Dairy Session I

"New Concepts in the Interactions of Nutrition and Reproduction"

Moderator: **Gala Beckendorf**

Impacts of Early Postpartum Metabolism on Follicular Development and Fertility

Jack H. Britt
Department of Animal Science
Box 7621, North Carolina State University
Raleigh, NC 27695-7621

Introduction

High producing dairy cows often experience low rates of conception during the target breeding period of 60 to 120 d postpartum (Faust *et al.,* 1988; Harrison *et al.,* 1990). Low fertility is costly for dairy producers because of extra expenditures for semen and insemination and because of reduced income from excessive days open (Britt, 1985). Cows that fail to conceive to AI between 60 and 120 d postpartum often are bred to clean-up bulls of unknown genetic merit. This not only affects long-range potential income for producers, but it reduces the number of records available for AI progeny testing programs.

Review of experimental data and careful study of published papers reveal that not all high producing healthy cows experience low fertility during the breeding period (Butler and Smith, 1989; Fonseca *et al.,*1983; Helmer and Britt, 1986; Staples *et al.,*1990). On a within-herd basis, the association between level of production and fertility is often weak (Fonseca *et al.,* 1983). This leads one to suspect that factors other than high production *per se* are responsible for low fertility. It is with this concept in mind that we have begun to examine various biological pathways that might account for differences in fertility in otherwise healthy high-producing cows.

This paper will focus on one potential pathway—namely the latent effect of early postpartum metabolism on quality of follicles destined to ovulate during the breeding period. Such follicles influence fertility in two ways: 1) through the quality grade or viability of the ovulated oocytes, and 2) through the amount of progesterone secreted by corpora lutea (CL) formed from these follicles (Fonseca *et al.,* 1983). This paper examines the possibility that quality of a follicle is dependent on the conditions under which it begins its initial development many weeks prior to ovulation.

Time Course of Follicle Development in the Cows

Lussier *et al.* (1987), through an elegant experiment with cattle, showed that more than 40 days elapse between the time that a follicle begins to form an antrum and when it reaches ovulatory size. By extrapolation of these data we can then project that it takes at least 60 to 80 days for bovine follicles to grow from the early preantral stage (primary or secondary follicle) to mature ripe stage ready for ovulation. (Graafian follicle, Figure 1). There are no good experimental data from which one can estimate accurately the rate of growth of preantral follicles in cattle, but studies with other species have shown that the preantral growth phase may be considerably longer than period of growth after antrum formation. For example, recent studies with gilts in our own laboratory (Morbeck *et al.,* 1991) indicate that it takes approximately 100 days from activation of a resting primordial follicle until the follicle reaches an ovulatory size.

If follicles are exposed to adverse conditions such as severe negative energy balance, heat stress or postpartum disease during their initial stages of growth, this could affect gene expression, resulting in impaired or altered development. Such an impairment could result in formation of dysfunctional mature follicles, which produce poor oocytes and result in the formation of weakened corpora lutea. This is the postulate that this paper will take.

Oocytes in developing preantral follicles are highly active terms of synthesis of nucleic acids and protein and these active oocytes double in size during the preantral growth period (Roy and Greenwald, 1991). During this same period, the number of granulosa cells surrounding the oocyte increase linearly and thecal cells just outside the membrana granulosa are first observable by histological techniques (Erickson, 1966 Marion *et al.,* 1968). Microscopic cellular connections between the vitelline membrane of the oocyte and plasma membrane of granulosa

Figure 1. Illustration of predicted periods of growth for the first six follicles destined to ovulate during the postpartum period for a cow that ovulates first at 3 weeks postpartum and then every 3 weeks thereafter. In this model, inactive primordial follicles are activated and begin to grow at least 60 to 80 days before ovulation. The earliest growth may even occur earlier than this. Early growth is a slow process and follicles are only visible by histological methods.

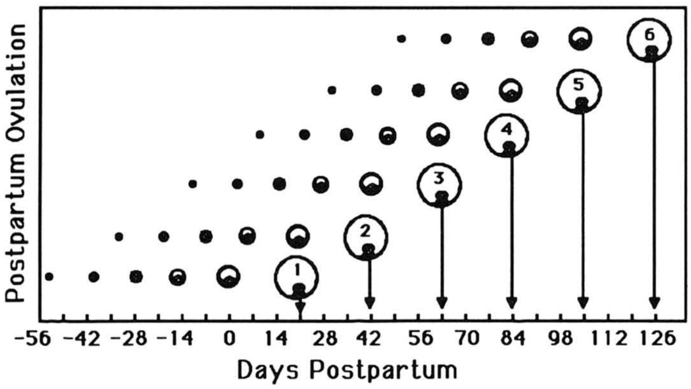
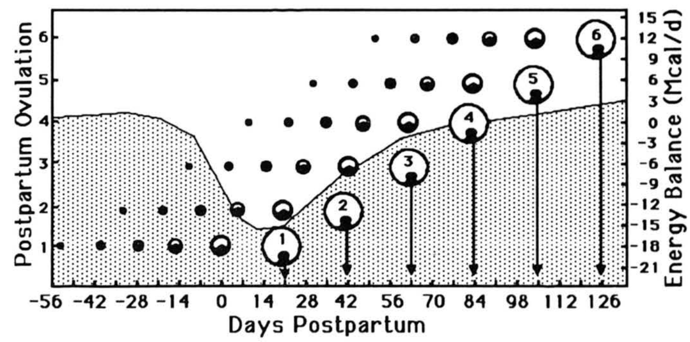


Figure 2. A model to illustrate how adverse conditions during folliculogenesis could influence fertility during the breeding period. The 1st and 2nd follicles begin development during the dry period, when metabolic conditions are desirable. In contrast, the 3rd, 4th, and 5th follicles begin development during the early postpartum period, when negative energy balance could be severe (shaded area). This predicts that 1st and 2nd follicles would differ little between cows that had large differences in energy balance but 3rd, 4th and 5th follicles would be altered by negative energy balance.



cell indicate that cellular components of developing follicles communicate, and thickening of the zona pellucida between the oocyte and its surrounding cells indicates that there is active secretion of glycoproteins. Evidence is now beginning to accumulate (Roy and Greenwald, 1991) that cells of these early follicles respond to growth regulators such as insulin-like growth factor I(IGR-I), fibroblast growth factor (FGF) and epidermal growth factor (EGF).

Although we suspect that adverse conditions can affect gene expression during folliculogenesis, and therefore exert a latent effect on fertility, we have little data to test this hypothesis directly. However, in a parallel system, the spermatozoa-producing seminiferous tubules of the testis, there is clear evidence that factors such as heat stress, altered nutrition or exposure to toxins can exert adverse effects on gene expression of developing germ cells, resulting several weeks later in ejaculation of poor quality sperm.

High producing cows which experience severe weight losses during the first 3 to 5 weeks after calving presumably subject their developing follicles to adverse metabolic conditions associated with the rapid weight loss. If our theory is correct, this presumably leads to the production of defective follicles during the breeding period and results in low levels of progesterone (Fonesca *et al.,* 1983: Villa-Godoy *et al.,* 1988; Harrison *et al.,* 1989; Spicer *et al.,* 1990) and associated low fertility (Butler and Smith, 1989). In contrast, cows that are able to consume sufficient dry matter during this period apparently have healthy follicles and maintain high fertility (Butler and Smith, 1989; Staples *et al.,* 1990).

Relatonship Between Adverse Conditions During Follicle Development and Conception Rates During the Breeding Period

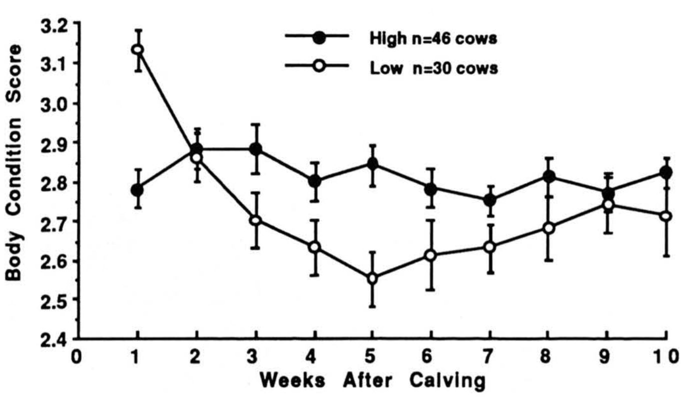
Our working hypothesis contrasts with much of the current thinking about causes of low conception rates because it puts more of the emphasis on conditions that existed 60 to 80 d before insemination than on the period immediately preceding insemination. Moreover, our hypothesis predicts that there would be a natural delay between when such adverse conditions subsided and restoration of normal fertility. We recognize that fertility is affected by concurrent environmental conditions (e.g. temperature stress, uterine infection, etc.), but we propose that it is influenced even more by conditions that existed 60 to 80 d earlier. These conditions are somehow "imprinted" upon follicles that began development during the adversity.

Such a latent effect can explain several phenomena. For example: 1) cows in southern and southwestern parts of the U.S. remain relatively infertile for up to 2 mo after the hot summer temperatures have subsided (Faust *et al,* 1988), 2) early postpartum clinical disorders affect fertility 60 to 80 d later, even though the clinical condition is corrected (Oltenacu *et al,* 1984), and 3) cows that have greater losses in body condition during the first 2 to 5 wk postpartum have substantially lower fertility at first AI (Butler and Smith, 1989).

In support of this hypothesis, we recently conducted a retrospective analysis of data collected several years ago in a study of Fonseca *et al.* (1983). In that experiment, high producing Holstein cows in two N.C. State University research herds were monitored intensively for approximately 150 days postpartum. Briefly, cows were bled twice weekly for assessment of progesterone concentrations, palpated twice weekly for evaluation of uterine involution and ovarian function, weighed weekly and body condition scored weekly. In addition, milk yield and reproductive performance were assessed (Fonseca *et al.*,1983).

For the retrospective analyses, we utilized data from 76 Holstein cows. These cows were selected on the basis of having complete data available. Body condition scores of these cows were summarized for the first 10 weeks postpartum and it was found that the greatest change in score between any two measurements was between weeks 1 and 5. Therefore, cows were allotted to 'high' and 'low' groups based solely on change in body condition between weeks 1 and 5 (Figure 3). The low cows had the greatest decline (below the mean for the entire group) in scores between weeks 1 and 5 whereas the high cow had the least decline (above the mean for the entire group). Only change in body condition score was used to allot cows to these two groups; no other criteria was considered.

Figure 3. Changes in body condition score during 10 weeks after calving for cows sorted solely on change in BCS during the first 5 weeks after calving. Data were from a study conducted by Fonseca *et al.* (1983). Orginally the body condition of cows was scored on a 1 to 9 scale, but the scores have been converted to a 1 to 5 scale to be consistent with scores systems in use today. Note that the higher conditioned cows at calving lost more condition during the first 5 weeks (Low) whereas those with lower scores at calving lost less condition. Milk production did not differ between the two groups of cows (see Table 1).



Once the cows were allotted to the high and low groups, their ovarian function was assessed by determining days to first ovulation, days between ovulations, and progesterone secretion (average, peak, luteal phase mean) during each estrous cycle. Conception rate was assessed for services occurring at various times during the breeding period. A summary of some of our findings are shown in Table 1.

We recognize that retrospective analysis of data cannot be used to test a scientific hypothesis for which the experiment was not designed. However, the data in Table 1 reveal that cows that had greater losses in body condition during the first 5 weeks postpartum had lower fertility, even though milk yields were similar between high and low cows. The low cows had their first postpartum cycle about 6 d later than the high cows, consistent with the results of Butler *et al.* (1981) and Canfield and Butler (1991); however, this 6-d delay is not enough to account for the 37% difference in conception rate at first service.

To further assess whether our hypothesis had merit, we compared progesterone concentrations between high and low cows for the first five estrous cycles. Results are shown in Figure 4. This figure shows the average progesterone concentration during peak luteal function (days 6-13 of first cycle; days 10-17 of all other cycles). Note that the two groups of cows did not differ in peak progesterone secretion during the first two cycles (P=.71, P=.47), but differences occurred during cycles three (P=.13), four (P=.06) and five (P=.08). This supports the concept that CL function (progesterone secretion) seems to be more related to conditions that existed during initial development of the destined ovulatory follicle than to conditions that existed closer to the actual time of ovulation.

Figure 4. Progesterone secretion during peak luteal function of postpartum cycles 1-5 for cows sorted solely on basis of body condition score during weeks 1-5 after calving (see Figure 3). Cows classified as low had a greater loss in body condition score. Blood samples were collected twice weekly from calving until at least 50 days after each cow was diagnosed pregnant. The 'P' values for each cycle represent the probability that progesterone concentrations between 10 and 17 days after estrus differed between groups at that cycle.

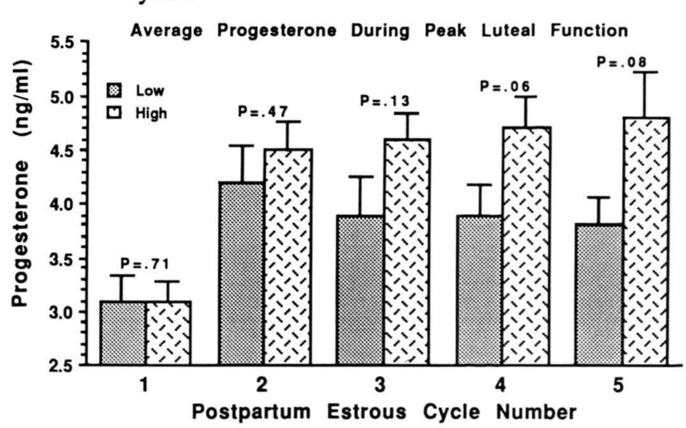


Table 1. Results of retrospective analysis of data from high-producing Holstein cows sorted solely on basis of change in body condition scores between weeks 1 and 5 postpartum.

Trait	High Cows	Low Cows
No. cows[a]	46	30
Body condition score change[b]		
Weeks 1 to 5	+.06	-.58*
Weeks 5 to 10	-.02	+.17*
Days postpartum to ovulations:		
First	17.2	23.3*
Second	35.8	44.3*
Third	58.7	64.4*
Fourth	78.4	86.1*
Fifth	102.0	110.1*
Milk yield		
Average during first 70 d postpartum (lbs)	58	60
Average yield for 305 d lactation (lbs)	17,941	18,198
Days postpartum to first AI	84.9	82.9
Conception rate (%)		
First service	62*	25*
All services	61*	42*
3rd ovulation	63	27
4th ovulation	67	50
5th ovulation	53	44

[a]Primiparous, n = 45; multiparous, n = 31.

[b]Body condition scores are on a 1 (thin) to 5 (fat) scale. These were converted from the original 1 to 9 scale to make them consistent with current day scoring systems.

*$P < .05$

What Can Be Done to Alleviate Low Fertility Associated With Loss of Body Condition

It is clear from the analyses of our data and from data of Butler and Smith (1989) that a decrease in BCS of more than 1.0 point during the first 5 weeks after calving leads to poorer fertility at first service. Villa-Godoy and co-workers (1988) indicated that energy balance during the first 9 days after calving affected progesterone secretion during the first three postpartum cycles, and that the strength of the relationship was greater between the 9-day energy balance and progesterone secretion during the third cycle (~ 60-80 days postpartum) than with the first (23-40 days postpartum) or second (40-60 days postpartum) cycles. Again this indicates that events that occur early postpartum influence fertility much later.

There are several steps that could be taken to reduce the loss in body condition during the early postpartum period and therefore to improve fertility later on. Feeding changes need to be made during the late dry period so that cows do not enter the severe negative energy balance stage before calving. Adjustment to the lactation ration should begin during the last 2-3 weeks of the dry period, but care should be taken to avoid over-consumption. Fresh cows should be provided with the best quality, freshest feed on the farm. Feeding total mixed rations three to four times per day will probably stimulate intake in these fresh cow, and these rations should be specifically formulated for fresh cows.

Cows with high BCS at calving are the ones that lose the most after calving, probably because their appetite is reduced by the fatness at calving. There is still considerable debate about the ideal score at calving, and the ideal score probably varies from herd to herd. Generally cows that calve with a BCS of about 3.25 to 3.75 probably perform best in terms of health, peak milk and fertility; but these performance traits are really dependent on voluntary feed intake after calving.

Summary

This paper has provided a hypothesis to explain the latent effect of postpartum losses in body condition on follicular development, corpus luteum formation and function, and fertility in high producing dairy cows. Retrospective analysis of existing data support the concept presented here as do results reported by others. Nevertheless, one must view this hypothesis with caution until more direct experimentation can be done to reveal potential mechanisms and pathways through which such effects might be mediated.

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Abstracts

Long-acting antibiotic formulations in the treatment of calf pneumonia: a comparative study of tilmicosin and oxytetracycline

R. Laven, A. H. Andrews

Veterinary Record (1991) **129**, 109-111

The treatment of an outbreak of acute pneumonia in 50 four-to-eight-month-old Friesian and Friesain cross calves is described. At the first visit (day 0) 16 calves received 20 mg/kg bodyweight of oxytetracycline dihydrate intramuscularly and 15 received 10 mg/kg of the macrolide tilmicosin subcutaneously. The remaining 19 in-contact animals were not considered ill enough to be included in the trial and received 20 mg/kg of oxytetracycline dihydrate. The rectal temperature, demeanour, respiratory rate and respiratory effort of each calf was assessed on days 1, 2, 3, 9, 14, 21 and 28, and calves which had not responded were given repeat injections of the same antibiotic. All the calves recovered from the outbreak and of the 19 calves treated strategically, three required a second injection. Among the calves with clinical pneumonia, fewer treatments ($P < 0{\cdot}01$) were required by those treated with tilmicosin. The rectal temperatures of both groups decreased ($P < 0{\cdot}05$) after the first injection, but on day 3 the decrease was greater ($P < 0{\cdot}05$) in the group treated with tilmicosin. Respiratory rates varied widely but respiratory effort was less ($P < 0{\cdot}05$) on day 2 in the calves treated with tilmicosin. When long-acting antibiotic injections are used to treat enzootic pneumonia it is suggested that a second visit should be made on day 3 to assess the animals' response to treatment.

Effect of clenbuterol administered during the expulsive stage of bovine parturition on uterine activity and the fetus

F. H. Jonker, G. C. van der Weijden, M. A. M. Taverne

Veterinary Record (1991) **129**, 423-426

The effect of an intravenous injection of 0·3 mg clenbuterol on myometrial activity, fetal heart rate and fetal outcome was studied in three groups of animals: six heifers in active labour at term, in which fetal oversize was diagnosed just before the drug was injected and whose calves were delivered by caesarean section within 50 minutes; four cows at the start of the explusive stage of parturition which had been induced with flumethasone on day 270 of gestation; and in four parturient cows which had had electrodes implanted on the myometrium at least one week before calving was induced with flumethasone on day 270. Electrocardiograph electrodes were placed on the calf and an intrauterine pressure catheter was inserted between the calf and the uterine wall upon rupture of the amniotic membrane. Clenbuterol induced a significant decrease of myometrial activity for at least 20 minutes. Recovery was most rapid in the heifers in which an obstetrical examination had taken place during active labour. There was no significant effect on basal fetal heart rate but decreases in heart rate were absent as long as uterine contractions were inhibited. The fetal outcome, judged either before or at birth by blood pH, base-excess and P_{CO_2} and by a clinical examination, was not adversely affected.