OFFICIAL

PROJECT: NE-161 (Rev.)

TITLE: ASSOCIATION OF FERTILITY WITH TEMPORAL CHANGES IN OVARIAN FUNCTION OF DOMESTIC RUMINANTS

DURATION: OCTOBER 1, 1996 TO SEPTEMBER 30, 2001

STATEMENT OF THE PROBLEM AND JUSTIFICATION:

Reproductive inefficiency is a major cause of reduced productivity for both dairy and beef cattle. Reproductive loss occurs primarily through fertilization failure and early embryonic mortality; estimates of loss from first inseminations are about 15% fertilization failure and 20% embryo death (Hawk, 1979). Reproductive problems have a major impact in the Northeast, where dairy cattle constitute a major part of the agricultural enterprise. Furthermore, as annual milk production has risen, the problems of poor fertility have worsened (Butler and Smith, 1989; Lean et al., 1989). Scientific knowledge of the basis for these early reproductive failures is either lacking or incomplete.

The problems of fertilization failure and embryonic death involve a multitude of cellular functions and their products. The resolution of these problems necessitates research on many physiological mechanisms: regulation of gonadotropin secretion, follicular growth and maturation, corpus luteum formation and development, gamete transport, utero-embryonic interactions, and fetal-uterine regulation of corpus luteum lifespan. In broadest terms these require understanding the cascade of events leading to ovarian follicular development, ovulation and the subsequent luteal phase during which pregnancy is established.

The complexity of the mechanisms involved in the successful establishment of pregnancy emphasizes the importance of an organized regional approach with the combined resources of several contributing stations. This proposal describes the key areas to be studied and the step-wise regional cooperation that will be applied to orchestrate these efforts. Initial collaborative efforts will focus on the examination of naturally-occurring patterns of development and function of the preovulatory follicle for their effects on oocyte maturation, fertilization and embryo survival. The effects of altered metabolism on these parameters will be investigated. Finally, we will examine how differences in luteal function affect preovulatory follicle development and subsequent fertility. There will be direct inter-station cooperation in these experiments in which animal resources will be pooled by utilizing the same experimental protocol at several stations, with analyses of different components subdivided among the stations.

RELATED CURRENT AND PREVIOUS WORK:

Information about the processes involved in follicular development in a variety of species has been accumulating. In dairy cows, the presumptive ovulatory follicle can be identified as the largest follicle in the ovaries only a few days prior to estrus (Dufour et al., 1972). However, follicular growth and atresia occur throughout the luteal phase (Rajakoski, 1960; Spicer and Echternkamp, 1986). The most recent studies of follicle dynamics in cattle

have shown that a large follicle emerges from a cohort of smaller follicles about once every 7 to 10 days and apparently is dominant over the small follicles (Fortune et al., 1988; Sirois and Fortune, 1988; Ginther et al., 1989; Savio et al., 1990).

Early workers reported that follicles were larger (Ulberg et al., 1951; Zimbelman 1963; Zimbelman and Smith, 1966), persisted (Trimberger and Hansel, 1955), or became atretic (Guthrie et al., 1970) when cows received minimal doses of progestogen required to suppress estrous behavior and ovulation. Conversely, follicles were smaller when doses of progesterone were increased (Nellor and Cole, 1957). With the application of ultrasonography to examine patterns of follicular development in cattle, persistence of follicles during conditions of low progesterone or progestogens has been documented in several studies (Sirois and Fortune, 1990; Cupp et al., 1992; Savio et al., 1993b).

Lowered fertility in dairy cows has been associated with lower concentrations of progesterone in milk (Meisterling and Dailey, 1987) or blood (Folman et al., 1973). Several recent studies have confirmed that low fertility is common under conditions of low progesterone, increased frequency of secretion of LH, prolonged preovulatory increases in estradiol, and with persistent follicles (e.g. Wehrman et al., 1993; Savio et al., 1993a, b; Stock and Fortune, 1993). During the current funding period, this cooperative regional research project has completed an experiment to compare fertility in cows that ovulate a growing vs. a persistent follicle. Persistent follicles were induced using the progesterone releasing, intravaginal devices, CIDR-B. The study was conducted at 7 different experiment stations and included a total of 187 cows. Fertility was significantly lower in animals that ovulated a persistent follicle (pregnancy rate = 23.6%) than those that ovulated a growing follicle (pregnancy rate = 58.2%). Further, fertility was higher in CIDR-treated heifers than in CIDR-treated cows. Thus, although ovulation of a persistent follicle results in lower fertility in both cows and heifers, the effect is most dramatic in lactating cows.

From this study and the work of others, it is clear that hormonal manipulation of the estrous cycle, as is often used in estrous synchronization schemes, may alter patterns of follicular development. This alteration can lead to reduced fertility. It is now of interest to determine if naturally-occurring differences in patterns of follicular development also lead to greater, or reduced, fertility. In cattle, the development of large antral follicles occurs in a wave-like fashion; a cohort of follicles is recruited contemporaneously to grow larger than 4 to 5 mm in diameter. One follicle is selected to be the dominant follicle, but this follicle will undergo atresia with the rest of the cohort if the corpus luteum has not regressed. Most cows have two or three waves of follicular development within an estrous cycle (Sirois and Fortune, 1988; Savio et al., 1988; Knopf et al., 1989). It is unknown whether fertility differs in cows with two vs. three waves of follicular development, or if individual cows tend to have the same pattern of follicular development in subsequent cycles. Therefore, the first objective of the proposed project will be to evaluate fertility after two vs. three waves of follicular development. It is essential that this experiment be performed as a regional research project, so that sufficient numbers of two- and three-wave cows are obtained for statistical analysis. The study will also determine if the duration of the last follicular wave, rather than the number of waves, affects subsequent fertility.

Differentiation of dominant follicles is a two-step process: recruitment of healthy gonadotropin-sensitive follicles and selection of the follicle(s) that becomes dominant while the others undergo atresia (Driancourt and Fry, 1988). Both endocrine and paracrine controls are involved in the differentiation of the follicles toward ovulation. Recruitment of each follicular cohort is preceded by an elevation in plasma FSH. Selection is correlated with a decrease in FSH concentrations together with a change in balance between the paracrine stimulators and inhibitors found in follicular fluid. Dominance of ovulatory follicles is maintained perhaps by high estradiol production linked to the stimulatory action of paracrine regulators and by the high sensitivity of the large dominant follicle to gonadotropins.

In addition to gonadotropins, a variety of other endocrine, autocrine and paracrine regulators may be involved in follicular development. Insulin exerts actions on ovarian tissues which are very similar to those of pituitary gonadotropins (Poretsky and Kalin, 1987). Insulin stimulates thecal cell androgen production (Hernandez et al., 1988; Caubo et al., 1989) via high affinity receptors (Hernandez et al., 1988) and increases granulosal cell metabolism (Weber and LaBarbera, 1988). Perhaps most importantly, insulin has been shown to synergize with FSH to facilitate morphological differentiation of granulosal cells and enhance LH receptor binding (Amsterdam et al., 1988). It is not clear whether all the effects of insulin are direct or are mediated by interaction with receptors for insulin-like growth factors (IGF). There is considerable overlap in activities and associations between insulin and IGF-I.

Although the possible relationship with ovarian IGF-I or its receptors has not been established, insulin was more mitogenic than gonadotropins in bovine follicles grown in perfusion culture (Peluso and Hirschel, 1987). Also, insulin enhanced ovulation rate during negative energy balance in heifers (Harrison and Randel, 1986). Ovulation enhancement has also been demonstrated following long-acting insulin treatment in well-fed gilts (Cox et al., 1987). During negative energy balance in early lactation, the rapid increase in utilization of glucose for milk lactose production results in lower plasma concentrations of both glucose and insulin as compared to later stages of lactation (Hart et al., 1978; Smith et al., 1976), after first ovulation has occurred. Plasma IGF-I levels closely parallel insulin during early lactation (Ronge et al., 1988), suggesting a direct relationship.

Despite the clear relationships between insulin and energy balance or follicular function, very little is known regarding the effects of negative energy balance on patterns of follicular development. It has been suggested that severe negative energy balance may affect initial follicular growth in early postpartum cows (Britt, 1992). Energy balance does seem to be related to rate of follicular growth prior to initiation of estrous cycles in postpartum cows (Lucy et al., 1991) and is correlated negatively with the interval to first ovulation (Butler et al., 1981). Miyoshi et al (1995) found that plasma insulin could be elevated in postpartum cows by daily drenching with propylene glycol. Although animal numbers were insufficient in this trial to produce significant differences in fertility, conception rates tended to be greater in the propylene glycol treated cows (57.1% vs. 33.0%). Therefore, the second objective of the proposed project will be to determine if alterations in plasma insulin during negative energy balance affect patterns of follicular development and fertility.

OBJECTIVES:

1. Examine naturally-occurring patterns of development and function of the preovulatory follicle for their effects on oocyte maturation, fertilization and embryo survival.
2. Compare naturally-occurring and induced differences in metabolism for their effects on oocyte maturation, fertilization and embryo survival.
3. Examine how differences in luteal function affect preovulatory follicle development and subsequent fertility.

PROCEDURES:

Objective 1: Examine naturally-occurring patterns of development and function of the preovulatory follicle for their effects on oocyte maturation, fertilization and embryo survival.

Our collaborative project (NE-161, Relationships Between Patterns of Follicular Development and Fertility) showed that low levels of progesterone delivered by a Controlled Internal Drug Releasing (CIDR) device during the first follicular wave in heifers and lactating dairy cows resulted in the development of a persistent follicle (Ahmad et al., submitted). Furthermore, the fertility rate of heifers and cows ovulating a persistent follicle, as opposed to a growing follicle, was greatly reduced (i.e., pregnancy rate: persistent follicle = 23.6%, growing follicle = 58.2%). Previous studies (Savio et al., 1993a, b; Stock and Fortune, 1993; Wehrman et al., 1993) have also concluded that prolonged follicular growth is associated with lowered or impaired fertility. Various naturally-occurring patterns of follicle growth are exhibited in cattle (i.e., most estrous cycles consist of two or three follicular waves, although four waves have been reported; Fortune et al., 1988; Pierson and Ginther, 1988; Savio et al., 1988; Sirois and Fortune, 1988; Knopf et al., 1989). In heifers that exhibit two waves/cycle, the ovulatory follicle is observed approximately 10 days before ovulation, while in those that exhibit three waves/cycle, the ovulatory follicle is observed approximately 5.9 days before ovulation. In four-wave cycles, the ovulatory follicle is observed approximately 7 days before ovulation (Sirois and Fortune, 1988). Thus, it is of interest to determine whether follicles that naturally have a longer period of growth (i.e., in two-wave cows) ovulate oocytes that are less fertile than those with shorter periods of growth (i.e., in three- and four-wave cows).

1a. Do different naturally-occurring patterns of follicle development affect oocyte maturation, fertilization and embryo survival?

To address this question, ultrasound exams will be performed to monitor follicle development in lactating cows. Plasma progesterone concentrations will be determined and pregnancy status assessed. Specifically, seven research stations [Connecticut (CT), Maine (ME), New Hampshire (NH), New York (NY), Ohio (OH), Pennsylvania (PA), West Virginia (WV)] will pool resources to contribute to this objective. Each station will follow the protocol outlined below. Ultrasound exams will be performed in lactating Holstein cows that

demonstrate a natural estrus between 45 to 70 days postpartum (n = minimum of 20 cows per research station; assuming a 20% difference in conception rate, a total of 30 cows/station is required to achieve significance at $P<0.05$). Following a spontaneous estrus (day = 0), ultrasound exams will commence on day 5 postestrus, and continue every other day until the next estrus. Then, daily ultrasound exams will be performed to confirm ovulation. Following each ultrasound exam, a blood sample will be collected. Semen from bulls (selected for high proof for milk and fertility) will be used to breed cows according to the AM/PM rule. Pregnancy status will be determined on days 30-35 with ultrasonography.

Besides follicle diameters, the size and location (ipsilateral or contralateral to ovulatory follicle) of the corpus luteum will also be noted during ultrasound exams. This information provides the basis for objective 3. Also, each research station will report the incidence of "aberrant" patterns of follicle development, i.e., cystic follicles, failure to ovulate or short cycles. Together, results from this objective will elucidate which naturally-occurring pattern(s) of follicle development leads to successful oocyte maturation, fertilization and embryo survival.

A preliminary trial was conducted at the New Hampshire and Ohio research stations. Twenty eight lactating Holstein cows were scanned, and the results are summarized below (Table 1). The number of cows exhibiting two waves predominated over those exhibiting three or four waves. Five of 28, or 18% of cows exhibited "aberrant" waves, i.e., no ovulation, short cycle or cystic follicles. In addition, the pregnancy rate in two wave cows was lower than for three or four wave cows. The data so far appear to support our hypothesis that two-wave cows are less fertile than three-wave cows. However, the low number of three-wave cows require the cooperation of seven research stations to pool resources (i.e., increase animal numbers), which will also enable us to make inferences regarding fertility rates.

Table 1. Preliminary data for NE-161 joint project. Lactating Holstein cows were scanned every other day. Pregnancy was diagnosed between d 30-35 post-breeding with ultrasonography.

# waves/cycle	#cows/total (%)	#cows pregnant/total (%)	ovulatory follicle (mm ± SE)
2	18/28 (64)	10/18 (56%)	16.47 ± 0.52
3	4/28 (14)	3/4 (75%)	14.88 ± 1.60
4	1/28 (4)	1/1 (100%)	*15.5
"aberrant"	5/28 (18)	---	---

*follicle diameter, one cow.

1b. Ultrasound Exams.

Transrectal ultrasonography will be performed using a 5 or 7.5 MHz probe. Each ovary will be scanned several times and in at least two different planes. Individual follicles (≥ 5mm) will be followed and their diameters recorded. Diameters are determined using a calibrated caliper resident in the ultrasound monitor. These measurements represent the size of the antrum. Since follicles are not perfect spheres, the diameter of the follicle is calculated as the average of the antral length and width. Each research station will provide data on the number of waves, as well as the size of the ovulatory follicle, for their cows.

1c. Progesterone Assays

A blood sample (10 ml) will be collected into heparinized tubes following each ultrasound exam. Following centrifugation, the plasma will be kept frozen at -20°C until analysis by radioimmunoassay (RIA). To maintain consistency, all research stations will send their plasma samples to the Connecticut research station for analysis. The extraction protocol and RIA have been previously characterized (Beal et al., 1980).

1d. Data Collection

The spreadsheet below (i.e., the headings) has been developed to facilitate data entry and analysis. Each research station will record their data and then send a final spreadsheet to the New Hampshire research station where the data will be summarized and analyzed. Differences in fertility will be tested by chi-square analysis (Steel and Torrie, 1980), and hormonal data subjected to repeated measures ANOVA (Gill and Hafs, 1971) or ANOVA (Steel and Torrie, 1980).

Cow ID Number	Number of Waves	Preg? Y or N	Sire Registration Number

Ovulatory Follicle		Growth Rate	Cycle	Corpus Luteum		Aberrant
diameter (mm)	duration (d)	(mm/d)	Length	Contralateral	Ipsilateral	"waves"

Objective 2: Compare naturally-occurring and induced differences in metabolism for their effects on oocyte maturation, fertilization and embryo survival.

2a. Initial investigations will utilize treatments known to affect ovarian function/fertility and will determine whether treatment groups differ in secretion or degradation of progesterone and estradiol-17ß. This work will be initiated at two stations (NY and WV).

Studies at Cornell will be conducted in lactating and nonlactating cows to assess the effects on steroid metabolism of lactation and associated changes in feed intake. During the first 3-4 weeks of lactation in dairy cows, milk production increases much more quickly than feed intake, resulting in a large negative energy balance. By day 60 of lactation, milk production has passed its peak, feed intake has increased by about one-third from that after parturition and energy balance has been nearly restored. Several metabolic factors have been related to differences in follicular development in postpartum cows (Beam, 1995). In addition the results from the previous regional project suggested that plasma progesterone may be lower in cows than in heifers and that plasma progesterone was lower during the first week after AI in cows that failed to become pregnant. Such differences in plasma progesterone are related to poor embryo development (Maurer and Echternkamp, 1982) and a lower pregnancy rate (Butler et al., 1996). Blastocysts can stimulate progesterone output from luteal cells *via* interaction with the endometrium (Thibodeaux et al., 1994). Increased progesterone in circulation is important for full inhibitory action of development of the luteolytic mechanism during maternal recognition of pregnancy (Lamming and Mann, 1995). In this latter critical period after AI (d 14-17), higher estradiol concentrations in plasma are negatively associated with pregnancy (Pritchard et al., 1994).

Studies at West Virginia will be conducted in ewes. In earlier studies, short-term flushing with high protein diets has been shown to increase ovulation rate and/or litter size (Thomas et al., 1987; Smith, 1988; Smith and Stewart, 1990). In unpublished work performed in West Virginia, Israel and Sardinia, (S. Landau et al.), corn gluten meal increased ovulation rate, whereas soybean oil meal did not affect ovulation rate, but decreased, or tended to decrease, conception rates. Other studies have shown that higher protein diets (Thomas et al., 1987) or higher intake increased metabolic clearance rate, or reduced circulating concentrations, of progesterone (Williams and Cumming, 1982; Parr et al., 1993; McEvoy et al., 1995) or of estrogen (Payne et al., 1991; Adams et al., 1994). McEvoy et al. (1995) presented evidence that dietary-induced reductions in circulating concentrations of progesterone impaired subsequent development of ova in ewes. Conversely, higher concentrations of estradiol have been predictive of greater early embryonic death in a series of studies in beef cattle (Breuel et al., 1993; Ahmad et al., 1995), and were associated with the persistent follicles in which oocytes undergo premature development (Mihm et al., 1994; Revah and Butler, 1996). Thus it is of interest to determine whether effects of diet on steroid metabolism are generic, or are specific to estradiol or progesterone and whether these effects are mediated in part by changes in blood flow (such as were produced by changes in feed intake in gilts; Prime and Symonds, 1993).

How do changes in steroid metabolism (production and degradation) affect patterns of follicular development, oocyte maturation and embryo survival?

The studies at Cornell will compare steroid metabolism in lactating cows *vs.* non-lactating heifers consuming moderate or high dietary intakes. On day 12 postpartum (moderate intake), lactating cows (n = 4) will receive an intra-vaginal progesterone-releasing device (CIDR) and two estradiol implants (Compudose, ear implants). This period is chosen prior to formation of the first corpus luteum such that the CIDR provides the only source of plasma progesterone. The estradiol implants are expected (Taft and Deaver, unpublished) to result in turn-over of any large dominant follicles, thus becoming the sole source of plasma estradiol (~ 10 pg/ml). The CIDR and estradiol implants will be removed 48 hr after insertion, when plasma steroid concentrations will have reached steady-state equilibrium. Blood samples will be collected at -60, -30 and 0 min before implant removal to establish baseline concentrations and at 15, 30, 45, 60, 90, 120, 180, 240, 300 and 360 min, thereafter, to calculate the disappearance rate ($t_{1/2}$, time to 50% of baseline mean) of both progesterone and estradiol (Hawkins et al., 1995). At 60-70 days postpartum (high dietary intake), the same cows will be studied starting on day 11 of the estrous cycle. Blood sampling catheters will be placed as described below for experiments in ewes to allow calculation of blood flow and production and degradation of progesterone from the corpus luteum on day 12 of the cycle. Upon completion of the 6-hr sampling schedule, a CIDR will be placed intravaginally, two estradiol implants will be placed in one ear, and prostaglandin $F_{2\alpha}$ (25 mg Lutalyse im) will be injected to cause luteolysis. After 48 hours all implants will be removed for calculation of progesterone and estradiol disappearance rate as described above. To compare the effects of lactation, a group of non-lactating heifers (14 months of age, n = 4, high intake) will be studied in the same manner as the lactating cows from day 11 of the cycle including calculation of steroid disappearance rate. To complete these studies for the effects of dietary intake, an experiment will be conducted in feed-restricted (moderate intake) heifers (n = 4). The heifers will be fed

individually *ad libitum* from day 2-6 of the estrous cycle and at 66% of their mean individual intake from days 7-14. On day 12, the treatment protocol for progesterone and estradiol disappearance rate will be followed. The level of feed restriction is chosen to provide a similar relative magnitude difference in intake as for the difference observed in lactating cows between days 12 and 60 postpartum.

At West Virginia, studies in ewes will examine effects on steroid metabolism of dietary treatments known to affect ovulation rate and/or fertility. Specifically, three isocaloric dietary supplements will be fed once daily to ewes on a maintenance ration, beginning 7 days before estrus (mating), during the luteal phase. Control ewes will receive ground corn, and treated ewes will receive high protein from either corn gluten meal (high in branched chain amino acids, high rumen bypass protein) or soybean oil meal (lower in branched chain amino acids and rumen bypass protein). On day 3 of supplement feeding, sampling catheters will be inserted in the posterior vena cava via the saphenous vein (Benoit and Dailey, 1991) and into the right ventricle via the jugular vein. A second saphenous vein catheter, in the opposite saphenous vein, will be used for infusion of para-aminohippurate (PAH), so that blood flow and production and degradation rates of steroids can be calculated from concentrations of PAH, estradiol and progesterone in frequent samples. Samples will be collected at 30 minute to 2 hour intervals for 6 hours after feeding, during a period of high progesterone, low estradiol on day 4 of feeding and during a period of low progesterone, high estradiol on day 6 of feeding. These concentrations will be achieved by treating the ewes with prostaglandin F_2 on day 5 of the feeding period.

2b. In the early postpartum period, lactating dairy cows enter a state of negative energy balance. Negative energy balance is associated with decreased reproductive performance and with delayed follicular development (Lucy et al., 1991). One characteristic of negative energy balance is reduced plasma insulin concentration. Using daily drenches of propylene glycol to elevate insulin, Miyoshi et al. (1995) demonstrated an increase in cumulative plasma progesterone concentration of the first estrous cycle. This was associated with a tendency toward a longer first luteal phase (13.1 days vs. 7.1 days; treatment vs. control, respectively) and a higher conception rate to first AI (57.1% vs. 33%).

This study will confirm and extend the study of Miyoshi et al. As a cooperative regional research project, sufficient animals will be available for a fertility study. This experiment is designed to determine if transient increases in insulin alter the pattern of follicular development and enhance fertility in postpartum cows.

Do propylene glycol or increases in insulin affect the pattern of follicular development, oocyte maturation and embryo survival?

In the study on which this experiment is based, cows were drenched with 500 ml propylene glycol daily from days 7-42 postpartum. Because this is very labor intensive, the practicality of this procedure for on-farm use is questionable. Therefore, a preliminary trial will be conducted to determine if propylene glycol can be administered as a feed additive or if insulin can be administered directly. Also, the duration of treatment will be shorter and will

focus on the period during preovulatory follicle development. This preliminary trial will be conducted early in 1996, prior to the initiation of the renewed project.

Four stations (MA, NH, NY, OH) will collaborate on this experiment. At each station, 30 cows (days 40-55 postpartum) will be assigned to one of three groups: control or propylene glycol (treatment begins on day 10 or day 16). Among stations, this will allow for a minimum of 40 control and 80 treated cows, [if we assume there is a 20% difference in conception rates, a total of 132 cows (n=11 cows/group x 3 groups x 4 stations) is then required for the study]. Cows will be observed for estrus twice daily and randomly allotted at spontaneous estrus (day=0) among the three groups.

Beginning on day 5 after estrus, ultrasonography will be conducted and a blood sample collected every other day until the start of treatment. During the treatment period, ultrasonography will be conducted daily and blood samples collected twice daily. Ultrasonography will allow for determination of the growth pattern of the dominant follicle, the size of the follicle prior to ovulation, and the approximate time of ovulation relative to estrus. Size of the corpus luteum will also be monitored. One-half of the cows in the treatment group (n=minimum of 10) will be treated with propylene glycol beginning on day 10. The remaining treatment cows (n=minimum of 10) will be treated with propylene glycol beginning on day 16. Propylene glycol (500 ml) will be administered daily by drenching, unless a suitable alternative means of delivery is determined in the preliminary study. Treatment will continue until ovulation occurs. All cows will be artificially inseminated to a high fertility bull upon standing estrus, according to the AM/PM rule. Pregnancy will be diagnosed on day 30-35 with ultrasonography. Plasma samples will be assayed for progesterone and estrogen, all samples to be assayed at one station. This will provide information about the endocrine environment that exists as the follicle develops in control vs. propylene glycol treated cows. The acute insulin response to the propylene glycol drench is already known (Miyoshi et al., 1995). Twice daily blood samples will also be used for insulin analysis, to provide information on chronic, or baseline alterations in insulin concentration. From previous studies it is known that this short duration of propylene glycol treatment will not significantly alter energy balance.

Direct administration of insulin will be tested as an alternative to elevating plasma insulin concentrations by propylene glycol drenching. Dr. Nancy Cox (Mississippi State University) has treated lactating beef cows with long-acting insulin (Lente Insulin, Eli Lilly) twice daily for five days. Plasma glucose concentrations fluctuated between 30 and 45 mg/dl. Lactating animals are much more sensitive to insulin than non-lactating animals. Careful dose-titration studies will be performed in lactating dairy cows to establish a dose that enhances plasma insulin levels and glucose uptake by tissues (i.e., lowers blood glucose by 25-35 percentage points) for 12 hours each day. If feasible, this treatment will be used in place of the more laborious propylene glycol drenches in the protocol described above. A preliminary trial will also determine the route of administration (either intramuscular or subcutaneous) and time of treatment (e.g. day 10 of the cycle).

Objective 3: Examine how differences in luteal function affect preovulatory follicle development and subsequent fertility.

Objective 3 will examine how the location and the function of the CL affects concurrent follicular development. Experiments proposed to satisfy objective 3 can be categorized into three broad areas. The first area will examine whether the location of the CL affects follicular development. The second and third areas will examine if increased or decreased output of luteal progesterone affects patterns of follicular development.

3a. Does the location of the CL affect follicle development?

Ultrasonographic and fertility data obtained in experiments conducted to satisfy objectives 1 and 2 will be analyzed to determine if follicle development is affected when the CL is located either on the ipsilateral or contralateral ovary. Seven research stations (CT, ME, NH, NY, OH, PA, WV) will contribute to this objective. We will determine if the numbers of 2 vs. 3 wave patterns of follicular growth are affected by the local presence of the CL. Additionally, we will determine if fertility and follicle development are affected when the ovulatory follicle is on the same ovary as the CL vs. the contralateral ovary.

3b. Do increased or decreased concentrations of systemic progesterone affect follicle development and subsequent fertility?

These experiments will utilize two methods to alter luteal output of progesterone to determine the effects on concomitant follicle development and subsequent fertility. The first, twice daily SC injections of oxytocin on days 3 and 4 of the estrous cycle, have been reported (Milvae and Hansel, 1980) to inhibit luteal function and lower concentrations of plasma progesterone. In those experiments the length of the estrous cycle was not affected by oxytocin treatment. In the proposed experiments heifers will be treated with oxytocin (0.33 USP units/kg body wt) or saline on days 3 and 4 of the estrous cycle [n = minimum of 5/group; if we assume there is a 20% difference in conception rates, a total of 80 heifers (n=8 heifers/group x 2 groups x 5 stations) is then required for the study].

In the second experiment, levels of progesterone will be elevated by the insertion of a CIDR on days 8 through 17 of the estrous cycle. This time period coincides with the period of reduced progesterone levels in the oxytocin treated group.

Five research stations (CT, MA, ME, NH, OH) will contribute to this objective. In both experiments, nonlactating heifers will be scanned by ultrasound every other day beginning on day 5 of the estrous cycle. Concentrations of plasma progesterone will be determined in plasma collected at the time of ultrasonography. Animals will be bred by AI with semen from a bull of high fertility at the estrus following alterations in luteal function. Results from these experiments will determine if changes in plasma levels of progesterone alter patterns of follicle development and subsequent fertility.

EXPECTED OUTCOMES:

Information to be obtained in these studies will significantly increase our understanding of follicular development in cattle. Characterizing the patterns of follicular growth and their

correlation to the viability and developmental competence of the oocytes they contain is essential to improving fertility in cattle. These results will provide the framework from which new methods to regulate follicular development to insure that the ovulatory follicle contains a viable oocyte capable of fertilization and developing into a viable embryo.

Metabolic changes induced by the initiation of lactation and the continued production of high levels of milk adversely affect reproduction in cattle. Determining plasma levels of progesterone and estradiol-17β in lactating cattle during the post-partum breeding period will provide basic information concerning turnover of these substrates. Comparing these data with results obtained from cattle in similar stages of lactation but treated to increase insulin availability (known to increase glucose uptake) will determine the role of this "metabolic hormone" in the reproductive process. Understanding these changes could result in the development of simple, novel methods for increasing the efficiency of glucose metabolism and, thereby, fertility during the critical re-breeding phase of the lactation cycle.

Development of new management programs that improve fertility during the post-partum period will decrease the calving interval, number of services required per conception, and the labor required to detect estrus. Costs of long calving intervals (those greater than 380 days) have been estimated between $3.00 and $5.00 per day. Assuming an average loss of $4.00 per day and assuming that new methods decrease the national calving interval by 5 days, we estimate producers would save $200,000,000 annually (10,000,000 cows x 5 days x $4.00/day).

The excellent publication records of the participants in this regional project will facilitate the dissemination of this information to the scientific community. Publication of the results in peer reviewed journals and their presentation at national and international meetings on animal reproduction and management insures that the results will receive widespread review in the research community. Just as important, is the fact that all participants are associated with very active cooperative extension programs that facilitate on-farm assessment of the efficacy of new management methods and dissemination of the information to the producers. The unique combination of productive, creative researchers and a highly effective system that advocates for adoption of new technology by producers ensures that the objective of this project, improving fertility in cattle and increasing producer efficiency, will be met.

ORGANIZATION:

A regional Technical Committee will be organized in accordance with the Manual for Cooperative Regional Research (CSRS-OD-1082, 1992). The voting membership of the Regional Technical Committee shall include one representative from each cooperating Agricultural Experiment Station as appointed by the respective Director, a technical representative of each cooperating USDA-ARS research division and other participating organizations. Non-voting members shall consist of the Administrative Advisor and a consulting member representing CSREES.

All voting members of the Technical Committee are eligible for office. A chairperson, a secretary, and a third member of the committee will be elected for a 2-year term to compose

an Executive Committee. The Technical Committee will meet at least annually. The chairperson, in consultation with the administrative advisor, will notify members of the time and place of meetings. The chairperson is responsible for the preparation of the annual report of the regional project. The secretary records the minutes and other duties as assigned by the Technical Committee.

The Executive Committee may be delegated to conduct the business of the Technical Committee between meetings. Other subcommittees may be named by the chairperson as required.

ATTACHMENTS:

PROJECT LEADERS:

Connecticut -	*R.A. Milvae, Bovine Reprod. Phys., Storrs
Maine -	*J. Weber, Bovine Reprod. Phys., Orono
Massachusetts -	*R.T. Duby, Bovine Reprod. Phys., Amherst J. Robl, Bovine Reprod. Phys., Amherst
New Hampshire -	*P.C.W. Tsang, Bovine Reprod. Phys., Durham W. A. Condon, Bovine Reprod. Phys., Durham
New York -	*W. R. Butler, Bovine Reprod. Phys., Cornell-Ithaca J. E. Fortune, Bovine Reprod. Phys., Cornell-Ithaca
Ohio -	*J.L. Pate, Bovine Reprod. Phys., OARDC Columbus
Pennsylvania -	*L. C. Griel, Jr., Bovine Reprod. Phys., State College D. R. Deaver, Bovine Reprod. Phys. State College
West Virginia -	*E.K. Inskeep, Bovine Reprod. Phys., Morgantown R. A. Dailey, Bovine Reprod. Phys., Morgantown P. E. Lewis, Bovine Reprod. Phys., Morgantown
Administrative Advisor -	R. Haggett, West Virginia
CSREES Representative -	H. Glenn Gray

*Voting Member

RESOURCES:

Institution	SY	PY	TY	Objective(s)
Connecticut (CT)	0.2	0.25	0.0	1,3a,b
Maine	0.6	0.5	0.5	1,3a,b
Massachusetts	0.4	0.0	1.0	2b,3b
New Hampshire	1.2	2.0	1.9	1,2b,3a,b
New York (NYC)	0.25	0.0	0.55	1,2a,b,3a
Ohio	0.4	0.0	1.0	1,2b,3a,b
Pennsylvania	0.25	0.1	0.1	1,3a
West Virginia	1.0	2.0	1.4	1,2a,3a
	4.3	4.85	6.45	

CRITICAL REVIEW:

Work accomplished: During the period of the previous project, major progress was made in assessing patterns of ovarian activity affecting fertility in cattle. The first objective was to characterize patterns of development of the preovulatory follicle that are essential for oocyte maturation, fertilization and embryo survival. A major collaborative study was conducted at all the stations (CT, MA, NH, NY, OH, PA, WV). An experimental model was applied to lactating cows and non-lactating heifers that reliably resulted in development of persistent follicles for

Station	Theses/Dissertations	Abstracts
Connecticut	3	7
Massachusetts	2	18
New Hampshire	5	5
New York	5	23
Ohio	4	7
Pennsylvania	2	7
West Virginia	9	15

SIGNATURES:

Regional Project Title: **ASSOCIATION OF FERTILITY WITH TEMPORAL CHANGES IN OVARIAN FUNCTION OF DOMESTIC RUMINANTS**

Rosemary R. Haggett — 3/27/96

Administrative Advisor — **Date**

Yukiyo A. Yen — 7-11-96

Chair, Regional Association of Directors — Date

Doug E. Caton — 8-5-96

Administrator, — **Date**

Cooperative State Research, Education and Extension Service

REFERENCES:

Adams NR, Abordi JA, Briegel JR, Sanders MR. Effect of diet on the clearance of estradiol-17ß in the ewe. Biol Reprod 1994; 51:668-674.

Ahmad N, Beam SW, Butler WR, Deaver DR, Duby RT, Elder DR, Griel LC Jr., Fortune JE, Jones LS, Milvae RA, Pate JL, Revah I, Schreiber DT Jr., Townson DH, Tsang PCW, Inskeep EK. Relationship of fertility to pattern of follicular development and associated hormonal profiles in dairy cows and heifers. J Anim Sci; (submitted).

Ahmad N, Schrick FN, Butcher RL, Inskeep EK. Fertilization rate and embryo development in relation to persistent follicles and peripheral estradiol-17ß in beef cows. Biol Reprod 1995; 52:1129-1135.

Amsterdam A, May JV, Schomberg DW. Synergistic effect of insulin and follicle-stimulating hormone on biochemical and morphological differentiation of porcine granulosa cells in vitro. Biol Reprod 1988; 39:379-390.

Beal WE, Milvae RA, Hansel W. Oestrous cycle length and plasma progesterone concentrations following administration of prostaglandin F-2α early in the bovine oestrus cycle. J Reprod Fertil 1980; 59:393-396.

Beam SW. Follicular development in postpartum dairy cattle: Effects of energy balance and dietary lipid. Ph.D. Dissertation, Cornell University; 1995.

Benoit AM, Dailey RA. Catheterization of the caudal vena cava via the lateral saphenous vein in the ewe, cow and gilt: an alternative to utero-ovarian and medial coccygeal vein catheters. J Anim Sci 1991; 69:2971-2979.

Breuel KF, Lewis PE, Schrick FN, Lishman AW, Inskeep EK, Butcher RL. Factors affecting fertility in the postpartum cow: role of the oocyte and follicle in conception rate. Biol Reprod 1993; 48:655-661.

Britt JH. Impacts of early postpartum metabolism on follicular development and fertility. The Bovine Practitioner Proc. 1992; 24:39.

Butler WR, Calaman JJ, Beam SW. Plasma and milk urea nitrogen in relation to pregnancy rate in lactating dairy cattle. J Anim Sci 1966; (In press).

Butler WR, Everett RW, Coppock CE. The relationships between energy balance, milk production and ovulation in postpartum Holstein cows. J Anim Sci 1981; 53:742.

Butler WR, Smith RD. Interrelationships between energy balance and postpartum reproductive function in dairy cattle. J Dairy Sci 1989; 72:767-783.

Caubo B, DeVinna RS, Tonetta SA. Regulation of steroidogenesis in cultured porcine theca cells by growth factors. Endocrinology 1989; 125:321-326.

Cox NM, Stuart MJ, Althen TG, Bennett WA, Miller HW. Enhancement of ovulation rate in gilts by increasing dietary energy and administering insulin during follicular growth. J Anim Sci 1987; 64:507:516.

Cupp A, Garcia-Winder M, Zumudio A, Mariscal V, Wehrman M, Kojima N, Peters K, Bergfeld E, Hernandez P, Sanchez T, Kittock R, Kinder J. Two concentrations of progesterone (P_4) in circulation have a differential effect on pattern of ovarian follicular development in the cow. Biol Reprod 1992; 45(suppl 1):106 (abstract 222).

Driancourt MA, Fry RC. Differentiation of ovulatory follicles in sheep. J Anim Sci 1988; 66(Suppl 2):9-20.

Dufour J, Whitmore HL, Ginther OJ, Casida LE. Identification of the ovulatory follicle by its size on different days of the estrous cycle in heifers. J Anim Sci 1972; 34:85-90.

Folman Y, Rosenberg M, Herz Z, Davidson M. The relationship between plasma progesterone concentration and conception in post-partum dairy cows maintained on two levels of nutrition. J Reprod Fertil 1973; 34:267-278.

Fortune JE, Sirois J, Quirk SM. The growth and differentiation of ovarian follicles during the bovine estrous cycle. Theriogenology 1988; 29:95-110.

Gill JL, Hafs HD. Analysis of repeated measurements of animals. J. Anim Sci 1971; 33:331.

Ginther OJ, Kastelic JP, Knopf L. Composition and characteristics of follicular waves during the bovine estrous cycle. Anim Reprod Sci 1989; 20:187-200.

Guthrie HD, Lamond DR, Henricks DM, Dickey JF. Ovarian follicular changes in heifers treated with melengesterol acetate. J Reprod Fertil 1970; 22:363-364.

Harrison LM, Randel RD. Influence of insulin and energy intake on ovulation rate, luteinizing hormone and progesterone in beef heifers. J Anim Sci 1986; 63:1228-1235.

Hart IC, Bines JA, Morant SV, Ridley JL. Endocrine control of energy metabolism in the cow: Comparison of the levels of hormones (prolactin, growth hormone, insulin and thyroxine) and metabolites in the plasma of high- and low-yielding cattle at various stages of lactation. J Endocrinol 1978; 77:333-345.

Hawk HW. Infertility in dairy cattle. In: Hawk HW (ed.), Beltsville Symposia in Agriculture Research. Montclair, New Jersey: Allanheld, Osmun and Co.; 1979:19-29.

Hawkins DE, Niswender KD, Oss GM, Moeller CL, Odde KG, Sawyer HR, Niswender GD. An increase in serum lipids increases luteal lipid content and alters the disappearance rate of progesterone in cows. J Anim Sci 1995; 73:541-545.

Hernandez ER, Resnick CE, Holtzclaw WD, Payne DW, Adashi EY. Insulin as a regulator of androgen biosynthesis by cultured rat ovarian cells: cellular mechanism(s) underlying physiological and pharmacological hormonal actions. Endocrinology 1988; 122:2034-2043.

Knopf L, Kastelic JP, Schellenberger E, Ginther OJ. Ovarian follicular dynamics in heifers: test of two-wave hypothesis by ultrasonically monitoring individual follicles. Dom Anim Endocrinol 1989; 6:111-119.

Lamming GE, Mann GE. A dual role for progesterone in the control of cyclicity in ruminants. J. Reprod Fertil 1995; Suppl. 49:561-566.

Lean IJ, Galland JC, Scott JC. Relationships between fertility, peak milk yields, and lactational persistency in dairy cows. Theriogenology 1989; 31:1093-1103.

Lucy MC, Staples CR, Michel FM, Thatcher WW. Energy balance and size and number of ovarian follicles detected by ultrasonography in early postpartum dairy cows. J Dairy Sci 1991; 74:473.

Maurer RR, Echternkamp SE. Hormonal asynchrony and embryonic development. Theriogenology 1982; 17:11-22.

McEvoy TG, Robinson JJ, Aitken RP, Findlay PA, Palmer RM, Robertson IS. Dietary-induced suppression of pre-ovulatory progesterone concentrations in superovulated ewes impairs the subsequent in vivo and in vitro development of their ova. Anim Reprod Sci 1995; 39:89-107.

Meisterling EM, Dailey RA. Use of concentrations of progesterone and estradiol-17β in milk in monitoring postpartum ovarian function in dairy cows. J Dairy Sci 1987; 70:2154-2161.

Mihm M, Curran N, Hyttel P, Boland MP, Roche JF. Resumption of meiosis in cattle oocytes from preovulatory follicles with a short and long duration of dominance. J Reprod Fertil 1994; Abst Ser 13; abst 36.

Milvae RA, Hansel W. Concurrent uterine venous and ovarian arterial prostaglandin F concentrations in heifers treated with oxytocin. J Reprod Fertil 1980; 60:7-15.

Miyoshi S, Pate JL, Palmquist DL. Productive, reproductive, and metabolic responses to daily administration of propylene glycol in early lactation. J Dairy Sci 1995; 78(Suppl 1):306.

Nellor JE, Cole HH. The hormonal control of estrus and ovulation in beef heifer. J Anim Sci 1957; 15:650-661.

Parr RA, Davis IF, Miles MA, Squires TJ. Feed intake affects metabolic clearance of progesterone in sheep. Res Vet Sci 1993; 55:306-310.

Payne E, Smith JF, Cape BC, McGowan LT. Studies on the role of liver cytochrome P450 and estradiol metabolism in the effects of nutrition and Phenobarbital on ovulation rate in the ewe. Reprod Fert Dev 1991; 3:725-736.

Peluso JJ, Hirschel MD. Role of gonadotropins and insulin in controlling steroidogenesis and growth of antral bovine follicles in perifusion culture. Theriogenology 1987; 28:503-512.

Pierson RA, Ginther OJ. Follicular populations during the estrous cycle in heifers: III. Time of selection of the ovulatory follicle. Anim Reprod Sci 1988; 16:81-95.

Poretsky L, Kalin MF. The gonadotrophic function of insulin. Endocrine Reviews 1987; 8:132-141.

Prime GR, Symonds HW. Influence of plane of nutrition on portal blood flow and the metabolic clearance rate of progesterone in ovariectomized gilts. J Agric Sci 1993; 121:389-397.

Pritchard JY, Schrick FN, Inskeep EK. Relationship of pregnancy rate to peripheral concentrations of progesterone and estradiol in beef cows. Theriogenology 1994; 42:247-259.

Rajakoski E. The ovarian follicular system is sexually mature heifers with special reference to seasonal, cyclical and left-right variations. Acta Endocrinol 1960; 34(Suppl 52):7-68.

Revah I, Butler WR. Prolonged dominance of follicles reduces the viability of bovine oocytes. J Reprod Fertil 1996; (In press).

Ronge H, Blum J, Clement C, Jans F, Leuenberger H, Binder H. Somatomedin-C in dairy cows related to energy and protein supply and to milk production. Anim Prod 1988; 47:165-183.

Savio JD, Boland MP, Roche JF. Development of dominant follicles and length of ovarian cycles in postpartum dairy cows. J Reprod Fertil 1990; 88:581-591.

Savio JD, Keenan L, Boland MP, Roche JF. Pattern of growth of dominant follicles during the oestrus cycle of heifers. J Reprod Fertil 1988; 83:663-671.

Savio JD, Thatcher WW, Badinga L, de la Sota RL, Wolfenson D. Regulation of dominant follicle turnover during the estrous cycle in cows. J Reprod Fertil 1993a; 97:197-203.

Savio JD, Thatcher WW, Morris GR, Entwistle K, Drost M, Mattiacci MR. Effects of induction of low plasma progesterone concentrations with a progesterone-releasing intravaginal device on follicular turnover and fertility in cattle. J Reprod Fertil 1993b; 98:77-84.

Sirois J, Fortune JE. Lengthening the bovine estrous cycle with low levels of exogenous progesterone: a model for studying ovarian follicular dominance. Endocrinology 1990; 127:916-925.

Sirois J, Fortune JE. Ovarian follicular dynamics during the estrous cycle in heifers monitored by real-time ultrasonography. Biol Reprod 1988; 39:308-317.

Smith JF, Stewart RD. Effects of nutrition on the ovulation rate of ewes. In Oldham CM, Martin GB, Purvis IW, Reproductive Physiology of Merino Sheep, Univ of Western Australia, Perth 1990; pp 85-101.

Smith JF. Influence of nutrition on ovulation rate in the ewe. Aust J Biol Sci 1988; 41:27-36.

Smith RD, Hansel W, Coppock CE. Plasma growth hormone and insulin during early lactation in sows fed silage based diets. J Dairy Sci 1976; 59:248-258.

Spicer LJ, Echternkamp SE. Ovarian follicular growth, function and turnover in cattle: A review. J Anim Sci 1986; 62:428-451.

Steel RGD, Torrie JH. Principles and Procedures of Statistics, 2nd ed. New York, NY: McGraw Hill, Inc; 1980.

Stock AE, Fortune JE. Ovarian follicular dominance in cattle: Relationship between prolonged growth of the ovulatory follicle and endocrine parameters. Endocrinology 1993; 132:1108-1114.

Thibodeaux JK, Broussard JR, Godke RA, Hansel W. Stimulation of progesterone production in bovine luteal cells by co-incubation with bovine blastocyst-stage embryos or trophoblastic vesicles. J Reprod Fertil 1994; 101:657-662.

Thomas DL, Thomford PJ, Crickman JG, Cobb AR, Dziuk PJ. Effects of plane of nutrition and phenobarbital during the pre-mating period on reproduction in ewes fed differentially during the summer and mated in the fall. J Anim Sci 1987; 64:1144-1152.

Trimberger GW, Hansel W. Conception rate and ovarian functions following estrus control by progesterone injections in dairy cattle. J Anim Sci 1955; 14:224-232.

Ulberg LC, Christian RE, Casida LE. Ovarian response in heifers to progesterone injections. J Anim Sci 1951; 10:752-759.

Weber SL, LaBarbera AR. Insulin, somatomedin-C, human chorionic gonadotropin, and forskolin enhance glucose oxidation by granulosa cells. Biol Reprod 1988; 38:812-816.

Wehrman ME, Roberson MS, Cupp AS, Kojima FN, Stumpf TT, Werth LA, Wolfe MW, Kittock RJ, Kinder JE. Increasing exogenous progesterone during synchronization of estrus decreases endogenous 17β-estradiol and increases conception in cows. Biol Reprod 1993; 49:214-220.

Williams AH, Cumming IA. Inverse relationship between concentration of progesterone and nutrition in ewes. J Agric Sci 1982; 98: 517-522.

Zimbelman RG, Smith LW. Control of ovulation in cattle with melengesterol acetate II. Effects on follicular size and activity. J Reprod Fertil 1966; 11:193-201

Zimbelman RG. Determination of the minimal effective dose of 6 -methyl-17 -acetoxy-progesterone for control of the estrual cycle of cattle. J Anim Sci 1963; 22:1051-1058.