

SYMPOSIUM: PHYSIOLOGY, LACTATION, AND REPRODUCTION

Mechanisms that Prevent and Produce Double Ovulations in Dairy Cattle¹

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ABSTRACT

This review integrates information on follicular and hormonal physiology and epidemiology into a novel physiological model for regulation of the ovulation rate in lactating dairy cows. First, the basic mechanisms that produce a single ovulation are examined. Follicular deviation is a critical new concept in our understanding of selection of a single dominant follicle. Follicular deviation is characterized by an abrupt deviation in the growth rates between the two largest follicles when the future dominant follicle reaches a diameter of 8.5 ± 1.2 mm (mean and SD). The mechanisms involved in this selection process are not completely defined but appear to involve acquisition of LH receptors on granulosa cells of the dominant follicle, increased estradiol production by the dominant follicle, and inhibition of circulating FSH concentrations. Second, lactation number and milk production were found to be critical epidemiological factors associated with increased ovulation rate and twinning in dairy cattle. Finally, high steroid metabolism is proposed as the critical link between high milk production and double ovulation. It is proposed that high milk production increases steroid metabolism due to increased blood flow to the digestive tract and subsequently to the liver. The liver represents the primary site of steroid metabolism, and blood entering the liver is cleared of steroids. At the time of selection of the dominant follicle, the normal increase in circulating estradiol concentrations and subsequent depression in circulating FSH is blunted due to estradiol metabolism. Thus, FSH remains elevated for a time sufficient to allow follicles to undergo the physiological changes necessary to proceed to ovulation.

(**Key words:** follicle, twinning, double ovulation, reproduction)

INTRODUCTION

The regulation of ovulation rate appears to be the primary mechanism for regulating litter size. Certain species such as rabbits and rodents have extremely high ovulation rates that correspond to large litter sizes. Regnier de Graaf (14) noted this relationship as early as 1672 when he wrote [explanations added]: "these globules [corpora lutea] do not exist at all times in the testicles of the females; on the contrary they are only detected after coitus [rabbit is induced ovulator], being one or more in number, according as the animal brings forth one or more foetuses from that congress."

Some species, such as humans and cattle, are primarily monovular and generally produce only one offspring per pregnancy. There has been substantial interest in regulating the twinning rate in cattle either through genetic selection (70, 72), hormonal treatments (45), embryo transfer (2, 9, 66, 67), or immunologic suppression of hormones (32, 48, 74). The twinning rate in dairy cattle seems to have increased recently, and this may have important practical implications (39). Nevertheless, research has yet to uncover the basic mechanisms that regulate ovulation rate in cattle. A number of recent reviews have discussed the regulation of follicular development and selection of a single dominant follicle in cattle (4, 5, 28, 69), and the reader should consult these reviews for further scientific literature on the basic and applied aspects. This review presents recent information on the mechanisms resulting in selection of a single dominant follicle and the factors involved in multiple ovulation and twinning in cattle. A final section proposes a mechanism that may explain the regulation of double ovulation rate in dairy cattle.

Selection of a Single Dominant Follicle

The final stages of bovine follicular development from 4 mm diameter to preovulatory size are distinguished by episodes of growth termed follicular waves. The clearest characterization of follicular waves was accomplished by tracking the growth of individual follicles by

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transrectal ultrasonography (23, 53, 61, 64). However, earlier techniques also suggested the presence of follicular waves; these include collection of ovaries at slaughter (56), measurement of estradiol concentrations in the ovarian artery (34) or follicular fluid (33), and grouping of follicles according to diameter based on ultrasonography (52, 53). Cattle generally show either two or three follicular waves during an estrous cycle. Follicular waves also occur during many anovulatory conditions including: pregnancy (27, 28), postpartum anovulation (62), and prepubertal anovulation (20). Thus, follicular waves seem to be a consistent pattern during the ever-fluctuating physiology that regulates the bovine ovary.

The hormone that has been most closely linked to follicular waves is FSH. The circulating concentrations of FSH increase at the onset of each follicular wave (1). For example, individual cows with two waves of follicular development have an FSH surge associated with the emergence of the first follicular wave at about the time of ovulation and have a second FSH surge associated with the emergence of the second follicular wave around d 10 of the estrous cycle. The first follicular wave is best studied because there is a relative synchrony in emergence of the first follicular wave related to ovulation of the dominant follicle. In this regard, the FSH surges associated with the second or third follicular wave of an estrous cycle were not distinguished until FSH concentrations from individual cows were normalized to the time of emergence of follicular waves (1). Evaluation of individual cows clearly showed multiple FSH surges during the estrous cycle or pregnancy; however, the asynchrony in the initiation of these follicular waves between different cows produced average results with indistinct FSH surges. It is now clear that analysis of follicular development and FSH concentrations must be correlated in individual cows in order to understand the underlying physiology.

The analysis of profiles from individual cows has been key to development of the concept of follicular deviation (28, 30). Deviation is evidenced by an abrupt change in the differences in diameter between the two largest follicles in a follicular wave. To allow objective assignment of the time of deviation we have used the following definition: time of deviation is the beginning of the difference in growth rates between the two largest follicles at or before the examination when the second-largest follicle reaches its maximum diameter (28). Many researchers continue to express results in relation to estrus, ovulation, or emergence of the follicular wave, although it is readily apparent that growth of follicles within an individual wave is highly variable among individual waves. Figure 1 illustrates the growth profile

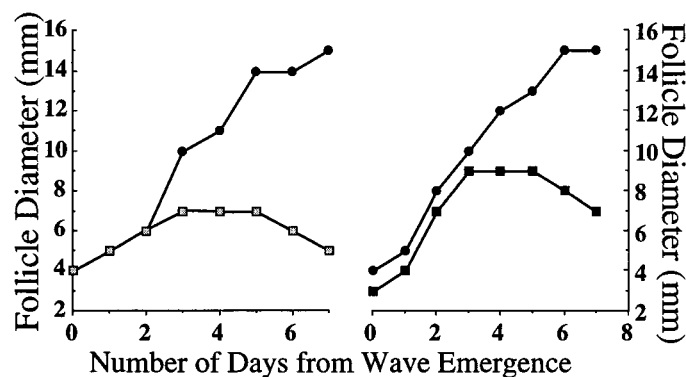


Figure 1. Growth patterns of the future dominant and the largest subordinate follicle in two different cows during the first follicular wave of an estrous cycle. (From Ginther et al. (30)).

for the dominant and largest subordinate follicle for the first follicular wave in two individual cows. The wave on the left shows a profile similar to about 24% of waves in which the future dominant follicle was a similar (21% of waves) or smaller (3%) size than the future subordinate follicle until the time of deviation (d 2 after wave emergence in this cow). On the right is shown a profile similar to most waves (76%) in which the future dominant follicle was larger than the future subordinate follicle at all times before deviation (d 3 after wave emergence). These profiles illustrate that follicular waves not only vary in the time from wave emergence until deviation (range of d 2 to 6) but also vary in the size of the dominant follicle on the day of deviation (6 to 12 mm). Thus, analysis of follicular growth profiles from individual waves indicates that there is a critical point in follicular growth when a single follicle is selected from the cohort of follicles (deviation).

Normalization of data in relation to deviation shows that the nadir of FSH concentrations occurs near the time of deviation (Figure 2; 28). Figure 2 also reveals that growth rates of the future dominant and the future largest subordinate follicles are similar before the time of deviation. This is important because before deviation any follicle of the wave has the functional capacity to become the dominant follicle, evidenced by the finding that a randomly selected follicle will become the dominant follicle if other larger follicles are removed by aspiration early in a follicular wave (25). Apparently, one follicle reaches the point of functional deviation before the other follicles. The associated decrease in FSH or other inhibitory actions of the dominant follicle does not allow the next follicle to reach a diameter associated with functional and morphological deviation, and it subsequently becomes atretic.

Near the time of deviation, follicular estradiol production increases; this may be critical for the decrease in FSH concentrations. In earlier results, before the concept of follicular deviation, there was a clearly distinguished increase in estradiol that occurred from only one of the ovaries during the early estrous cycle (33). A technique was developed (29) to evaluate the changes in follicular estradiol production that allowed sampling of a small amount of follicular fluid from follicles without altering subsequent growth of the sampled follicles. Follicular fluid estradiol concentrations were similar in future dominant and future largest subordinate follicles (~100 ng/ml) until the time of deviation. After deviation, there was a much greater estradiol concentration in the dominant follicle (~600 ng/ml) than in the subordinate follicle (~50 ng/ml). In another study (40), serum estradiol concentration significantly increased from just before deviation (0.5 pg/ml) to 32 h later (1.6 pg/ml). In addition, the concentrations of androgen and estradiol have been found to increase at about d 5 of the cycle in the utero-ovarian vein draining the ovary containing the dominant follicle but not the opposite ovary (21), which is near the expected time of deviation. Thus, follicular production of estradiol increases near the time of deviation, and this may be critical for the reduced FSH concentration at this time. Nevertheless, reservation is required concerning the hypothesis that estradiol production by the future dominant follicle is involved in deviation by reducing FSH concentrations. To date, estradiol involvement is based on temporal relationships only; the required functional studies have not been performed.

Why does a specific follicle become dominant and not another follicle in the wave? In 75% of the waves, the

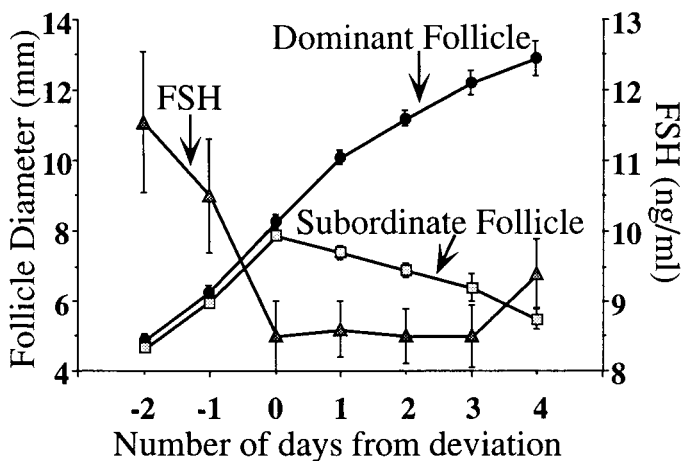


Figure 2. Average size of the dominant and largest subordinate follicles and serum FSH concentrations during the first follicular wave of the estrous cycle ($n = 13$). Data were normalized to the day of follicular deviation. (Reprinted from Ginther et al. (28)).

first follicle to emerge during the wave becomes the dominant follicle. Therefore, the dominant follicle often has a slight size advantage over the other follicles in the wave, and this may allow it to reach the point of deviation earlier than the other follicles of the wave. Morphological size, as determined by transrectal ultrasound, is being used as a measure of the functional stage of development in the follicle. Interestingly, if a 5-mm follicle is selected randomly to remain, whereas all other follicles are eliminated by follicular aspiration, the remaining follicle will become a dominant follicle (25). Thus, all follicles of the wave appear to have the capacity to become the dominant follicle; however, the future dominant follicle may have a slight developmental advantage that allows it to reach a critical diameter before the other follicles. The decrease in FSH concentrations or other systemic changes associated with deviation then appear to “slam the door” so that other follicles cannot proceed to a similar critical developmental stage.

Follicular deviation is probably a rapid event because the largest subordinate follicle is only 6 to 8 h of development behind the dominant follicle until the time of deviation. For example, emergence of the future dominant follicle occurred an average of 6 (30) or 7 h (40) before emergence of the future largest subordinate follicle (28). In addition, follicular aspiration provides evidence of an approximately 8-h interval between the emergence of each follicle (25). In one of the treatment groups, all follicles ≥ 5 mm were aspirated every 8 h for 6 d. The serum FSH concentrations in this group were extremely high compared with the nonaspirated group. On average, one follicle emerged every 8 h during the course of that experiment, in spite of the persistently elevated FSH concentrations. The upper part of Figure 3 shows a simplified model, based on those data and previous models (28, 30). In that model, follicles are present in a hierarchy of development. The future dominant follicle is probably the most mature follicle of the wave and is about 8 h ahead of the next follicle in the wave. The future dominant and largest subordinate follicles grow at the same rate but the larger follicle reaches the critical stage of differentiation just before the next smaller follicle and deviation occurs. At deviation, FSH concentrations are not adequate for the next follicle to continue through the point of deviation. Thus, only one follicle proceeds as the dominant follicle.

Why can the dominant follicle continue to grow after deviation, whereas the other follicles undergo atresia? Low FSH concentrations after deviation could continue to be stimulatory to the dominant follicle either due to increased FSH receptors or FSH responsiveness. The number of FSH receptors does not seem to differ between the dominant and largest subordinate follicle (8)

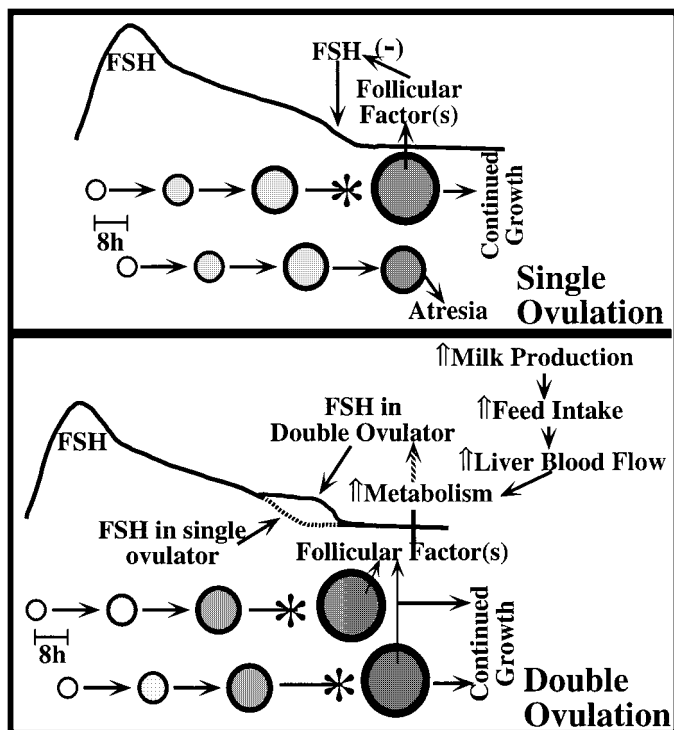


Figure 3. Upper panel is a simplified model of follicular deviation. The future dominant follicle emerges ~8 h earlier than the future largest subordinate follicle. Under the influence of follicle stimulating hormone (FSH) the two follicles grow at the same rate but the larger follicle or future dominant follicle reaches the point of deviation (*) before the future subordinate follicle due to the initial advantage in the follicular hierarchy. The subordinate follicle regresses (deviates) because of declining FSH supply caused by inhibition of FSH by follicular factor(s). However, changes in the dominant follicle, such as increased responsiveness to LH, allow continued growth in the presence of the low FSH. In the lower panel is shown a possible mechanism that leads to double ovulations in lactating dairy cows. The high milk production and associated dry matter intake ($r = 0.88$; 31) leads to high liver blood flow and hormonal metabolism. The increased metabolism of the FSH-depressing follicular factor delays the decrease in FSH allowing the largest subordinate follicle to continue development until it also undergoes the changes that normally occur only in the dominant follicle (i.e., requires less FSH for further development).

and there is no increase in FSH receptor mRNA near this period (77). However, a dramatic increase in mRNA and protein for the LH receptor near the time when deviation would be expected has been reported in most (5, 8, 28, 77) but not all studies (22). The concept of deviation was not yet recognized and was not considered in those studies; other reference points were used. Recently we found evidence of LH responsiveness in the follicle after deviation using the endpoint of ovulation to an LH surge. Treatment of cows with 40 mg of purified LH caused ovulation of 80% of follicles that were 10 mm in diameter but did not cause ovulation of follicles 8.5 or 7 mm in diameter (60). In that study cows that

had follicles that were 10 mm in diameter but did not ovulate after the treatment with 40 mg of LH had not yet undergone follicular deviation (Fricke, Sartori, and Wiltbank, unpublished results). Thus, responsiveness to LH, as evidenced by ovulation to an LH surge, occurs after follicular deviation. Responsiveness to LH pulses may allow the dominant follicle to continue to grow, whereas the subordinate follicles cannot grow due to low circulating FSH concentrations. Other changes within the follicle, such as decreased expression of IGF-binding proteins (46), also are probably important to continued development of the dominant follicle in a low FSH environment.

Regulation of Twinning and Double Ovulation

The process that allows cows to produce two calves rather than one has been of interest for many years. In dairy cattle, twin births have generally been viewed as undesirable due to increased problems in the dam and calf leading to increased costs (6, 18, 49). As early as the 1920's a number of factors were recognized as possible regulators of twinning rates including: age of dam (36), season (13), and genetics (43). Numerous other factors also have been found to be associated with twinning, including use of antibiotics or reproductive hormones, ovarian cysts, days open, and peak milk production (35, 39, 49, 51, 59). This review will focus on two factors, lactation number and milk production, that we believe are critical determinants of twinning rate in dairy herds. Detailed information on the relationship of other factors with twinning can be found elsewhere (35, 39, 49, 58, 59).

Parity of the dam is clearly associated with an increase in twinning rate (Table 1). In all studies, the largest increase in twinning rate is between first calving and second calving. For example, in high producing dairy cattle (7, 59), there was an increase from a twinning rate of about 1% in heifers to 6 to 7% in second-parity cows. There is also generally a slight increase in twinning rate after second parity.

A number of studies have examined the relationship of milk production and twinning rate in dairy cattle. Syrstad (68) noted that cows with twins had greater milk production than cows with singletons. Wood (76) found that cows with twins produced more milk, fat, and protein than cows with single calves at every parity group. Higher milk production in twin-bearing cows is similar to results of Wood (75) and Kay (37) but differs from Chapin and Van Vleck (11) who found that cows with twins had lower milk production. Nielen et al. (49) and Kinsel et al. (39) found that high cumulative milk production or peak milk production were strongly associated with increased likelihood of twinning. Kinsel et

al. (39) stated “the single largest contributor (>50%) to the recent increase in the rate of twinning is the increase in peak milk production.” Thus, in almost all studies high twinning rate was associated with increased milk production, examined either as peak or cumulative milk production.

Most twins are due to multiple ovulations. The monozygotic and dizygotic twinning rates have been examined in some studies by evaluating the sex ratio of the calves using the methods of Bonnier (10). A skewing of the sex ratio toward a greater percentage of like-sexed twins has been used to calculate the fraction of monozygous twins compared with dizygous twins. The percentages of twins that were monozygous were calculated to be 7.4% (19), 13.5 to 25% depending on breed (35), and 13.6% (59). However, as mentioned by Ryan and Boland (59), these rates seem extremely high, considering the high double ovulation rate in lactating cows. Numerous other factors could lead to a skewing of the sex ratio toward a greater percentage of like-sexed calves. For example, the time from insemination until ovulation may alter the gender ratio of calves (55).

Thus, no definitive studies have been performed to establish the monozygous twinning rate in dairy cattle but, from the information currently available, it is likely that the great majority of twins in dairy cattle are due to multiple ovulations.

The double ovulation rate in dairy cattle has been determined in only a limited number of studies. There are two older studies from the laboratory of L. E. Casida that used rectal palpation to detect ovulations (38, 41). In the second study, all suspected double ovulations were confirmed by palpation by a second individual. The overall ovulation rate in the second study was lower (4%) than in the first (13%), and the authors suggested that this decrease may be due to the added confirmation of double ovulation. There was no effect of time postpartum or of sire family on multiple ovulations. In contrast, season (highest in May) and previous double ovulation were associated with higher ovulation rates (38). In the

second study, sire line was again found to not significantly influence ovulation rate; although parity, outbreeding (compared with inbreeding), and presence of ovarian cysts were found to be associated with increased ovulation rate (41). A German study analyzed double ovulation rate in the Red-White dual-purpose breed of East-Flanders by evaluating 4400 reproductive tracts at a slaughterhouse (73). There was a 5% double ovulation rate in cows, compared with 3.8% in heifers. The German study also evaluated 38,297 calvings and found a twinning rate of 3.77% in cows and 1.04% in heifers. Because twinning rate was 3.63 times as frequent in cows than heifers, whereas double ovulation rate was only minimally different (1.32 times as frequent), the authors concluded that capacity for double ovulation was not limiting for twinning rate in heifers. Therefore, uterine capacity was speculated to play an important role in determining twinning rate. However, it should be noted that heifers at the time of slaughter are likely to have greater BCS, age, size, and feed consumption than heifers at the normal time for breeding. As discussed below these changes could have dramatically altered the ovulation rate of the heifers in this study. Echterkamp et al. (16) evaluated 444 crossbred beef cows for 3516 estrous cycles and determined that embryonic and fetal survival were not statistically different between single and double ovulating cows. They suggested that “the primary constraint to achieving multiple births in cattle is ovulation rate.” In addition, they reported that ovulation rate increased about 0.01% per month of age. An ongoing study from our laboratory (n = 125 ovulations during normal estrous cycles evaluated at this time) currently shows a very high double ovulation rate (40.0%) in lactating Holstein cows (DIM = 40 to 110 d; milk production > 40 kg/d) but a 1.7% double ovulation rate in breeding age heifers (12 to 14 mo of age; Sartori and Wiltbank, unpublished results).

We recently completed a study in which we evaluated double ovulation rate in 240 dairy cows (24). All cows had ovulation synchronized with the Ovsynch protocol

Table 1. Effect of parity on twinning rate (%) or relative risk (R)¹ of twinning in dairy cattle.

Ref	No. of Calvings	Parity						All parities
		1	2	3	4	5	6	
7	19,497	1.3	6.0	9.4 ²
18	19,755	0.9	2.1	3.5	3.4	3.7	3.2	2.5
19	7387	1.3	4.4	5.3	4.6	5.8	6.0	4.6
39	52,362	1.0	2.9	3.2	3.9	3.3	4.1 ²	2.4
44	8521	R = 0.2	R = 1.5	R = 1.6	R = 1.6	R = 2.0 ^b	...	5.8
49	11,951	0.8	2.7	4.1	4.5	4.9	4.8 ^b	3.2
51	937	0.7	5.0	4.2	5.0	7.0	6.7 ^b	4.2
59	24,843	1.0	7.0	7.5	7.9	9.1 ^b	...	4.2

¹Relative risk (R) of twinning for each parity group is calculated against all others pooled together.

²Includes all cows ≥ the parity listed.

Table 2. Effect of milk production and lactation number on the incidence of double ovulation in lactating dairy cows (n = 196) (from Fricke and Wiltbank (24)).

Lactation	Milk production		Overall
	Low (≤ 40 kg/d)	High (> 40 kg/d)	
1	7.4	22.2	9.5 ^x
2	4.0	14.3	10.8 ^y
3	8.7	27.8	20.3 ^z
Total	6.9 ^a	20.2 ^b	13.3

^{a,b}Different ($P < 0.05$).

^{x,y,z}Linear increase $P = 0.09$.

(54) that uses two treatments with GnRH and one treatment with PGF_{2 α} . Ovulation was evaluated by transrectal ultrasound at the time of the second GnRH injection and 48 h after this injection. Table 2 shows the double ovulation rate at different lactation number and different levels of milk production. The mean milk production was determined 3 d before ovulation and averaged 40.5 \pm 0.8 kg/d. The cows were segregated by whether they were below or above the mean value. Double ovulation rate in cows that were high milk producers was ~20% compared with ~7% in low producers. This difference was similar regardless of lactation number. Thus, these data are consistent with milk production being the greatest predictor of ovulation rate and twinning rate (39) in dairy cattle. It seems likely that ovulation rate is more closely linked than twinning rate to the physiological effects of milk production. In addition, the current availability of transrectal ultrasonography allows the ovulation rate to be accurately and readily obtained. The possible physiology involved in this relationship is discussed in the next section.

Potential Mechanism Producing Multiple Ovulations

Many physiological situations can result in an increase in ovulation rate, and these increases may be due to differing mechanisms or multiple situations leading to a common underlying mechanism. The first question is whether the two ovulatory follicles come from the same follicular wave. We have evaluated a total of 27 multiple ovulations that occurred during a normal estrous cycle in dairy cows (Sartori and Wiltbank, unpublished). We found that all except two of the ovulated follicles came from the same follicular wave (92.6%). In contrast, multiple ovulations in sheep (26, 63) were often associated with multiple ovulatory follicular waves.

What hormonal mechanisms would allow two follicles to arise from a follicular wave rather than only one? A complete answer to this question would provide substantial insight into the critical mechanisms involved

in selection of a single dominant follicle. Based on the model presented earlier, there are a number of possible scenarios that could result in a lack of follicular deviation. An initial possibility that we have considered is that the future dominant and subordinate follicles may be at a similar stage of differentiation such that both reach the point of deviation at approximately the same time. This would require a disruption of the normal follicular hierarchy that results in about an 8-h interval between each follicle in a follicular wave. This is obviously a fairly speculative concept, and there is currently a lack of published data that would refute or support a disruption of follicular hierarchy due to milk production.

Another possible mechanism is that FSH concentrations do not diminish sufficiently at the time of deviation to prevent the subordinate follicle from further development. We have preliminary data that supports this scenario. Circulating FSH concentrations were compared in six heifers that had codominant follicles in a follicular wave as compared to six heifers that had only a single dominant follicle. The heifers with codominant follicles had greater ($P < 0.05$) FSH concentrations than single follicle heifers at 8 h before follicles reached 8.5 mm and at the time the largest follicle reached 8.5 mm (average size at which deviation occurs). However, FSH concentrations were similar for heifers with codominant or single dominant follicles at 8 h after the follicle reached 8.5 mm and at all other analyzed times. These results are preliminary but are consistent with the possibility that a slightly greater FSH concentration could lead to codominant follicles. In contrast, no difference in FSH concentrations was detected in twin-bearing versus control beef cattle (15). However, the differences we observed in FSH concentrations were very short-lived and only detected when FSH was assessed every 8 h and normalized to the time the largest follicle reached 8.5 mm in diameter. Thus, the differences in FSH concentrations that may lead to double ovulation are not large and occur only for a short time near the normal time of deviation.

We have devised a physiological model to account for a relationship between high milk production and increased ovulation rate. The central point of this model is that metabolism of the follicular factor that causes depressed FSH is elevated in high producing lactating dairy cows. For the purposes of this review, we will assume that estradiol is this critical factor, although another factor may be more important and could be regulated in a similar manner as described below. High milk production is closely associated with high DMI ($r = 0.88$; 31). The high DMI is likely to result in high blood flow to the digestive tract due to requirements to digest and absorb increased nutrient load. Anatomic

cally, all blood flow from the gut must pass through the liver. We speculate that the digestive tract blood flow is so great during the extremely high blood flow associated with lactation that there is greatly increased liver blood flow. Increased liver blood flow would be expected to increase steroid metabolism because blood that passes through the hepatic circulation is essentially cleared of steroid (50). Other hormonal factors also might be metabolized at an increased rate with elevated DMI. Thus, high DMI would result in increased metabolism of follicular factors that are critical for the final depression in FSH that occurs near follicular deviation. We have considered the possibility that solubilization of estradiol in the milk fat and removal with the milk also may be a mechanism for removal of steroids in high producing dairy cows. At this time, the role of hormone elimination in milk cannot be discounted but a simple calculation indicates that the amount of estradiol eliminated in milk (~1 pg of estradiol removed per day assuming 20 pg/ml concentration in milk and 50 L of milk produced) is minor compared to the amount removed by liver metabolism (~240 μg of estradiol removed per day assuming 10 pg/ml in blood and 1000 L of liver blood flow per hour).

There are substantial data that are consistent with acute feeding increasing liver blood flow and steroid metabolism. We have previously reported that feeding leads to increased metabolism of progesterone in lactating dairy cows (71). This is similar to the feed-induced increase in progesterone and estrogen metabolism previously reported in sheep (50) and pigs (57). In Figure 4, liver blood flow was measured by continuous infusion with bromosulphophthalein. Steroid metabolism was measured by the continuous infusion of estradiol and progesterone (data not shown). It is clear that acute feeding resulted in dramatically increased liver blood flow as measured by bromosulphophthalein metabolism. The basal flow rate was 957 L per hour, so there was more than a 60% increase in blood flow following feeding. Similarly, estradiol and progesterone clearance rates were about twofold greater in fed compared with unfed cattle (data not shown). Dry cows appear to have a lower liver blood flow and a reduced response of liver blood flow to acute feeding (Sangsrivavong and Wiltbank, unpublished results). Thus, blood flow and steroid metabolism may be greatly increased in lactating dairy cows due to a chronic high feed intake as well as acute feeding of a large meal.

The bottom portion of Figure 3 compares and contrasts the possible physiology leading to double ovulation compared with single ovulation (upper portion). It is hypothesized that high feed consumption and high hormone metabolism would result in a transient elevation of circulating FSH and physiological selection of a

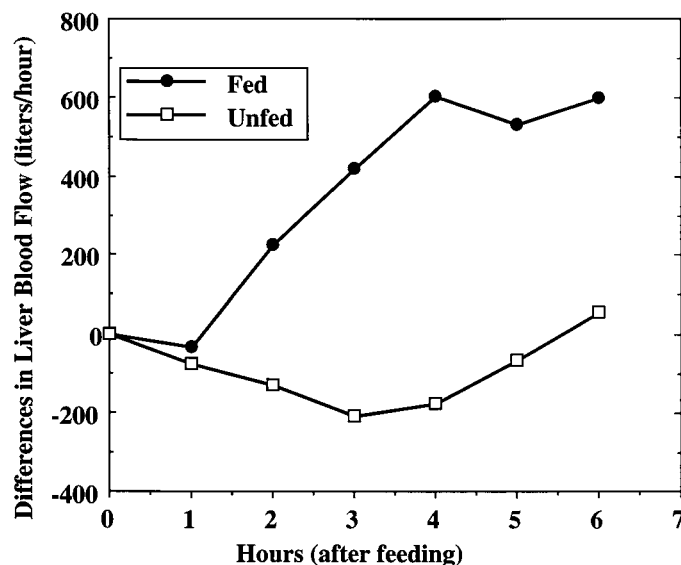


Figure 4. Changes in liver blood flow after feeding of lactating dairy cows ($n = 2/\text{group}$). Feed was removed 12 h before the blood flow measurements. Blood flow was measured using metabolism of bromosulphophthalein (BSP). Continuous infusion of BSP was initiated 1 h before treatments and cows were either given a lactating dairy cow diet ad libitum or continued to have no feed. Blood samples were removed every 15 min to evaluate serum BSP concentration and this was converted to liver blood flow using the methods of Clarkson (12). Extraction rate of BSP by the liver is 80% (3) and 45% hematocrit. The basal blood flow (0 h) averaged 957 L/h.

second dominant follicle. This scenario is consistent with the obvious links between high milk production, increased hormone metabolism, and double ovulation. Nevertheless, substantial research will be required to confirm, modify, or refute this working physiological model.

The present model should not be used to discount other possible physiological scenarios underlying double ovulation. For example, the growth hormone/IGF-I system has been found to have numerous reproductive effects (for review see 42), and increased circulating concentrations of IGF-I have been associated with increased ovulation rate (16, 65). Nevertheless, elimination of liver IGF-I secretion in mice was associated with a decrease to about 30% in circulating IGF-I but no change in litter size (78). Thus, the data on whether circulating or follicular IGF-1 causes increased ovulation rate in cattle must be considered inconclusive at this time. Other interesting situations with increased double ovulation occurred after removal of a single ovary (47) or after aspiration of the dominant follicle (25). After aspiration there was a greater surge in FSH than observed during normal FSH surges (25), suggesting that FSH may be involved in increased ovulation rate after follicular aspiration. No differences in FSH concentration were observed after unilateral

ovariectomy. Nevertheless it is possible that small changes in FSH concentrations near the time of deviation could be involved as described above. Ovariectomy would be expected to reduce circulating inhibin concentrations due to the elimination of half the small follicle population. More definitive studies in cattle will be required to better define the physiological scenario that results in high double ovulation rate in dairy cattle.

CONCLUSIONS AND PRACTICAL IMPLICATIONS

The proposed working model should provide a stimulus for discussions and research projects of those interested in practical or basic aspects of follicular development. Although the proposed model of increased double ovulation rate remains to be confirmed it provides a possible basis for understanding many practical situations in which double ovulation rate may be higher. For example, the increase in multiple births associated with "flushing" of sheep or pigs with a high amount of feed may be due to increased liver blood flow and estradiol metabolism. It may be possible to focus the stimulation of liver blood flow on the critical time near deviation in order to increase ovulation rate and fecundity with minimal manipulation. This may allow development of practical methods that allow synchronized double ovulation; particularly for use in beef cattle. Increased prolificacy could improve profitability of beef cattle operations (17). Additionally, there may be methods that would allow decreased ovulation rate by acute inhibition of estradiol metabolism near the time of deviation. This might be possible either by reducing feed intake or liver enzyme activity at a critical time during a synchronized follicular wave. The methodology to decrease twinning rate could improve dairy cattle performance because of a reduction in the reproductive and metabolic problems associated with twin births. Until this methodology becomes available, it seems likely that ovulation rate will continue to increase as milk production continues to increase in lactating dairy cattle populations.

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