals. Consequently, the predicted low repeatability of follicle numbers during waves would imply strongly that the potential heritability of this trait, like other female fertility traits, also would be low.

For all the aforementioned reasons, the potential use of numbers of follicles growing during follicular waves as a trait associated with fertility has not been studied, despite overwhelming scientific evidence from numerous laboratories that number of follicles is associated positively with fertility.

Pursley shared information from his thesis work at the University of Wisconsin. He had collected ultrasound data on separate follicular waves in several post-calving Holstein cows. The data showed that the variation in peak numbers of follicles during a wave was high among the cattle, but somewhat similar between the two waves within individuals. This information, albeit preliminary, was exciting to me because it was clearly contrary to some of the aforementioned reservations about follicle numbers during waves and fertility, especially the idea that follicular growth is random. Indeed, something simple to do like counting follicles, something contrary to scientific dogma, and something in my area of expertise with a potential positive association with fertility was all the motivation needed to start a new research project.

**Follicular Growth Variability**

David Burns, a graduate student in my laboratory, was trained by Pursley to use ultrasound to count follicles. A group of approximately 50 Holstein heifers and cows (lactating and non-lactating) were secured. These animals were used to view, count, and draw the location of follicles on each ovary onto maps.

Each animal in the study was injected with prostaglandin F2α (PG) to attempt to synchronize ovulation and initiate a new estrous cycle. After PG, each animal was subjected to twice daily ultrasound analysis, which continued until 2 to 3 days after the next ovulation. During each ultrasound session, Burns recorded number and size of all follicles equal to or greater than 3 mm in diameter on each ovary. Because some animals have two (usually cows) or three (usually heifers) waves, the scanning regimen was sufficient to analyze two to four follicular waves per animal. In total, 160 waves were analyzed.

Each cow was classified arbitrarily as having a low, intermediate, high, or very high average peak number of follicles counted during each follicular wave. The results demonstrated clearly the high variation of follicle numbers during waves among cows but surprisingly little variation in peak number of follicles within individuals. Indeed, after statistical analysis of all 160 waves in heifers and cows, the overall variation in peak numbers of follicles per wave among animals was very high (8 to 56 follicles per wave) while repeatability of peak numbers per wave within individuals was a remarkable 0.95 (1 is perfect).

My research associate Fermin Jimenez-Krassel conducted a similar project with different animals to be sure Burns’s results were repeatable. Much to our delight Jimenez-Krassel’s ultrasound studies produced results nearly identical to Burns’s. Moreover, another follow-up study conducted in Ireland last year at the University College Dublin by Alex Evans, Pat Loernegan and Fabian Ward generated results in beef heifers nearly identical to those produced by Burns and Jimenez-Krassel in dairy cattle. Three different studies in two different breeds of cattle conducted by three different ultrasound technicians in two different countries strengthens confidence in the results.

**Potential Importance to the Dairy Industry**

The high variation of follicle numbers during follicular
waves among animals and repeatability within individuals means that cattle can be reliably classified based on numbers of follicles growing during ovarian follicular waves. For example, ultrasound can be used to identify cows that consistently have either low or very high numbers of follicles growing during their follicular waves.

I indicated earlier in this article that total numbers of follicles are associated positively with fertility in a variety of species including cattle. Because the variation in numbers of follicles growing during follicular waves can be measured, it is possible to firmly establish in field trials if cows with high numbers of follicles growing during waves are more fertile than cows with low numbers.

Our new way to evaluate cattle could have important genetic implications. For example, if number of follicles growing during waves is genetically regulated, then the very high variation in numbers of follicles growing during waves among animals and very high repeatability within individuals implies that substantial genetic progress could be made with traditional animal breeding programs.

However, I don’t want to put the cart before the horse. Whether our relatively simple new way to evaluate cattle can ever be used in animal breeding schemes to improve fertility, or lead to development of new technologies to improve reproductive efficiency remains to be seen. We are at work answering this question and will share our new results with you soon.

Figure 1. Model depicting the transient increase in secretion of follicle stimulating hormone (FSH) from the pituitary gland into blood preceding each of the three waves of growth of ovarian follicles during a 21-day estrous cycle in cattle. Dotted circles depict death of follicles.