ANALYSIS OF EARLY LACTATION REPRODUCTIVE CHARACTERISTICS IN HOLSTEIN COWS.

by

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(ABSTRACT)

Ultrasound-guided transvaginal follicular aspiration was used to obtain oocytes from cows to study follicular development and oocyte morphology. Follicular aspiration was conducted once during wk 1 to 12 postpartum on 120 lactating cows with 6 groups, separated by biweekly intervals. Approximately one half of the aspirated cows at each session were from the early groups (wk 1-2, 3-4, or 5-6) and the other half from the later groups (wk 7-8, 9-10, or 11-12). On the day of aspiration the number of follicles on each ovary, and their sizes, small (2-5 mm), medium (6-10 mm) and large (≥ 11 mm), were recorded. The collected oocytes were morphologically classified into 4 grades, with 4 = excellent, 3 = good, 2 = fair, and 1 = poor. Blood samples from the jugular vein and follicular fluid samples from the largest follicle were collected in order to perform hormone and metabolite assays. Environmental data were obtained from the local airport. There was a significant ($P < .01$) quadratic days pre- and postpartum by parity interaction for BCS. Body condition score for older cattle was the lowest at 90 d prior to calving and changed the least amount over time, while youngest cattle had the highest initial BCS at d 90 prior to calving and had the greatest change in BCS over time. Body condition score was the highest during summer calving season ($3.3 \pm .06$) compared to BCS during winter calving season ($2.6 \pm .06$). But the loss in BCS was greater for cows that calved in summer ($-0.53 \pm .06$) compared to cows that calved in winter ($-0.07 \pm .08$). Increased serum NEFA concentrations with simultaneous decreases in serum insulin concentrations for younger cattle implied a more negative EB status than for older cattle. The total number of follicles and total number of oocytes retrieved was significantly ($P < .001$) affected by a linear days postpartum by parity interaction with younger cattle having linear increases compared to decreases in the total number of follicles for older cattle. Oocyte quality score was affected by the quadratic days postpartum by parity interaction ($P < .01$) and calving season ($P < .01$). Younger cattle had higher initial quality scores compared to older cattle, but older cattle had higher quality oocytes towards the end of the 12 wk period compared with younger cattle. Younger cattle had higher E_2 and IGF-I concentrations in follicular fluid associated with a higher number of total follicles and number of oocytes, compared to older cattle. However, oocyte quality of younger cattle seemed to be reduced and oocytes were less competent than for older cattle. Cattle in 3rd and greater lactation showed very little change in BCS and hormone and metabolite measures during early lactation, with no apparent decrease in oocyte quality, despite the aging effect on follicle numbers. This study demonstrated that conditions related to early

lactation have a negative effect on oocyte quality and endocrine measures of dairy cattle and that animals of different ages are differentially affected.
Keywords: Follicle, Oocyte, Parity, Cattle, Calving Season, Quality

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CHAPTER I

INTRODUCTION

Low fertility is costly for dairy producers because of extra expenses for semen and insemination and because of reduced income from excessive days open. Cows that fail to conceive after 4 to 5 services, normally within 60 to 160 d postpartum, are often 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 progeny testing programs.

Several traits are included in a cow's reproductive performance that is frequently measured by calving interval or components of calving interval such as days open. Calving interval is a combination of days open and length of gestation. Duration of gestation cannot be altered appreciably without affecting the survival of the neonate. Therefore, variation of calving interval results from changes of days open. Days open is a function of interval from parturition to first insemination, rates of conception at first and subsequent inseminations, and intervals between successive inseminations. Intervals between successive inseminations are dependent on the rate of estrus detection (Fonseca et al., 1983).

Bovine follicles take at least 60 to 80 d to grow from the early preantral (primary follicle) to the mature stage (Graafian Follicle) and become ready to ovulate. Therefore, if the developing follicles are exposed to adverse condition such as severe negative energy balance (EB), drastic changes in bodyweight (BW) and body condition, heat stress, or postpartum disease during the initial stages of follicular growth, subsequent oocytes may have impaired or altered gene expression that will effect their developmental competence. The impairments can result in formation of dysfunctional mature follicles, which produce poor quality oocytes (Lussier et al., 1987).

In the last few decades milk yield in dairy cows has increased significantly because of genetic selection and this increased milk production has been associated with reduced fertility. High producing cows which experience severe weight losses during the first 3 to 5 wk after calving subject growing follicles to adverse metabolic conditions associated with rapid weight loss and body condition score (BCS) reduction (Roy and Greenwald, 1991).

The negative effects of high milk yield on conception rate may be due to a delay or failure of early resumption of ovulation in the postpartum period. However, the correlation between milk yield and days to first ovulation only become significant after 40 d when most cows have already ovulated, suggesting that factors other than milk yield are involved in determining the interval to first ovulation. Not all high producing healthy dairy cows experience low fertility during the breeding period on a within-herd basis. Therefore, there must be factors other than high milk production responsible for low fertility (Butler and Smith, 1989).

The objectives of this study were to characterize the impact of parity on the changes in: 1) oocyte quality following follicular aspiration; 2) the developmental

competence of bovine oocytes after in vitro maturation and fertilization; and 3) the hormonal profiles in serum and follicular fluid during the first 12 wk postpartum.

REVIEW OF LITERATURE

Our knowledge in vitro maturation (IVM) and fertilization (IFV) of bovine oocytes has been expanding rapidly since first applied in the 1960's and 70s. Today, these techniques are employed worldwide, although only about 30% of the bovine oocytes matured and fertilized in vitro usually develop to the morula and blastocyst stages. Although proper culture conditions after initial cleavage seem essential, the process of oocyte maturation and fertilization also presents special requirements for the formation of a zygote. Oocyte quality and the use of chemically defined culture media for IVM also need to be more clearly elaborated. Apart from oocyte maturation, the success of IVF depends on the donor of the spermatozoa, the method used for selecting motile and morphologically normal spermatozoa, and the method used to enable proper sperm capacitation.

There is still a clear difference between ova obtained from in vivo maturation and oocytes matured under in vitro culture conditions (Sirad and Blondin, 1996). Differences in subsequent development may be a result of the culture conditions used or the initial intrinsic competence of the oocyte. However, studies conducted over an extended period of time indicated that a specific signal or component was missing for full development rather than inadequate or toxic culture conditions (Hytell et al., 1986). Oocyte competency is required before chromatin condensation occurs and possibly may be affected by specific mRNA accumulation. Differences in developmental competence between in vivo and in vitro cultured oocytes were expressed 4 to 5 d post fertilization (Sirad and Blondin, 1996). These findings are supported by the results of Sirad and Coenen (1994) who suggested that competent oocytes must store important factors either in the form of proteins or in the form of stable mRNA. Machatková et al. (1996) implied that there was a high probability that the developmental capacity of in vitro fertilized oocytes was related to the actual stage of the estrous at the time of oocyte collection.

Follicular Development

Ovarian antral follicular development in cattle proceeds through stages of follicular recruitment, selection and dominance. Dominant follicles control development of other follicles through the production of factors that act locally and/or systemically (Savio et al., 1988). Development of anovulatory dominant follicles was interrupted by the negative feedback of progesterone from the corpus luteum (CL) on the secretion of luteinizing hormone (LH; Thatcher et al., 1992).

In vivo, bovine oocytes undergo final maturation in the period between the LH surge and ovulation, approximately 30 h (Leibfried-Rutledge et al., 1987). Furthermore, Walker et al. (1995) reported that ovulation, detected by intrarectal ultrasonography, occurred 27.6 h after the first standing event. However, oocyte nuclear maturation (development to metaphase II) appears to be accelerated in vitro. The accelerated maturation with current culture conditions in vitro might explain why bovine oocytes matured in vivo developed to the morula and blastocyst stage at a higher frequency than embryos produced in vitro (Leibfried-Rutledge et al., 1987). Oocytes should be at metaphase II before fertilization with the first polar body evident (Staigmiller, 1988). Generally, 24 h is sufficient for in vitro maturation of bovine oocytes from antral

follicles. No developmental differences were found when bovine oocytes were matured for 18 or 24 h (Prokofiev et al., 1992), which is in contrast to results of Monaghan et al. (1993) who reported greater cleavage with 24 h versus 18 h of oocyte maturation. Gliedt et al. (1996) found no differences in developmental competence when oocytes were matured for 24 h compared to a longer period of 28 h. Heparin has been identified as a sperm capacitation factor in the bovine oviduct (Parrish et al., 1989). Cleavage rates increased significantly when heparin was added to the fertilization medium (Gliedt et al., 1996).

Onset of the first wave follicular of an estrous cycle is detected as a group of 4-mm follicles just before the day of ovulation. The peak of the follicle-stimulating hormone (FSH) surge occurs at or near the time when the future dominant follicle of the resulting follicular wave has a mean diameter of only 4 mm. The initial decline in FSH concentrations after its peak occurs when the future dominant follicle and its largest companions are approximately 6 mm. Wandji et al. (1996) showed that FSH and bovine fibroblastic growth factor (FGF) supported the survival and growth in vitro of granulosa cells of bovine preantral follicles. The selective effect of epidermal growth factor (EGF) on the growth of bovine preantral follicles is consistent with previous studies (Wandji et al., 1992). Epidermal growth factor-like binding activity does not appear in granulosa and theca cells until the end of the secondary preantral stage and then increases during the antral stage.

During the next few days, one of the follicles becomes dominant. A second follicular wave emerges at about 10 d postovulation and, if present, a third follicular wave starts at 16 d. The ovulatory follicle originates from the final wave (Ginther et al., 1989a; Lucy et al., 1992; Ginther et al., 1989b; Roche et al., 1991). Some of the factors that seem to affect these three waves each estrous cycle include dietary intake, parity and lactational status (Ginther et al., 1989a). Moreover the diameter attained by the dominant follicle is affected by stage of the estrous cycle and pregnancy (Lucy et al., 1992). Development of a dominant follicle is closely associated with the regression of subordinate follicles, and new growth of small follicles occurs only once the large dominant follicle has ceased growing (Savio et al., 1988). Smith et al. (1996) demonstrated that developmental competence of oocytes was not significantly affected by the presence or absence of a dominant follicle. Therefore, they suggested that the developmental capacity of oocytes from small follicles was not compromised by the endocrine milieu of the follicle. The emergence of a large dominant follicle in all cows during the second week postpartum demonstrated that development of pre-ovulatory-size dominant follicles is not the limiting factor in the reproductive recrudescence in lactating cows.

Early Postpartum Energy Balance

The general relationship between dietary energy intake and energy utilization is defined as EB and is described by the following equation: daily EB = NE_I (consumed) - NE_I (required). The NE_I requirement includes both maintenance and production components. Computation of EB reflects the metabolic status of the cow more accurately than does simply measuring milk yield. During early lactation the deficiency in dietary energy intake relative to the energy utilized for milk production results in negative EB, and this condition may persist for several weeks. Dairy cows in negative EB lose BW when body reserves are mobilized as energy resources to support lactation. Negative

energy balance usually reaches its maximum during the wk 1 and 2 of lactation and recovers at a variable rate (Butler and Smith, 1989). During negative EB in early lactation, the rapid increases in utilization of glucose for milk lactose production results in lower plasma concentrations of both glucose and insulin as compared with that of later stages of lactation. The relative lack of insulin would enhance lipolysis in adipose tissues, thereby further increasing appetite via increased availability of free fatty acids for hypothalamic oxidation. The increased feed consumption from these effects and other additive stimuli in early lactation would likely be associated with increased release of β-endorphin within the hypothalamo-hypophyseal unit. Elevated β-endorphin would be expected to inhibit the gonadotrophin-releasing hormone (GnRH) pulse system. At the same time the GnRH system and, hence, LH patterns may be depressed directly by low insulin and secondarily by the increased production and utilization of ketones as a consequence of mobilization of body adipose reserves (Hart et al., 1978).

A biological or physiological explanation for the relationship between fat percentage and EB is related to mobilization of fat reserves. Cows in negative EB mobilize more body fat reserves and produce more glycerol for energy resources, which leads to increased non-esterified fatty acids (NEFA) concentrations in blood. These NEFA are taken up by the liver and can be oxidized for additional energy supply or esterified into triglycerides, which can lead to either ketosis or fatty liver disorder. However, a fraction of the triglycerides are transformed to very low density lipoproteins, which can be taken up by the mammary gland. Because deposition of very low density lipoproteins is maximum in early lactation a higher fat percentage in early lactation can be caused by decreased milk yield because of a lack of glucose during maximum milk fat production. Hence, changes in milk fat percentage can indicate subclinical ketosis during early lactation in dairy cattle (Bauman and Currie, 1990).

Butler and Smith (1989) concluded that the negative effects of high milk yield on conception rate were due to a delay or failure of early resumption of ovulation in the postpartum period, thereby allowing fewer ovulatory cycles before insemination and thus resulting in lower fertility. However, they reported that the correlation between milk yield and days to first ovulation, only became significant after the period (40 d) when most cows had already ovulated, and suggested that factors other than milk yield alone are involved in determining the interval to first ovulation. Previous studies (Fonseca et al., 1983; Staples et al., 1990) reported that not all high producing healthy cows experience low fertility during the breeding period. Fonseca et al. (1983) found that the association between level of milk production and fertility was not very strong on a within-herd basis.

Yield of milk and consumption of dietary energy have been implicated as causes of low fertility in lactating dairy cows. Cows experienced a negative EB even when the calories offered were not limiting, thus EB and variation in EB were spontaneous (Villa Godoy et al., 1988). The amount of feed offered is not predictive of the amount of the feed ingested and is not predictive of EB. To examine the definitive relationships between reproductive events and energy status, the sources of variation for energy status should be EB or feed intake and not the amount of feed offered. If feed offered to cows is adequate, indigestion of calories, not yield of milk, is the major determinant of EB in postpartum cows (Villa Godoy et al., 1988). Cows with normal ovarian activity increased their feed intake more rapidly than cows with abnormal ovarian activity

(Staples et al., 1990). A slow increase in dry matter intake postpartum is commonly observed for cows that have low milk production and low reproductive rates. Cows fed less then 100% of their NRC requirements took more days to form a follicle of at least 10 mm in diameter than cows fed 100% and more. An increased loss of BW during the first weeks postpartum coincided with decreased ovarian activity until the 8^{th} wk in lactation, providing evidence that cows do not have to be in positive EB to ovulate, but cows in a more positive EB were the first cows to show ovarian activity. The impact of severe negative EB in early lactation can potentially influence ovarian activity later in the lactation. Cows that had experienced a negative EB during the first 3 wk postpartum failed to cylce until after 9 wk postpartum compared with cows that had a positive EB during early postpartum (Staples et al., 1990).

Postpartum reproductive function in dairy cattle seems directly dependent on the availability of nutrient energy relative it its utilization for lactation (Butler and Smith., 1989). Data collected by Stevenson and Britt (1979) indicated that high levels of milk production and associated negative EB delayed initiation of postpartum ovarian activity in dairy cattle. In dairy cows, negative EB is directly related to the postpartum interval to first ovulation. The recovery or improvement in EB from a severe negative state, at the onset of lactation, toward a positive state may provide an important signal for initiation of ovarian activity (Butler and Smith, 1989). However, ovulation did occur when cows were still in a negative EB, but were approaching zero EB.

In dairy cattle, luteal function is associated with three events that determine fertility. First is the detection of estrus, next is the rate of conception, and finally, embryonic survival rate affects CL function. Villa Godoy et al. (1988) determined the relationship between EB and luteal function in dairy cows. Overall, their findings showed that daily EB had high variability among cows as well as among days within cows. They found a correlation between luteal function and milk progesterone (P_4), while during the first estrous cycle postpartum EB did not have an effect on milk P_4. While cows in negative EB had CL in their second and third cycles postpartum that had a normal life span, the CL had reduced function due to reduced luteal development, decreased secretory activity per luteal cell or a combination of these factors. Thus, negative EB may not have an immediate influence on the CL function of cows. Factors that mediate adverse effects of negative EB appear to occur after or persist longer than the nadir of EB. Early postpartum occurrences of nadir or severe negative EB at nadir are components of EB that potentially limit luteal function in dairy cows.

Cows in severe negative EB in the first weeks of lactation secrete less P_4 into their milk during their second and third cycles than cows in zero and slightly negative EB (Villa-Godoy et al., 1988). However, no correlation was found between the EB and the amount of P_4 secreted into milk during the first estrous cycle postpartum. Anestrous cow ate less feed, produced less milk, and lost more BW, resulting in a more negative energy status than cycling cows. Also, anestrous cows and cows showing CL activity between d 40 and 60 obtained more energy from body reserves for milk production in the first weeks of lactation than cows cycling prior to d 40 (Staples et al., 1990).

Body Condition Score

Changes in body condition (BC) during early lactation follow changes in EB (Villa-Godoy et al., 1988). The energy requirement of a dairy cow is met through a combination of dietary intake and mobilization of body reserves. The mobilization of

body reserves leads to a decrease in BW and BC. Therefore, BC scoring, although subjective, can be a useful tool for relating suboptimal reproductive performance to inadequate nutrition in early lactation (Butler and Smith, 1989; De Vries et al., 2000). Both pre- and postpartum restriction of dietary energy decreased BW, BCS, and longissimus muscle area after parturition (Perry et al., 1991). There was an interaction between pre- and postpartum level of energy based on changes in subcutaneous fat after parturition. Cows receiving lower levels of energy pre-partum had longer intervals from parturition to first ovulation, agreeing with studies done by (Wiltbank et al., 1962). Cows that received high energy prepartum diets followed by low energy diets postpartum ovulated earlier with a substantial lost in BC. Cows on low energy diets pre-partum and high energy postpartum diets failed to ovulate until they had regained an adequate amount of BC, which occurred much later in their lactation (Perry et al., 1991). This study agreed with Kiracofe (1980) who found that uterine and cervical involution were unaffected by dietary energy levels before and after parturition. Also, uterine involution was not associated with duration of postpartum anestrous. According to Fonesca et al. (1983), Villa-Godoy et al. (1988), and Spicer et al. (1990) high producing cows experience a severe weight loss during the first 3 to 5 wk after calving. The adverse effect of this weight loss on developing follicles can be detrimental and lead to defective follicles and low levels of P_4 that are associated with low fertility (Butler and Smith, 1989). But, cows that consume sufficient dry matter during this period apparently have healthy follicles and maintain high fertility (Staples et al., 1990). A decrease in BCS of more than 1.0 point during the first 5 wk after calving leads to lower fertility at first service (Britt, 1992; Butler and Smith, 1989).

Body condition and BW increased linearly with prepartum high energy diets. Moreover, over consumption of energy prepartum did not impair milk production when high energy total mixed rations were fed postpartum (Boisclair et al., 1986). However, over conditioning of the dairy cows during the late prepartum period lead to an increased number of days from parturition to conception due to a higher incidence of embryonic mortality (Flipot et al., 1988).

According to Domecq et al. (1997b) the loss of BC in multiparous cows contributed to the failure of conception at first AI service. There was a positive correlation between higher total milk yield at 120 d of lactation and conception at first AI service. Energy balance during the dry period and early lactation, as monitored by BCS scores, was more important to conception at first AI service than were health disorders or other risk factors. Cows that are at risk for failure to conceive at first AI can be identified by monitoring BCS at dry-off, parturition and during early lactation.

Body condition score at calving appears to have little influence on milk yield. However, changes in BCS, which are related to BCS at calving, have influenced milk yield (Domecq et al., 1997a). The rate of increase in milk yield in early lactation was important to total yield and may more accurately reflect the dynamic biological changes experienced by the cow. The rate of increase in milk yield may be associated with BCS or changes in BCS. An increase in BCS during the dry period was related to an increase in milk yield and milk yield acceleration, and a higher BCS at dry-off was associated with a decrease in milk yield and milk yield acceleration than a low BCS. A dry period length that is <58 d or >58 d was negatively associated with milk yield and milk yield acceleration after parturition (Domecq et al., 1997b).

The initiation of a follicular wave, including recruitment and dominant follicle selection, occurs regardless of the typical early postpartum negative EB (Beam and Butler 1997). Cows that were on a high energy diet had an increase in the number of follicles larger than 15 mm. Their results showed that although the mean daily EB did not appear to have a significant role in follicular function, the day of EB nadir was positively correlated with the day of first ovulation. The improvement of EB from its most negative value also enhanced follicular competence, since first wave dominant follicles that emerged after the EB nadir exhibited greater apparent steroidogenic output and ovulation rate than dominant follicles that emerged before the EB nadir. Furthermore, dominant follicle diameter and plasma estradiol levels were increased after the EB nadir in cows that experienced multiple waves of follicular development prior to first ovulation. The day of the EB nadir appeared to be a more important factor in ovarian activity than the degree of negative EB.

Cows experiencing multiple ovulation's CL were the cows that had a higher average EB than cows that had a single ovulation or no ovulation. Cows on a low-energy diet seem to have a higher concentration of NEFA and a lower concentration of insulin-like growth factor (IGF-I) than cows on a high energy diet. Plasma concentrations of NEFA are good indicators of adipose tissue mobilization for energy, and the increase of NEFA in cows on a low energy diet reflect the dietary induction of a negative EB. The daily growth rate and total growth of the dominant follicle were affected by the fluctuation in energy intake, both the rate and total growth were higher for cows on a high energy diet compared to cows on a low energy diet. A decrease in energy levels caused a reduction in the IGF-I concentration, possibly the reason for a decrease in growth rate of the developing follicle. Lactational status seems to have an influence on ovarian follicular responses. Dry cows (non-pregnant) tended to have smaller dominant follicles during the first follicular wave, and a lower frequency of larger follicles (>15 mm) through out their estrous cycles (Thatcher et al., 1992).

Lucy et al. (1991) have shown a decrease in the number of smaller follicles and increase in the number of larger follicle as days postpartum increased. This pattern is consistent with the concept of follicular recruitment and selection leading to terminal follicular growth and dominance (Hafez et al., 1987). Smaller follicles (3 to 5 mm) that grow and move into larger classes of follicles (10 to ≥15 mm) with increasing time postpartum are not replenished during the first 25 d postpartum. The number of medium size follicles (6 to 9 mm) stayed unchanged, which probably represented a transitory class with follicles moving into and out of this diameter range. Energy balance seems to modify these population changes and affects the average number of follicles per cow for the first 25 d postpartum. As positive EB increases, average number of small follicles decreases, and the average number of larger follicles increases. This theoretically leads to an earlier ovulation in cows with a more positive EB. A higher ovulation rate was associated with a greater postpartum positive EB to 25 d postpartum and follicles did not respond to changes in positive EB thereafter. Once a cow starts having regular estrous cycles, positive EB does not influence her follicular dynamics. However, the theory is only partially correct because, Lucy et al. (1990) found that cycling cows placed in deficit energy during final follicle maturation had slowed follicle growth. Lucy et al. (1991) concluded that positive EB influences follicular growth early in the postpartum period. The effects of positive EB seem to influence day to first ovulation by influencing growth

of follicles from small into larger sizes. After estrous cycles have been initiated, positive EB does not influence follicular growth or numbers of follicles within different size classes. Pre-and postpartum energy levels have a significant influence on the size and the number of follicles. When low nutritional levels are combined with poor body condition, the growth of small follicles to larger follicles is suppressed, with a substantial decrease in the number of follicles present. Perry et al. (1991) also found that cows that were adequately fed and in a good body condition had an increase in the number of small follicles growing into larger follicles. There is about a 40 d period between the time that a follicle begins to form an antrum and when it reaches ovulatory size (Lussier et al., 1987). Britt (1992) suggested that it must take at least 60 to 80 d for bovine follicles to grow from the early preantral stage (primary and secondary follicle) to mature into a Graafian follicle. Therefore, the exposure of follicles to adverse conditions such as negative EB, heat stress, and or postpartum disease during initial stage of growth and development might influence gene expression that could result in altered or impaired development. Dysfunctional mature follicles can have a negative influence on the quality of oocytes and CL.

The presence of a CL significantly reduced the recovery rate and quality of the oocytes collected (Das et al., 1996). The differences between P_4 concentrations measured in the blood and the detection of CL through rectal palpation or ultrasonography might be because even though a CL is morphologically present, it might not produce a significant amounts of P_4 (Gutiérrez et al., 1996). The functional status of CL changes during the estrous cycle. At the beginning of the cycle the CL forms gradually and the production of P_4 increases progressively. Therefore, in some cases a CL might be physically present before the peripheral concentrations of P_4 reach the value of 1 ng/ml that is generally used as the criteria for an active CL (Hernández-Cerón et al., 1993). At the end of the estrous cycle there is a rapid decline in P_4 concentrations, which is not accompanied by a significant decline in CL diameter. Therefore, the CL is physically present, but not functionally (Gutiérrez et al., 1996).

The concentration of progesterone in the latter days of the luteal phase prior to insemination seems to be more important than the number of ovarian cycles completed in determining fertility (Meisterling and Dailey, 1987).

Energy Balance, Metabolites and Endocrine Measures

Another link between reproduction and nutrition involves the responsiveness of the ovary to metabolic hormones and growth factors. Cows in negative EB early postpartum have increased growth hormone (GH) and decreased concentrations of IGF-I, which when insufficient, impair ovarian function. Plasma concentrations of NEFA and β-hydroxybutyrate can be indicators of the overall energy status of dairy cows (Nebel and McGilliard., 1993).

An increase in EB is associated with an increase in concentrations of IGF-I in serum during early lactation. This increase in concentration of IGF-I is associated with increased P_4 secretion during diestrus of the first and second postpartum estrous cycles. Increased milk yield is associated with decreased serum IGF-I concentrations and EB. Thus, reduced ovarian activity that accompanies negative EB may be due to a decrease in concentration of IGF-I in serum (Spicer et al., 1990). These findings supported Villa-Godoy et al. (1988) who found that reduced ovarian activity during early lactation might be linked to a negative EB. Specifically, they observed that milk P_4 concentrations during diestrus of the second and third estrous cycles were positively correlated with EB.

The P_4 concentrations in serum during diestrous of the first and second estrous cycles were significantly greater in the positive EB than negative EB cows (Spicer et al., 1990). In comparison Villa-Godoy et al. (1988) reported that P_4 secretion during diestrous was increased in positive EB cows during the second and third estrous cycles postpartum. Yung et al. (1996) confirmed that negative EB decreased serum IGF-I and weight and P_4 content of the CL. However, the decreased serum IGF-I was not responsible for the decreased luteal development associated with negative EB in heifers. Collectively, these results suggest that negative EB had an adverse effect on luteal function.

Ronge et al. (1988) have shown that the concentration of IGF-I in serum increased with the time postpartum in lactating dairy cattle with maximal concentrations observed by 12 wk of lactation. In addition to decreases in IGF-I in serum, negative EB was also associated with reduced blood glucose and high NEFA and ketone bodies, suggesting that insufficient gluconeogenesis, ketogenesis and enhanced fat mobilization occurred during negative EB. Spicer et al. (1990) revealed an inverse relationship between milk production and IGF-I secretion. Similarly, Ronge et al. (1988) observed a negative correlation (r = -.5 to -.7) between milk production and IGF-I secretion. Overall, Spicer et al. (1990) have shown that increased concentrations of IGF-I in serum occur in conjunction with positive EB during early lactation in dairy cows, and both are associated with increased luteal function during the first and second postpartum estrous cycles.

Insulin-like Growth Factor-I

Insulin-like growth factor-I has been postulated as one of several intra-ovarian regulators of follicular growth and differentiation (Adashi et al., 1985; Reed and James, 1989). In vitro studies have established that ovarian granulosa cells can secrete IGF-I and that IGF-I is stimulatory to granulosa cell mitosis and steroidogenesis. Insulin-like growth factors (IGF-I and IGF-II) possess a high degree of amino acid sequence of homology with insulin, and similar tertiary structures. Insulin and the IGFs bind to cell-surface receptors, and their actions are receptor-mediated. The insulin and IGF-I receptors are structurally very similar, both consisting of tetrameric receptor made up to two alpha and two beta chains linked by disulfide bonds. The IGF-II receptors have a very different structure, with a relatively long extracellular domain and a short cytoplasmic domain. Insulin binds with high affinity to the insulin receptor, and with lower affinity to the IGF-I receptor, while IGF-I binds with high affinity to its own receptor, and with lower affinity to insulin receptor. The IGF-II receptor binds IGF-II with high affinity, IGF-I with low affinity, and does not bind insulin (Kiess et al., 1988).

A further intricacy in elucidating the roles of the insulin family of peptides is the association of IGF with binding proteins. At least six insulin-like growth factors binding proteins (IGFBP) have been described, and the three well characterized forms, (IGFBP-1, -2 and -3) have binding affinities for the ligand that are as high, or higher than the receptor (Heyner et al., 1993).

Under normal circumstance circulating IGF-I concentrations will increase in response to an increase in GH concentrations (Breier et al., 1988). However, during feed deprivation the concentration of GH and NEFA will increase, but will have no effect on the IGF concentrations (Lapierre et al., 1995), because the response mechanism of IGF-I to GH are completely abolished during the time of feed deprivation (Breier et al., 1988). The increased circulating GH during feed deprivation resulted from decreased clearance rather than an increased secretion (Lapierre et al., 1995). However, IGF-I concentrations were lower when cows were in a negative EB during early lactation than in later lactation, when cows were in

positive EB (Vicini et al., 1991). During the dry period, insulin concentrations were similar to early lactation concentrations, whereas concentrations of GH were similar to those occurring in late lactation. The IGFBP-I concentration usually appears to be inversely correlated with insulin concentration in serum. However, the availability of glucose to peripheral tissues in relation to insulin concentration seems to be a determinant of IGFBP-I concentration (Lapierre et al., 1995). Most circulating IGF seems to bind to the IGFBP-3 that appears to be the major binding protein in circulation. IGFBP-3 concentration is usually affected by factors that also regulate IGF-I production: age, nutritional status, and GH. Basal concentration of IGFBP-2 was the highest during the early lactation when GH was high, and lowest during the dry period when GH was lowest. Conversely, the concentration of IGF-I was high during the dry period. Ronge et al. (1988) reported an elevation in serum IGF-I during the dry period, with a subsequent decline around parturition, followed by an increase after parturition. They did not observe any changes in the serum IGF-II concentration throughout the dry period or early lactation.

Furthermore, serum IGFBP concentration seemed to follow similar profiles for serum IGF-I concentrations. The changes in serum IGF-I concentrations during the periparturient period are likely the result of at least two processes: 1) decrease and increase in metabolic demand due to the cessation and onset of lactation, respectively, and 2) changes in transport of IGF-I by the mammary gland. Serum IGF-I was positively correlated with energy and protein balances in dairy cows when such balances were positive due to low milk yield (Ronge et al., 1988). McGuire et al. (1992) concluded that the concentrations of IGF-I and IGFBP-2 are more sensitive to nutritional status than IGFBP-3.

Insulin inhibits the rate of adipose tissue lipolysis and stimulates the rate of lipogenesis, the net effect is to direct NEFA to adipose tissue and retain it there (Rose et al., 1996). An elevated GH concentration due to low energy intake has an antagonistic effect on the action of insulin, possibly by acting at an intracellular site. This ability is part of the proposed mechanism by which GH is thought to increase the milk yield of lactating cows by altering the response of tissues to homeostatic signals so that nutrients are directed towards the mammary gland and away from body tissue deposition (Breier et al., 1991).

Beam and Butler (1997) reported that there was a greater insulin to GH ratio for cows that ovulated during the first week postpartum compared to the cows that had not ovulated during the first week postpartum, suggesting that levels of insulin and GH during the very early stages of follicular recruitment may be important to later follicular cell function. Insulin is known as one of the effectors of follicle function in several species including cattle, with in vitro effects on granulosa cell steroidogenesis, mitosis, and morphological differentiation (Spicer et al., 1993).

Hammond et al. (1988) provided evidence that IGF-I concentrations in follicular fluid increase with follicular size and that the concentrations of IGF-I in follicular fluid were positively correlated to P_4 concentrations across individual follicles.

Adashi et al. (1985) and Hammond et al. (1988) suggested that IGF's are intra-ovarian or paracrine regulators of follicular growth and differentiation. A paracrine role for IGF's in ovarian function was further supported by the observations that granulosa cells can secrete immunoreactive IGF's and/or IGF-II in vitro and that ovarian tissues contain mRNA for IGF's (Murphy et al., 1987; Hammond et al., 1988).

There is a positive correlation ($r = .69$) between the concentration IGFs in follicular fluid and serum. Therefore IGF-I derived from serum can be considered as a possible source of intraovarian source of IGFs. Echterncamp et al. (1990) also showed that small follicles contained significantly lower IGF-I concentrations than large follicles,

indicating local control of IGF-I levels. These results reflect local biosynthesis, alterations in diffusion barriers between blood and follicular fluid, or changes in IGF-I binding proteins. Insulin-like growth factor I of ovarian origin plays a role in the regulation of folliculogenesis and is a mediator of genetic components of multiple ovulations in cattle. Adashi et al. (1985) provide evidence that IGF-I stimulates the proliferation of follicular cells and enhanced gonadotrophinstimulated steroidogenesis in both follicular and luteal cells. Although the liver is the proposed major source for IGF-I measured in blood, mRNA for IGF-I has been identified in ovarian tissue from cattle and other mammalian species (Spicer et al., 1993; Izadyar et al., 1997b).

The primary determinants of blood IGF-I concentrations are nutrition and body condition (Vicini et al., 1991). Well-fed cows and heifers have greater IGF-I concentrations, while under-fed cows had reduced IGF-I and smaller CL (Yung et al., 1996). However the relative contribution of absorption vs. intrafollicular secretion of IGF-I to concentrations of IGF-I in bovine follicular fluid is unknown (Echternkamp et al., 1990). Binding activities of IGFBP-2 and other IGFBPs were high in follicular pools from small or medium follicles and in follicular fluid of individual large atretic follicles, but were markedly reduced in the follicular fluid of large pre-ovulatory dominant follicles (Echternkamp et al., 1994). Transcription of IGFBP-2 mRNA was identified in both the granulosa and theca layers of antral follicles. Follicular fluid profiles of IGFBPs varied with stages of ovarian follicular development. This suggested that possibly IGFBPs play a possibly regulatory role in the selection of the dominant or ovulatory follicle(s) by modulating the biological activity of the IGFs within the follicle. Both IGFBPs and IGF-I/II are produced by the same follicular cell types and therefore IGFBPs may regulate the action of IGF-I in an autocrine/paracrine fashion at the cellular level as opposed to endocrine regulation. This hypothesis is consistent with the findings of Echternkamp et al. (1990) in that there was a negative correlation between the IGF concentrations in follicular fluid and the binding activity of IGFBPs and a positive correlation between IGF concentrations and E_2 concentration in follicular fluid. These findings were supported by Spicer et al. (1993) that showed that the IGF-I concentrations were higher in follicular fluid of large estrogen-active follicles than in the follicular fluid from small to medium estrogen-inactive follicles.

The expression of IGF-I mRNA by the bovine granulosa cells may serve as evidence for a combination of intra-and extra-follicular contributions observed when GH was administered in cycling cattle causing an increase IGF-I and a decrease in IGFBPs concentrations in both serum and follicular fluid (Stanko et al., 1994). However, according to Spicer et al. (1993), the possibility of direct stimulation of intraovarian IGF-I production by GH cannot be excluded. Echternkamp et al. (1994) concluded that the reduction in binding activity of the IGFBPs in estrogen-active preovulatory follicles was consistent with the proposed autocrine/paracrine regulatory role of IGFBPs on the biological activity of IGF-I and/or IGF-II and their effects on mitosis and steriodogenesis in ovarian follicular cells. Thus, fluctuations in endogenous FSH release during the bovine estrous cycle may modulate follicular development and atresia by attenuating the release of IGFBPs, which in turn then modulate endogenous IGF on its cognate cell surface receptors and normal hormonal action.

Additionally, IGF-I levels were approximately 40% higher during the first 2 wk pospartum for cows that ovulated compared to cows that did not ovulate (Beam and

Butler, 1997). Also, plasma IGF-I was higher in the cows that ovulated at the first day postpartum, before the establishment of follicular dominance and subsequent increases in peripheral E_2. Therefore, the higher IGF-I did not result from greater dominant follicle E_2 production, but proceeded and possibly contributed to differences in follicular function. Insulin-like growth factor-I is similar to insulin, a well known effector of follicle cell function in vitro, with stimulation of both theca (Spicer et al., 1996) and granulosa cells (Spicer et al., 1993). Both E_2 and FSH increase IGF-I receptors in small (1 to 5 mm) bovine follicles, and numbers of IGF-I receptors are 15-fold greater in large (>8 mm) follicles compared to small follicles (Spicer et al., 1994).

Stewart et al. (1996) found increased theca LH-binding sites and low amounts of IGFBp2 in follicular fluid related to establishment of the dominant follicle during the first follicular wave of the bovine estrous cycle.

Gonadotropins are the primary regulators of nuclear maturation in oocytes, although the somatic control of mammalian oocyte maturation involves steroid signals that regulate the synthesis of proteins specifically associated with this event (Osborn and Moore, 1983). Lorenzo et al. (1997) used denuded oocytes to study the possible effect of cumulus cells on IGF-I production and their influence on oocyte maturation and steroid production in culture media and found that denuded oocytes were unable to secrete steroids under the culture conditions. Culture media supplementation with growth factors appeared to contribute to the maturational requirements of the oocytes of various species. Singh et al. (1995) demonstrated that some growth factors can be synthesized by the ovary. While Hammond et al. (1988), Echternkamp et al. (1990), and Adashi et al. (1988) demonstrated the presence of IGF-I receptors in granulosa cells, Yoshimura et al. (1996) observed the specific binding of IGF-I to the ovarian membrane. Hainaut et al. (1991) postulated that oocyte maturation with IGF-I was iniated upon activation of the membrane receptor for this IGF-I and required tyrosine dephosphorylation of p34, the kinase component of maturation promoting factor (MPF). Lorenzo et al. (1997) provided evidence that IGF-I supplementation had a significant effect on E_2 and androstenedione (A) production by cumulus-oocyte-complexes during oocyte maturation in culture. Funston et al. (1995) suggested these steroids could play an important role in oocyte maturation, since in bovine pre-ovulatory follicular fluid levels of both steroids, E_2 and A, increase, while testosterone (T) levels decrease as ovulation approaches. The necessity for a decrease in testosterone levels was supported of Racowsky (1983) who found that high levels of testosterone were incompatible with oocyte maturation because of shunting the cAMP into the aromatase pathway. This shunting reduced the availability of the nucleotide within the cumulus cells for transfer to the oocyte to maintain meiotic arrest. The actual concentrations and mutual relationships of these and other steroids during the pre-ovulatory and peri-ovulatory period are believed to be of biological importance for normal oocyte maturation. Some studies have shown that perturbation of steroid equilibrium in this critical period results in inferior oocyte maturation (Andersen, 1993).

Steroid binding was present in granulosa cells (GC) and thecal membranes of follicles of all sizes, but P_4 plasma concentration binding to GC membranes decreased significantly with increasing follicle size, perhaps indicating developmental regulation of GC membrane non-genomic P_4 in the preovulatory bovine follicle (Rae et al., 1998). They suggested that these membrane steroid receptors might be involved in the autocrine/paracrine regulation of follicular function by P_4. The E_2:P_4 status of the cumulus cell may regulate

the androgenic modulation of cAMP-dependent meiotic arrest (Racowsky, 1983). Andersen (1993) had shown that viable oocytes were only the ones exposed to an environment with high E_2 and/or a high E_2:T ratio. Lorenzo et al. (1997) showed that when IGF-I was present in the culture media, E_2:T ratios were higher and therefore increased the capability of oocytes to mature and to be fertilized. They reported that IGF-I is a stimulator of nuclear maturation of oocytes, although these stimulatory effects are only possible in oocytes surrounded by cumulus cells. Their results shown that under the influence of IGF-I, cumulus cell-surrounded oocytes may produce significant amounts of either E_2 or A during the maturation period. In contrast Izadyar et al. (1997b) concluded that the stimulatory effect of GH on bovine oocyte maturation is a direct effect which is cumulus-dependent and not mediate via IGF-I.

Regulation of Ovarian Function

Regulation of follicular growth and ovulation as well as steriod production by the ovary depends principally on gonadotropins. However, non-steroid systemic hormones and autocrine and paracrine factors contribute to the regulation of ovarian function. Systemic hormones include as prolactin (PRL), GH, IGF-I and insulin. The ovary also contains many locally-produced peptides such as IGF-I and IGF-II and their binding proteins, IGFBPs, transforming growth factor α (TGF-α), TGF-β, inhibin, activin, follistatin, oxytocin, and ubiquitin which appear to be part of complex intra ovarian paracrine and autocrine regulatory system (Borromeo et al., 1996).

Borromeo et al. (1996) provided evidence for the presence of immunoreative bGH and bPRL in follicular fluid, found a positive correlation between bGH and P_4 concentration in follicular fluid, examined variability of introvarian bPRL hormones, and found a close relationship between P_4, E_2, and T patterns in follicular fluid. Steriod hormone content in follicular fluid reflects the synthetic capacity of the granulosa cells and theca layers. Luteinized cells have no androgen-producing capacity, whereas the granulosa cells under FSH stimulation, aromatize androgens of theca origin to estrogen. With increased luteinization there was a significant increase in P_4 concentration and decreases in E_2 and T concentrations. There was an increase in IGF-I concentration in cystic follicular fluid as luteinization proceeded. Immunoreactive GH receptors are present in bovine ovaries especially on large luteal cells and P_4 production increased significantly with an increase in GH. Bovine oocytes, cumulus cells, and mural granulosa cells express mRNA of the GH receptor (Izadyar et al., 1997b). It is known that PRL plays an important role in the control of ovarian folliculogenesis, luteogenesis, hormone production and oocyte maturation (Sirotkin et al., 1994). Synthesis of PRL and related substances was demonstrated not only in the pituitary, but also in other tissues like lymphocytes, the uterus, placenta, ovary, and the epididymis. In contrast, Ohwaki et al. (1992) did not detect PRL gene expression in human CL or luteal cells. However, they found a significant correlation between the levels of PRL in the plasma and in the follicular fluid, and that gonadotrophin stimulation of the ovary may alter the PRL permeability from plasma into follicular fluid.

The addition of bovine GH to culture media accelerates in vitro maturation of cumulus-enclosed bovine oocytes, induces cumulus expansion, and promotes early embryonic development in terms of enhancement of the number of blastomeres (Izadyar et al., 1996). The stimulatory effect of GH on oocyte maturation is exerted via GH receptors located in the cumulus cells and not mediated by IGF-I (Idazadyar et al.,

1997b). Maturation of denuded oocytes in the presence of GH caused a transient retardation in the process of germinal vesicle breakdown (GVBD) (Izadyar et al., 1997a). The effect of GH on the in vitro maturation of bovine oocytes is mediated by the cAMP signal transduction pathway, probably via a G-protein-coupled mechanism. The stimulatory effect of FSH on cumulus expansion is also known to be mediated by the cAMP signal transduction pathway (Dekel et al., 1983). Although both GH and FSH use cAMP as second messenger, the effect on nuclear maturation exerted by the hormones was different. Growth hormone accelerated nuclear maturation (Izadyar et al., 1997a), while FSH retarded nuclear maturation of mammalian oocytes (Armstrong et al., 1991). Despite these opposite effects on nuclear maturation, both FSH and GH, when added during in vitro maturation, promote subsequent fertilization and embryo development (Izadyar et al., 1996).

Luteinizing Hormone and 17β-Estradiol

Reestablishment of a normal LH pulse pattern is the key factor responsible for ovarian follicular development and the initiation of postpartum ovarian activity (Butler and Smith, 1989). In most cattle pituitary secretion of LH increases during the first wk 2 to 3 wk after parturition (Edgerton et al., 1973). Luteinizing hormone acting on the ovarian follicles induces waves of follicular growth, which leads to the selection and ovulation of a dominant follicle between 15 and 25 d after calving (Stevenson and Britt, 1979; Edgerton et al., 1973). Negative EB primarily appears to interfere with the ability to the hypothalamo-hypophyseal axis to develop the pulsatile LH pattern necessary for ovarian follicular development and ovulation (Lucy et al., 1990), which contributes to on-farm inefficiency through economic losses associated with postpartum anestrus. Possible modulators of these events are opiate peptides acting at the level of the hypothalamus to decrease the release of GnRH, and therefore, LH (Butler and Smith, 1989). Secondarily, the energy deficit and low insulin concentrations during this period may limit the responsiveness of the ovary to gonadotropin stimulation. Lucy et al. (1991) reported that glucose remained low during the early postpartum period (<30 d) and increased thereafter. These changes were not influenced by positive EB, supporting the general concept that glucose concentration in postpartum cattle is low and not influenced by changes in overall energy status. Alternatively plasma insulin was influenced by positive EB, with higher EB being associated with greater plasma insulin.

The interval to first ovulation in the postpartum period depends upon recovery of the normal functions of the pituitary-ovarian axis and the genital tract. Subsequent fertility is conveyed from the onset of first ovulation and completion of multiple cycles before insemination (Butler and Smith, 1989). Timing and magnitude of negative EB apparently interact to determine the extent to which negative EB alters hypothalamic secretion of GnRH, as well as its effect on gonadotropin secretion and, therefore, ovarian secretion of P_4 (Nebel and McGilliard., 1993).

Estradiol produced by ovarian follicles, is the primary hormone stimulating estrous behavior in cattle (Hafez et al., 1987). Reduced IGF-I secretion caused by negative EB could alter ovarian follicular E_2 production, thereby suppressing expression of estrus (Spicer et al., 1990). IGF-I has a stimulatory effect on the granulosa cell E_2 production (Adashi et al., 1985). Oocyte maturation in vivo is induced by pre-ovalutory LH surge, but the maturation process must be mediated through granulosa cells, since oocytes lack receptors for LH (Eppig, 1991).

The E_2 and LH levels in bovine follicular fluid change with follicular development in vivo, and the follicular fluid in developing follicles stimulates oocyte maturation. Meiotic resumption inhibitor and stimulator are present in bovine follicular fluid, and their levels change according to the developmental stage of the follicles (Kim et al., 1996). Adding increased concentrations of follicular fluid to the in vitro maturation medium decreased maturation and subsequent development. These findings indicate that some factors in the follicular fluid function either positively or negatively in the resumption of oocyte maturation according to hormonal background. It appears that coagulation of the cumulus cell mass by fibrin-like substance in the follicular fluid occurs when follicular fluid was at a high concentration in the maturation medium. Therefore, increasing the concentration of follicular fluid added to the maturation medium increases inhibitory factors such as adenosine and hypoxanthine. These findings are supported by the results of Sirard (1990) who found that when oocyte complexes are cultured in whole follicular fluid from small follicles (<5 mm), oocyte maturation decreased and nuclear maturation was inhibited. Kim et al. (1996) concluded that the cumulus cell-surrounded oocyte was stimulated in vitro in the same manner as it was by the LH surge-like system in follicular fluid in vivo and by hormones such as E_2 and LH. Therefore, any hormones or proteins that can counteract the follicular inhibitory action and induce meiosis resumption are synthesized in the cumulus oocyte complex.

As follicular size increased, follicular fluid lipoprotein, phospholipid, cholesterol and protein increased (Brantmeier et al., 1987). Bovine follicular fluid contains only high density lipoproteins (HDL) and concentrations increase with follicular maturation. Progesterone production by bovine granulosa cells in culture is stimulated by low and high density lipoproteins (Savion et al., 1982). The increase in high density lipoprotein could be a potentially important source of cholesterol for P_4 production after the LH surge (Brantmeier et al., 1987).

In Vitro Development of Oocytes

Cumulus cells are metabolically coupled to oocytes via heterologous gap junctions, and they play a role as a modulator for the effect of hormones and growth factors during in vitro maturation of bovine oocytes (Izadyar et al., 1997b). The thickness of the cumulus layers varies in accordance to follicular size. Cumulus integrity will vary with the health status of follicles, but not necessarily in a synchronous fashion (Leibfried and First, 1979). The thicker the cumulus mass surrounding the oocytes, the better the chances for development. A reduced number of cumulus layers might be the consequence of mechanically aspirating oocytes from early antral follicles or the loss of external layers caused by early atresia (Lonergan et al., 1992).

When oocytes are aspirated from follicles, their ability to synthesize proteins is not affected, but they lose the capacity to make RNA in less than 2 h unless meiotic arrest is artificially maintained (Sirard and Coenen, 1994). The follicular cells do not die immediately after the animal is slaughtered. There was a high survival rate of follicular cells in cell culture (Hytell et al., 1989), suggesting that the cells might be in the process of dying and acting in a similar fashion as post-LH granulosa cells by breaking gap junctions, by secreting vasoactive products to help CL formation, and/or by releasing stress signals.

Environmental hormonal composition is critical for acquisition of developmental capacity of the maturing oocytes. Cumulus expansion failed to occur when cumulus-

oocyte complexes were cultured in medium without gonadotropins. It is of great importance that the in vitro conditions are compatible with those in vivo to ensure optimal functioning of the cumulus-oocyte complex that prevails in vivo. Prolactin may be a prerequisite for successful fertilization and subsequent embryonic development of oocytes matured in vitro, suggesting a physiological role for transient hyperprolactinemia in the acquisition of developmental competence of oocytes. Prolactin, as well as estrogen, appears to be an important constituent in the process of oocyte maturation, promoting embryonic development. The pre-ovulatory environment within the follicle may influence cytoplasmic maturation of the oocyte, thereby affecting its potential for full and normal development (Yoshimura et al., 1989).

Bovine oocytes failed to mature or had a low maturation rate when cultured in the absence of cumulus cells (Kobayashi et al., 1994). The frequency of fertilization of denuded oocytes was significantly less than after maturation of cumulus cell-enclosed oocytes (Ball et al., 1983). Cumulus cells tightly adhered to oocytes become dispersed in response to the endogenous surge of gonadotrophins, growth factors, steroids factors secreted by the oocyte and other unknown molecules (Kobayashi et al., 1994; Lorenzo et al., 1994). These compounds could be contributing to maturational changes that occur in the oocyte, mediated by intracellular messengers such as cAMP, calmodulin or diacylglycerol (Lorenzo et al., 1994). Kobayashi et al. (1994) found that the maturation of bovine oocyte significantly improved in the chemical defined medium, TCM199, supplemented with LH and FSH or either EGF or TGF-α. A significant increase in the number of fertilized ova that developed to the blastocyst stage was also observed. However, there was no additive stimulation on cumulus expansion and oocyte fertilizability when EGF, TGF-α and gonadotrophins were added to TCM-199. Lorenzo et al. (1994) determined the relationship between growth factors and the regulation of nuclear and cumulus expansion. They found that a combination of the growth factors, EGF and IGF-I, significantly enhanced cumulus expansion, while IGF-I alone did not promote cumulus expansion. Growth factors only enhanced maturation in cumulus-oocyte complexes, but not denuded oocytes supporting the results Kobayashi et al. (1994). The growth factors in follicular fluid are a key factor in the regulation of intra-follicular bovine oocyte maturation. The role of IGF-I may be subtle in cytoplasmic maturation, providing its positive effects only in fertilization or early events related to embryogenesis (Lorenzo et al., 1994).

Blondin et al. (1996) suggested that low percentages of bovine embryos produced in vitro might not necessarily be due to the culture media and methods used but rather that immature oocytes might be developmentally incompetent even before they are used in an in vitro system. Incompetence might be due to incomplete nuclear or/and cytoplasmic maturation. Oocytes from follicles 3 mm or less did not develop past the 16-cell stage (Blondin and Sirad 1995). Culturing oocytes individually had a significant negative effect on the rate of development of embryo production compared with group culturing. Small follicles contained mostly oocytes with fully expanded or no cumulus layers and a very heterogeneous ooplasm. Medium size follicles contained oocytes mainly with compacted layers of cumulus with a homogenous to slightly granulated ooplasm, while the oocytes from large follicles had cumulus in which the outer layers were slightly expanded and the ooplasm slightly granulated (Blondin et al., 1996). Blondin and Sirad (1995) found a significant reduction in embryo development, with no

embryo developing to the 32-cell stage, with individual oocyte maturation, fertilization and culture. In contrast, Hazelberger et al. (1994) reported between 40 to 50% blastocysts yields when very good quality oocytes were matured, fertilized, and cultured individually.

Kane et al. (1992) reported that in vivo embryos develop in minute volumes of media and suggested that embryos would develop better in vitro in equally small volumes. The surface/volume ratio with the overlaid oil could be a major factor affecting the outcome of in vitro fertilization. However, Carolan et al. (1996) provided evidence of embryo development when oocytes were matured, fertilized and cultured in small droplets (>5 μl) of media, while there was an increase in development as the size of the droplets increased (5 to 20 μl). Ferry et al. (1994) suggested that the reduced efficiency associated with small droplets could be due to an increase in the surface/volume ratio, which increases the flow of the exchange, between the medium, the oil, and the atmosphere and the acceleration of transfer of toxic components from oil into culture medium. Another factor affecting embryo development in small droplets could be the limitation of energy substrates available to the oocytes in the droplet. Gardner et al. (1993) determined that the nutrient uptake of individual embryos and calculated that 20 μl of synthetic oviduct fluid (SOF) medium was the minimum volume of medium that could supply sufficient energy substrates to an embryo over a 6-d period. Carolan et al. (1996) showed clearly that with small modifications to the standard protocol for IVM/IVF/IVC procedures, with the main focus on the oocyte/embryo densities in the medium, bovine oocytes could develop to blastocyst stage when matured, fertilized and cultured individually.

Cystic Ovaries

Borromeo et al. (1996) described bovine ovarian cysts as structures having a diameter greater 2.5 cm in the absence of a CL on either ovary. A luteal cyst is a CL regularly formed after ovulation in which a central cavity persists for a few days and slowly becomes obliterated.

Savio et al. (1990) reported that the first dominant follicle after parturition in most dairy cows was either ovulated or developed into a cyst. Beam and Butler (1997) indicated that in addition to developing an ovulatory or cystic follicle, 40% of cows might experience multiple waves of non-ovulatory dominant follicle growth prior to first ovulation. The fate of the first-wave dominant follicle had a significant impact on the postpartum anovulatory interval that influenced the reproductive performance of lactating cows (Staples et al., 1990). These observations suggests that ovulation failure and subsequent atresia of the first postpartum dominant follicle may negatively impact the reproductive performance of lactating dairy cows to the same extent as does the early development of follicular cyst (Beam and Butler, 1997). The peripheral plasma E_2 concentrations were higher for cows that ovulated the first dominant follicle after parturition compared to those cows that did not ovulate and the first dominant follicle had turned cystic. This indicated that steroidogenesis during early follicle selection and dominance reflected later ovulatory competence. Also, the greater E_2 production by the ovulatory dominant follicles d 8 to 14 postpartum compared to the non-ovulatory dominant follicles occurred despite no differences in follicle diameter and suggests that early phases of dominant follicle growth are unrelated to ultimate estrogenic capability.

Persistent Follicles

Revah and Butler (1996) confirmed that oocytes recovered from follicles maintained under prolonged dominance undergo premature nuclear maturation in vivo. This would be expected to result in lower fertility after insemination. Stock and Fortune (1993) showed that infertility or low fertility cannot be attributed to luteal insufficiency, since CL that arise from the persistent follicles produced normal concentrations of P_4. Low P_4 concentration due to prolonged follicular dominance caused an increase in LH pulse frequency, while the LH surge was suppressed (Revah and Butler, 1996). Amad et al. (1995) reported that embryos obtained from cows that ovulated persistent follicles were comprised and could not reach the 16-cell stage.

Temperature

Pollard et al. (1996) have demonstrated that exposing ovaries and cumulus-oocyte-complexes to ambient temperature below 35°C during oocyte recovery may adversely affect subsequent embryonic development in vitro. The deleterious consequences of cooling oocytes below 35°C on subsequent embryonic development may result from the alteration of ooplasm during cooling, since transcripts and translational products within the ooplasm regulate early embryonic development in cattle. The loss of regulatory elements within the ooplasm by exposure of the ovary or cumulus-oocyte-complex to sub-physiological temperatures would ultimately limit the capacity of embryos produced from such oocytes to develop to the blastocyst stage. Alternatively, chromosomal degradation could induce similar reductions in development. Bovine ovaries, gametes and embryos need to be maintained at temperatures within the physiological range of 37°C to 39°C to maximize both quantity and quality of embryos produced by in vitro methods.

LITERATURE CITED

Adashi, E.Y., C.E. Resnick, A.J. D'Ercole, M.E. Svoboda, and J.J. van Wyk. 1985. Insulin-like growth factors as intraovarian regulators of granulosa cell growth and function. Endocr. Rev. 6:400-420.

Adashi, E.Y., C.E. Resnick, E.R. Hernández, M.E. Svoboda, and J.J. van Wyk. 1988. Characterization and regulation of a specific cell membrane receptor for somatomedin C/insulin-like growth factor-I in cultured rat granulosa cells. Endocrinology 122:194-201.

Ahmad, N., F.N. Schrick, R.L. Butcher, and E.K. Inskeep. 1995. Effect of persistent follicles on early embryonic losses in beef cows. Biol Reprod. 52:1129-1135.

Andersen, C.Y. 1993. Characteristics of human follicular fluid associated with successful conception after in vitro fertilization. J. Clin. Endocrinol. Metab. 77:1227-1234.

Araki, C.T., R.M. Nakamura, L.W.G. Kam, and N. Clarke. 1983. Effect of lactation on diurnal temperature patterns of dairy cattle in hot environments. J. Dairy Sci. 67:1752-1760.

Armstrong, D.T., X. Zhang, B.C. Vanderhyden, and F. Khamsi.1991. Hormonal actions during oocyte maturation influence fertilization and early embryonic development. Ann. NY Acad. Sci. 626:137-158.

Ball, G.D., M.L. Leibfried, R.W. Lenz, R.L. Ax, B.D. Bavister, and N.L. First. 1983. Factors affecting successful in vitro fertilization of bovine follicular oocytes. Biol Reprod. 28:717-725.

Bauman, D. E., and W. B. Currie. 1980. Partitioning of nutrient during pregnancy and lactation:a review of mechanics involving homeostasis and homeorhesis. J. Dairy Sci. 63:1514-1529.

Beam, S.W., and W.R. Butler. 1997. Energy balance and ovarian follicle development prior to the first ovulation postpartum in dairy cows receiving three levels of dietary fat. Biol Reprod. 56:133-142.

Blondin, P., and M.A. Sirad. 1995. Oocyte and follicular morphology as determining characteristics for development competence in bovine oocytes. Mol Reprod. Dev. 41:54-62.

Blondin, P., K. Coenen, L.A. Guibault, and M.A. Sirad. 1996. Superovulation can reduce the developmental competence of bovine embryos. Theriogenology 46:1191-1203.

Boisclair, Y., D.G. Grieve, J.B. Stone, O.B. Allen, and G.K. MacLeod. 1986. Effect of prepartum energy, body condition, and sodium bicarbonate on production of cows in early lactation. J. Dairy Sci. 69:2636-47.

Borromeo, V. S. Bramani, A. Berrini, G. Sironi, M. Finazzi, F. Cremonesi, and C. Secchi. 1996. Growth hormone but not prolactin concentrations in the fluid of bovine ovarian cysts are related to the cystic stage of luteinization. Theriogenology 46:481-489.

Brantmeier, S.A., R.R. Grummer, and R.L. Ax. 1987. Concentrations of high density lipoproteins vary among follicular sizes in the bovine. J. Dairy Sci. 70:2145-2149.

Breier, B.H., P.D. Gluckman, S. N. McCutcheon, and S.R. Davis. 1991. Physiological responses to somatotropin in the ruminant. J. Dairy Sci. 74 (Suppl. 2):20. (Abstr.)

Britt J.H. 1992. Impacts of early postpartum metabolism on follicular development and Fertility. Bov Proc. 24:39-43.

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

Carolan, C., P. Lonergan, H. Khatir, and P. Mermillod. 1996. In vitro production of bovine embryos using individual oocytes. Mol Reprod, Dev. 45:145-50.

Das, G. K., G.C. Jain, V.S. Sloanki, and V.N. Tripathi. 1996. Efficacy of various collection methods for oocyte retrieval in Buffalo. Theriogenology 46:1403-1411.

Dekel, N., and I. Sherizly. Induction of maturation in rat follicle-enclosed oocyte by forskolin. FEBS Lett 151:153-155.

De Vries, M.J., and R.F. Veerkamp. 2000. Energy Balance of Dairy Cattle in Relation to Milk Production. J. Dairy Sci. 83:62-69.

Domecq, J.J., A.L. Skidmore, J.W. Lloyd, and J.B. Kaneene. 1997a. Relationship between body condition score and milk yield in a large diary herd of high yielding Holstein cows. J. Anim. Sci. 80:101-112.

Domecq, J.J., A.L. Skidmore, J.W. Lloyd, and J.B. Kaneene. 1997b. Relationship between body condition score and conception at first artificial insemination in a large diary herd of high yielding Holstein cows. J. Anim. Sci. 80:113-120.

Echternkamp, S.E., L.J. Spicer, K.E. Gregory, S. F. Canning, and J.M. Hammond. 1990. Concentrations of insulin-like growth factor-I in blood and ovarian fluid of cattle selected for twins. Biol. Reprod. 43:8-14.

Echternkamp, S.E., H.J.Howard, A.J. Howard, A.J. Roberts, J. Grizzle, and T.Wise. 1994. Relationships among concentrations of steriods, insulin-like growth factor-I and insulin-like growth factor binding proteins in ovarian follicular fluid of beef cattle. Biol. Reprod. 51:971-981.

Edgerton, L.A., and H.D. Hafs. 1973. Serum luteinizing hormone, prolactin, glucocorticoid and progestin in dairy cows from calving to gestation. J.Diary Sci. 56:451-459.

Eppig, J.J. 1991. Intercommunication between mammalian oocytes and companion somatic cells. BioEssays 13:569-574.

Ferry, L., P. Mermillod, A. Massip, and F. Dessy. 1994. Bovine embryos cultered in serum-poor oviduct-conditioned medium need cooperation to reach the blastocyst stage. Theriogenology 42:445-453.

Flipot, P.M., G.L. Roy, and J.J. Dufour. 1988. Effect of peripartum energy concentration on production performance of Holstein cows. J. Dairy Sci. 71:1840-50.

Fonesca , F.A., J.H. Britt., B.T. McDaniel, J.C. Wilk, and A.H. Rakes. 1983. Reproductive traits of Holsteins and Jerseys. Effects of age, milk yield and clinical abnormalities on involution of cervix and uterus, ovulation, estrous cycles, detection of estrus, conception rate and days open. J. Dairy Sci. 66:1128-1147.

Funston, R.N., G.E. Seidel, and A.J. Roberts. 1995. IGF-I and IGF-I binding proteins (IGFBPs) in bovine serum and folliculur fluid before and after the preovulatory surge of LH. Biol. Reprod. 52(Suppl 1):152. (Abstr.)

Gardner, D.K., M. Lane, and P.A. Batt. 1993. Uptake and metabolism of pyruvate and glucose by individual sheep pre-attachment embryos developed in vivo. Mol. Reprod. Dev. 36:313-319.

Ginther, O.J., J.P. Kastelic, and L. Knopf. 1989a. Composition and characteristics of follicular waves during the bovine estrous cycle. Anim. Reprod. Sci. 20:187-200.

Ginther, O.J. L. Knoff, and J.P. Kastelic. 1989b. Temporal associations among ovarian events in cattle during oestrous cycles with two and three follicular waves. J. Reprod. Fertil. 87:223-230.

Gliedt, D.W., C.F. Rosenkrans, JR., R.W. Rorie, and J.M. Rakes. 1996. Effects of oocyte maturation length, sperm capacitation time and heparin on bovine embryo development. J,Dairy Sci. 79:532.535.

Gutiérrez, A.C., L.Zarco, C.S. Galina, I.Rubio, and H. Basurto. 1996. Predictive value of palpation per rectum for detection of th eCL in Zebu cattle as evaluated by progesterone concentrations and ultrasonography. Theriogenology 46:471-479.

Hart, I.C., J.A Bines, S. V. Morant, and J. L. Ridley. 1978. 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. Endocrinology. 77:333-345.

Hafez, E.S.E., 1987. Folliculogenesis, Egg Maturation and Ovualtion, In: Reproduction in Farm Animals. 5th ed. Lea & Febiger, Philadelphia, PA. p 130.

Hainaut, P., S. Giorgetti, A. Kowlaski, R. Ballotti, and E.van Obberghen. 1991. Anitbodies to phosphotyrosine injected into *Xenopus laevis* oocytes modulate maturation induced by insulin/IGF-I. Exp. Cell Res. 195:129-136.

Hammond, J.M., C.J. Hsu, J.M. Klindt, L. Ainsworth, B.R. Downey, and B.K. Tsang. 1988. Gonadotropins increase concentrations of immunoreactive insulin-like growth factor I in porcine follicular fluid in vivo. Biol. Reprod. 38:304-308.

Hazelberger, N.L., D.J. Hill, R.B. Stubbings, and J.S. Watson. 1995. Relationship of morphology and follicular fluid environment of bovine oocytes to their developmental potential in vitro. Theriogenology 43:509-522.

Hernández-Cerón, J., L. Zarco, and V. Lima-Tamayo. 1993. Incidence of delayed ovulation in Holstein heifers and its effects on fertility and early luteal function. Theriogenology 40:1073-1081.

Heyner, S., N. A. Shah, R.M. Smith, A.J. Watson, and G.A. Schultz. 1993. The role of growth factors in embryo production. Theriogenology 39:151-161.

Hytell, P., K.P. Xu, S. Smith, and T. Greve. 1986. Ultrastructure of in-vitro oocyte maturation in cattle. J. Reprod. Fert. 78:615-625.

Hytell, P., T. Greve, and H. Callensen. 1989. Ultrastructure of oocyte and fertilization in superovulated cattle. Developments in Ultrastructure of Reproduction. 296:287-297.

Izadyar, F., B. Colenbrander, and M.M. Bevers. 1996 in vitro maturation of bovine oocytes is in the presence of growth hormone accelerates nuclear maturation

and promotes subsequent embryonic development. Mol Reprod. Dev. 45:372-377.

Izadyar, F., B. Colenbrander, and M.M. Bevers. 1997a. Stimulatory effect if growth hormone on in vitro maturation of bovine oocytes is exerted through the cyclic adenosine 3'5'-monophosphate signaling pathway. Biol Reprod. 57:1484-1489.

Izadyar, F., H.T.A. van Tol, B. Colenvander, and M.M. Bevers. 1997b. Stimulatory effect of growth hormone on in vitro maturation of bovine oocytes is exerted through cumulus cells and not mediated by IGF-I. Mol Reprod Dev. 47:175-180.

Kane, M.T., E.W. Carney, and J.E.Ellington. 1992. The role of nutrients, peptide growth factors and co-culture cells in development of pre-implantation embryos in vitro. Theriogenology 38:297-313.

Kiess, W, G.D. Blickenstaf, M.M. Sklar, C.L. Thomas, S.P. Nissley, and G.C. Sahagian. 1988. Biochemical evidence that the type II insulin-like growth facotr rceptor is identical to the cation-independent mannose 6-phosphate receptor. J. Biol. Chem. 263:9339-9344

Kim, K.S., N. Mitsumizo, K. Fujita, and K. Utsumi. 1996. The effects of follicular fluid on in vitro maturation, oocyte fertilization and the development of bovine embryos. Theriogenology 45:787-799.

Kiracofe, G. H. 1980. Uterine involution: Its role in regulating post partum intervals. J. Anim. Sci. 51(Suppl. 2):16. (Abstr.)

Kobayashi, K., S. Yamashita, and H. Hoshi. 1994. Influence of epidermal growth factor and transformiong growth factor-α on in -vitro maturation of cumulus cell-enclosed bovine oocytes in a defined medium. J. Reprod. Fertil. 100:439-446.

Lapierre, H., G.Pelletier, T. Abribat, P. Gaudreau, P. Brazeau, and D. Peticlerc. 1995. The effect of feed intake and growth hormone-releasing factor on lactating cows. J. Dairy Sci. 804-815.

Leibfried, M.L., and N.L.First. 1979. Characterization of bovine follicular oocytes and their ability to mature in vitro. J. Anim. Sci. 48:76-85.

Leibfried-Rutledge, M.L., E.S. Critser, W.H. Eyestone, D.L. Northey, and N.L. First. 1987. Developmental potential of bovine oocytes matured in vitro or in vivo. Biol. Reprod. 36:376-380.

Lonergan, P. 1992. Studies on the in vitro maturation, fertilization and culture of bovine follicular oocyte. Ph.D Thesis, National University of Ireland, Dublin.

Lorenzo, P.L., M.J. Illera, J.C. Illera, and M. Illera. 1994. Enhancement of cumulas expansion and nuclear maturation during bovine oocyte maturation in vitro by the addition of epidermal growth factor and insulin-like growth factor I. J. Reprod. Fertil. 101:697-701.

Lorenzo, P.L., J.C. Illera, G.Silvan, C.J. Munro, M.J. Illera, and M Illera. 1997. Steroid-level response to insulin-like growth factor-I in oocytes matured in vitro. J.Reprod Immunol.35:11-29.

Lucy, M.C., J. Beck, M. Drost, and W.W. Thatcher. 1990. Plasma metabolites . growth factors, and IGF-I binding proteins during altered follicular growth in duced by shifting energy balance in lactating Holstein cows. J. Dairy Sci. 73 (Suppl.1):178. (Abstr)

Lucy, M.C., C.R. Staples, F.M. Michael, and W.W. Thatcher. 1991. Energy balance and Size and number of ovarian follicles detected by ultrasonography in early postpartum cows. J. Dairy Sci. 74:473-482.

Lucy, M.C. J.D. Savio, L. Badinga, R.L. de la Sota, and W.W. Thatcher. 1992. Factors that effect ovarian follicular dynamics in cattle. J. Anim. Sci. 70:3615-3626.

Lussier, J.G., P. Matton, and J.J. Dufour. 1987. Growth rates of follicles in the ovary of the cow. J. Reprod, Fertil.81:301-305.

Machatková, M., E. Jokešová, J. Petelíkova, and V. Dvoráçek. 1996. Developmental competence of bovine embryos derived form oocytes collected at various stages of the estrous cycle. Theriogenology 45:501-510.

McGuire, M.A., J.L. Vicini, D.E. Bauman, and J.J. Veenhuizen. 1992. Insulin-like growth factors and binding proteins in ruminnats and their nutritional regulation. J.Anim. Sci. 70:2901-2910.

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

Monaghan, P., C. Carolan, P.Lonergan, H. Sherif, H. Wahid, and I. Gordon. 1993. The effect of maturation time on the subsequent in-vitro development of bovine oocytes. Theriogenology 39:270 (Abstr).

Murphy, L.J., G.I. Bell, and H.G. Friesan. 1987. Tissue distrubition of insulin-like growth factor I and II messenger ribonucleic acid in the adult rat. Endocrinology 120:1279-1282.

Nebel, R.L., and M.L. McGilliard. 1993. Interactions of high milk yield and reproductive performance in dairy cows. J. Dairy Sci. 76:3257-3268.

Ohwaki, M., N. Suganuma, H. Seo, A. Nawa, F. Kikkawa, O. Narita, N. Matsui, and Y. Tomoda. 1992. Source of prolactin in human follicular fluid. Endocrin. Jpn. 39:601-607.

Osborn, J.C., and R.M. Moor. 1983. The role of steroid signals in the maturation of mammalian oocytes. J. Steroid Biochem. 19:133-137.

Parrish, J. J., J.L.Susko-Parrish, and N.L. First. 1989. Capacitation of bovine sperm by heparin: inhibitory effect of glucose and role of intracellular pH. Biol. Reprod. 41:683-688.

Pedron, O.F. Cheli, E. Senatore, D, Baroli, and R. Rizzi. 1993. Effect of body condition score at calving on performance, some blood parameters, and milk fatty acid composition in diary cows. J. Dairy Sci. 76:2528-2535.

Perry, R.C., L.R. Corah, R.C. Cochran, W.E. Beal, J.S. Stevenson, J.E.Minton, D.D. Simms, and J.R. Brethour. 1991. Influence of dietary energy on follicular development, serum gonadotropins, and first postpartum ovulation in suckled beef cows. J. Anim. Sci 69:3762-3773.

Pollard, J. W., A. Martino, N.D. Rumph, N. Songasen, C. Plante, and S.P. Leibo. 1996. Effect ambient temperatures during oocyte recovery on in vitro production of bovine embryos. Theriogenology 46:849-858.

Prokofiev, M.I., L.K. Ernest, N.M. Suraeva, I.S. Lagutina, N.N. Udavlennikova, A.Z. Kesyan, and A.I Dolgohatskiy. 1992. Bovine oocyte maturation, fertilization and further development in vitro and after transfer into recipients. Theriogenology 38:461-469.

Racowsky, C., 1983. Androgenic modulation on cyclic adenosine monophosphate (cAMP)-dependent meiotic arrest. Biol Reprod. 28:774-787.

Rae, M.T., G.S. Menzies, and T.A. Bramley. 1998. Bovine ovarian non-genomic progesterone binding sites: presence in follicular and luteal cell membranes. J. Endocrinol. 159:413-27.

Reed, M., and V.H.T. James. 1989. Regulation of steroid synthesis and metabolism by growth factors. Clin. Endocrin. 31:511-525.

Revah, I., and W.R. Butler. 1996. Prolonged dominance of follicles and reduced viability of bovine oocytes. J. Reprod. Fertil. 106:39-47.

Roche, J.F., and M.P. Boland. 1996. Turnover of dominant follicles in cattle of different reproductive states. Theriogenology 35:81-91.

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

Rose, M.T., Y. Obara, H. Fuse, F. Itoh, A. Ozawa, Y. Takahashi, K. Hodate, and S. Ohashi. 1996. Effect of growth hormone -releasing factor on the response to insulin of cows during early and late lactation. J. Dairy Sci. 79:1734-1745.

Roy, S.K., and G.S.Greenwald. 1991. In vitro effects of epidermal growth factor, insulin-like growth factor-I, fibroblast growth factor, and follicle-stimulating hormone on hamster follicular deoxyribonucleiec acid synthesis and steriodogenesis. Biol Reprod. 44:889-896

SAS® Institute, INC. 1999. SAS User's Guide: Statistics. Cary, NC, USA. Edition 6.12.

Savio, J.D., L. Keenan, M.P. Boland, N. Hynes, and J.F. Roche. 1988. Pattern of growth of dominant follicles during the oestrous cycle of heifers. J. Reprod. Fertil. 83:663-671.

Savio, J.D., M.P. Boland, N. Hanes, and J.F. Roche. 1990. Resumption of follicular activity in the early postpartum period of dairy cows. J. Reprod. Fertil. 88:569-579.

Savion, N., R. Laherty, D. Cohen, D. Lui, and D. Gospodarowicz. 1982. Role lipoproteins and 3-hydroxy-3-methylglutaryl coenzyme A reductase in progesterone production by cultured bovine granulosa cells. Endocrinology 110:13-22.

Sirard, M.A. 1990. Temporay inhibtion of meiosis resumption in vitro by adenylate cyclase stimulation in immature bovine oocyte. Theriogenology 33:457-767.

Sirard, M.A., and K. Coenen. 1994. The effect of hormones during in vitro meiotic inhibition with cyclohexamide on subsequent development of bovine oocytes. Biol Reprod. 50:136-141.

Sirard, M.A., and P. Blondin. 1996. Oocyte maturation and IVF in cattle. Anim. Reprod. Sci. 42:417-426.

Sirotkin, A.V., J. Nitray, A. Skokanova. V.P. Politou, J. Jurovicova, P. Marinchenko. 1994. Release of a prolactin-like substance by bovine granulosa cells in vitro: effects of ovarian steriods. Ann. Endocrinol. 55:279-282.

Smith, L.C., M. Olivera-Angel, N.P. Groome, B. Bhatia, and C.A. Price. 1996. Oocyte quality in small antral follicles in the presence or absence of a large dominant follicle in cattle. J. Reprod. Fertil. 106:193-199.

Spicer, L.J., W.B. Tucker, and G.D. Adams. 1990. Insulin-like growth factor-I in diary cows: Relationship among energy balance , body condition, ovarian activity, and estrous behavior. J. Dairy Sci. 73:929-37.

Spicer, L.J., E. Alpizar, S.E. Echternkamp. 1993. Effects of insulin, insulin-like growth factor-I, and gonadotropins on granulosa cell proliferation, progesterone production , estradiol production, and(or) insulin-like growth factor-I production in vitro. J. Anim. Sci. 71:1232-1241.

Spicer, L.J. E. Alpizar, and R.K. Vernon. 1994. Insulin-like growth factor-I receptors in ovarian granulosa cells:effect of follicle size and hormones. Mol. Cell. Endocrinol. 102:69-76.

Spicer. L.J., and R.E. Stewart. 1996. Interactions among basic fibroblast growth factor, epidermal growth factor, insulin, and insulin-like growth factor-I (IGF-I)on cell numbers and steroidogenesis of bovine thecal cells: role of IGF-I receptors. Biol Reprod. 54:255-263.

Staigmiller, R.B. 1988. In vitro methods for production of viable oocytes. J. Anim. Sci. 66 (suppl. 2) 54-60.

Staples, C.R., W.W. Thatcher, and J. H. Clark. 1990. Relationship between ovarian activity and energy status during the early postpartum period of high producing dairy cows. J. Dairy Sci. 73:938-47.

Stanko, R.L., W.S. Cohick, D.W.Shaw, R.W. Harvey, D.R.Clemmons, M.D.Whitacre, and J.D. Armstrong. 1994. Effect of somatotropin and/or Equine chorionic gonadotropin on serum and follicular insulin-like growth factor -I and insulin-like growth factor bindings proteins in cattle. Biol. Reprod. 50:290-300.

Stewart, R.E., L.J. Spicer, T.D.Hamilton, B.E.Keefer, L.J.Dawson G.L. Morgan, and S.E. Ecthernkamp. 1996. Levels of insulin-like growth-factor (IGF) binding proteins, luteinizing hormone and IGF-I receptors, and steroids in dominant follicles during the first follicular wave in cattle exhibiting regular estrous cycles. Endocrinology 137:2842-2850.

Stock, A.E., and J.E. Fortune. 1993. Ovarian follicular dominance in cattle: relationship between prolonged growth of the ovulatory follicle and endocrine parameters. Endocrinology132:1108-1114.

Stevenson, J.S., and J.H. Britt. 1979. Relationship among luteinizing hormone , estradiol, progesterone, glucocorticoids, milk yield, body weight and postpartum ovarian activity in Holstein cows. J. Anim. Sci. 48:570-577.

Thatcher , W.W. , J. Savio, C.R. Staples, M.C. Lucy, and R.L. de La Sota. Nutritional and hormonal factors influencing follicle development in cattle. 1992. Bov. Proc. 24:44-50.

Vicini, J.L., F.C. Buonomo, J.J. Veenhuizen, M.A. Miller, D.R. Clemmons, and R.J. Collier. 1991. Nutrient balance and stage of lactation affect responses of insulin, insulin-like growth factors I and II, and insulin-like growth factor-binding protein 2 to somatropin administration in dairy cows.J. Nutr. 121:1656-1664.

Villa-Goday, A., T.L. Huges, R.S. Emery, L.T. Chapin, and R.L. Fogwell. 1988. Association between energy balance and luteal function in lactating dairy cows. J. Dairy Sci. 73:2759-2765.

Walker, W.L., R.L. Nebel, and M.L. McGilliard. 1995. Characterization of estrus activity as monitored by an electronic pressure sensing system for detection of estrus. J. Dairy Sci. 78 (Suppl):307. (Abstr.).

Wandji, S.A., G. Pettlier, and M.A. Sirard. 1992. Ontogeny and cellular localization of ^{125}I-labeled basic fibroblast growth factor and ^{125}I-labeled epidermal growth factor binding sites in ovaries from bovine fetuses and neonatal calves. Biol. Reprod. 47:807-813.

Wandji, S.A., J.J.Eppig, and J.E. Fortune. 1996. FSH and Growth factors affect the growth and endocrine function in vitro of granulosa cells of bovine preantral follicles. Theriogenology 45:817-832.

Wiltbank, J.N., W.W. Rowden, J.E. Ingalls, K.E. Gregory , and R.M. Koch. 1962. Effect of energy on reproductive phenomena of mature Hereford cows. J. Anim. Sci. 21:219-225.

Yoshimura, Y., Y. Hosoi, A. Iritani, Y. Nakamura, S.J. Atlas, and E.E. Wallach. 1989. Developmental potential of rabbit oocyte matured in vitro: the possible contribution of prolactin. Biol Reprod. 41:26-33.

Yoshimura, Y., M. Ando S., Nagamatsu, M. Iwashita, T. Adachi, K. Sueoka, T. Miyazaki, N. Kuji, and T. Mamoru. 1996. Effects of insulin-like growth factor-I on follicle growth, oocyte maturation and ovarian steroidogenesis and plasminogen activator activity in the rabbit. Biol Reprod. 55:152-160.

Yung, M.C., M.J. van de Haar, R.L. Fogwell, and B.K. Sharma. 1996. Effect of energy balance and somatropin on insulin-like growth factor I in serum and on weight and progesterone of corpus luteum in heifers. J. Anim. Sci. 74:2239-2244.

CHAPTER II

ANALYSIS OF EARLY LACTATION REPRODUCTIVE CHARACTERISTICS IN HOLSTEIN COWS

(ABSTRACT)

Ultrasound-guided transvaginal follicular aspiration was used to obtain oocytes from cows to study follicular development and oocyte morphology. Follicular aspiration was conducted once during wk 1 to 12 postpartum on 120 lactating cows with 6 groups, separated by biweekly intervals. Approximately one half of the aspirated cows at each session were from the early groups (wk 1-2, 3-4, or 5-6) and the other half from the later groups (wk 7-8, 9-10, or 11-12). On the day of aspiration the number of follicles on each ovary, and their sizes, small (2-5 mm), medium (6-10 mm) and large (≥ 11 mm), were recorded. The collected oocytes were morphologically classified into 4 grades, with 4 = excellent, 3 = good, 2 = fair, and 1 = poor. Blood samples from the jugular vein and follicular fluid samples from the largest follicle were collected in order to perform hormone and metabolite assays. Environmental data were obtained from the local airport. There was a significant ($P < .01$) quadratic days pre- and postpartum by parity interaction for BCS. Body condition score for older cattle was the lowest at 90 d prior to calving and changed the least amount over time, while youngest cattle had the highest initial BCS at d 90 prior to calving and had the greatest change in BCS over time. Body condition score was the highest during summer calving season ($3.3 \pm .06$) compared to BCS during winter calving season ($2.6 \pm .06$). But the loss in BCS was greater for cows that calved in summer ($-0.53 \pm .06$) compared to cows that calved in winter ($-0.07 \pm .08$). Increased serum NEFA concentrations with simultaneous decreases in serum insulin concentrations for younger cattle implied a more negative EB status than for older cattle. The total number of follicles and total number of oocytes retrieved was significantly ($P < .001$) affected by a linear days postpartum by parity interaction with younger cattle having linear increases compared to decreases in the total number of follicles for older cattle. Oocyte quality score was affected by the quadratic days postpartum by parity interaction ($P < .01$) and calving season ($P < .01$). Younger cattle had higher initial quality scores compared to older cattle, but older cattle had higher quality oocytes towards the end of the 12 wk period compared with younger cattle. Younger cattle had higher E_2 and IGF-I concentrations in follicular fluid associated with a higher number of total follicles and number of oocytes, compared to older cattle. However, oocyte quality of younger cattle seemed to be reduced and oocytes were less competent than for older cattle. Cattle in 3rd and greater lactation showed very little change in BCS and hormone and metabolite measures during early lactation, with no apparent decrease in oocyte quality, despite the aging effect on follicle numbers. This study demonstrated that conditions related to early lactation have a negative effect on oocyte quality and endocrine measures of dairy cattle and that animals of different ages are differentially affected.

Keywords: Follicle, Oocyte, Parity, Cattle, Calving Season, Quality

INTRODUCTION

Ovarian antral follicular development in cattle proceeds through stages of follicular recruitment, selection and dominance. Dominant follicles control development of other follicles through the production of factors that act locally and/or systemically (Savio et al., 1988). Onset of the first follicular wave of an estrous cycle is detected as the appearnance a group of 4 mm follicles just before the day of ovulation (Wandji et al., 1996). During the next few days, one of the follicles becomes dominant. A second follicular wave emerges at about 10 d postovulation and if present, a third follicular wave starts at 16 d (Ginther et.al., 1989a; Lucy et al., 1991). Smith et al. (1996) reported that the emergence of a large follicle in all cows during the second week postpartum demonstrated that development of pre-ovulatory-size follicles is not the limiting factor in the reproductive recrudescence in lactating cows.

The general relationship between dietary intake and energy utilization is defined as EB and reflects the metabolic status of the cow. During early lactation the deficiency in dietary energy intake relative to the energy utilized for milk production results in negative EB and this condition may presist from several weeks to several months. The negative effects of high milk yield on conception rate were due to a delay in resumption of ovulation in the postpartum period, thereby allowing fewer ovulatory cycles before insemination and thus lower fertility (Butler and Smith, 1989). However, previous studies reported that not all high producing, healthy cows experience low conception at first AI service and that there might be factors other than milk yield alone affecting fertility (Butler and Smith, 1989; Fonseca et al., 1983; Staples et al., 1990).

Changes in BCS and BW during early lactation follow changes in EB because of the mobilization of body reserves to meet energy requirements (Villa-Godoy et al., 1988). Although subjective, BCS can be used to predict the energy status of the cow during early lactation (De Vries et al., 2000). Both pre- and postpartum restriction of dietary energy decreased BCS and BW (Perry et al., 1991). A decrease in BCS of more than 1.0 point during the first 5 wk postpartum leads to lower fertlity at first service (Britt et al., 1992; Butler and Smith, 1989). Beam and Butler (1997) noted that the initiation of a follicular wave occurs regradless of the early postpartum negative energy balance. However, the improvement of the EB from negative toward positive EB enhanced follicular competence, since the first wave of follicles that emerged after the EB nadir exhibited a greater apparent steroidogenic output and ovulation rate than the wave of follicles that emerged before EB nadir.

Another link between reproduction and nutrition involves the responsiveness of the ovary to hormones and metabolites (Nebel and McGilliard, 1993). Plasma concentrations of NEFA and IGF-I are good indicators of adipose tissue mobilization for energy, high plasma NEFA and low IGF-I concentrations reflect the dietary induction of a negative EB (Thatcher et al., 1992). The daily and total growth rate of the dominant follicle are affected by the fluctuation in energy intake, a low energy diet decreases the growth rate, mainly because of the decrease in IGF-I concentrations that are essential for follicular development (Adashi et al., 1985). Insulin inhibits the rate of adipose tissue lipolysis and stimulates the rate of lipogenesis, the net effect is to direct NEFA to adipose tissue and retain it there (Rose et al., 1996). Butler and Smith (1989) provided evidence that low energy and low levels of insulin concentrations during this period may limit the

responsiveness of the ovary to gonadotropin stimulation. Estradiol is the primary hormone stimulating estrous behavior in cattle (Hafez et al., 1987) and reduced IGF-I secretion could alter ovarian follicular E_2 production and thereby suppress estrous. The P_4 concentrations in serum were higher in cows with a positve EB compared to cows in negative EB (Spicer et al., 1990).

When low nutritional levels are combined with poor body condition the growth of small follicles is suppressed, with a substantial decrease in the number of follicles present (Lucy et al., 1991). Britt et al. (1992) suggested that it take at least 60 to 80 d for bovine oocytes to grow form early preantral stage to mature into Graafian follicle. And the exposure of follicles to adverse condition such as, negative EB, heat stress, and postpartum disease during the initial growing and developing phases might influence gene expression that could result in altered or impaired development. Dysfunctional mature follicles can have a negative influence on oocyte quality. In order to characterise the immediate and prolonged effects associated with the early postpartum period on the ovarian function of lactating cows, TVFA can be used to obtain oocytes from early postpartum cows. Immature oocytes recovered from such procedures can be successfully used for IVP (Pieterse et al., 1991; Boni et al., 1996).

The objectives of this study were to characterize the impact of parity on the changes in: 1) oocyte quality following follicular aspiration; 2) the developmental competence of Bovine oocytes after in vitro maturation and fertilization; and 3) the hormonal profiles in serum and follicular fluid during the first 12 wk postpartum.

MATERIALS AND METHODS

Treatment Groups

One hundred and twenty multiparous Holstein cows from the VA Tech Dairy Cattle Center were use. The cows were divided into 6 groups, 20 cows per group, with each group corresponding to 2 wk postpartum interval. The groupings began at parturition and group six corresponded to wk 11-12 postpartum. Ultrasound-guided transvaginal follicular aspiration was performed once per cow through wk 12. A group of 8-10 cows was aspirated at each session. Four to five cows were randomly selected from either group 1, 2 or 3 and the other 4-5 cows were from the later postpartum groups (4, 5 or 6).

Body condition scores for each animal were obtained at the time of dry-off for lactating cows and various times prior to calving for heifers, parturition, at 2 wk intervals after parturition, and on the day of aspiration. The scores were based on a five-point scale, 1 = emaciated cow, 5 = obese cow, with .25 increments and a healthy average was between 3 - 3.5 (Wildman et al., 1982).

On the day of aspiration BW was recorded. The milk record that was used for data analyses was a daily average for the week of aspiration. All cows were fed the same ration as formulated for the herd (Table 1).

Table 1. Dairy cattle ration formula for VA Tech Dairy Cattle Center

Ingredient	% of DM
Alfalfa Silage	34.6
Barley	19.3
High Moisture Corn	15.9
Corn Silage	11.8
Whole cottonseed	7.8
Soybean meal, 48% CP	6.1
Prolak[1]	2.3
Minerals/vitaminmix[2]	1.6
Limestone	0.3
Sodium Bicarbonate	0.3
Chemicals Components	
CP	17.7
ADF	21.4
NDF	31.4
Fat acids[3]	3.6
NE_L Mcal/kg	1.7

[1]Prolak (H. J. Baker & Bro., Inc., Atlanta, GA) contained: 60 % crude protein, 2.0% crude fiber, 5.0% crude fat 2.7% Ca, and 2.8% P.

[2]Mineral/vitmain mix (Southern Staes Cooperative, Richmond, VA) contained: salt 14.5 to 17.4% Ca, 6.5% P, 5.8% Cl., 3.2% S, 2.2% Mg, 3.5% K, 0.11% Mn, 0.13% Zn, 0.027% Fe, 44,000 UI vitamin D_3, and 550 vitamin E per kg.

[3]Total fatty acids (6:0 to 18:3[n-3])

Ultrasound-guided Transvaginal Follicular Aspiration (TVFA)

The following procedures were performed during TVFA (Gibbons et al.,1994; Kendrick et al., 1999). The cows were restrained in a squeeze-chute and feces were manually removed form the rectum. The cows were sedated with acepromazine maleate (44 mg/100 kg body weight, i.v.; Aveco Co., Inc., Fort Dodge, IA). The perineal was thoroughly scrubbed and disinfected (Nolvasan®; Fort Dodge Laboratories, Fort Dodge, IA) and followed by the lavage of the vaginal vault with a combination of 0.9% saline, 2% lidocaine and 3% iodine solution (120 ml) in a 10:1:1 ratio. A caudal epidural anesthesia was induced (6 ml; 2% Lidocaine Hydrochloride; Phoenix Scientific, Inc., St. Joseph, MO). Before initial sedation, blood samples (10 ml) were collected via jugular venipuncture, immediately placed on ice and stored at 4°C for 24 h to allow clotting. The samples were centrifuged at 2100 g for 30 min to separate serum and then stored at -20°C until assays were performed.

The ovaries were visualized by ultrasound (Aloka 500 V; Corimetrics Medical Systems, Inc., Wallingford, CT) with a 5.0 MHz convex-array transducer inside a vaginal probe with a dorsal-mounted needle guide. The transducer face was applied to the wall of the vaginal fornix (Ginther, 1997). The ovaries were rectally manipulated and positioned to align follicles with the built-in line on the ultrasound monitor representing the projected needle path. The number of follicles on each ovary as well as the size of each follicle as visualized on the ultrasound monitor was recorded. The presence of CL and its size was recorded at the same time. A 17 gauge (55 cm needle) with an echonic tip (RAM Consulting, Madison, WI) was inserted into the needle guide of the transvaginal probe. When the ovaries and the follicles were fully aligned, the needle was advanced until the tip of the needle became visible on the ultrasound monitor, indicating that the vaginal wall and peritoneum were penetrated. With the use of the needle guide-line the various follicles on the ovary were then penetrated and the follicular fluid and follicular contents were aspirated via 2 m Teflon tubing (2 mm o.d. x .5 mm i.d.; Fisher Scientific's Pittsburgh. PA). The tubing was attached to a needle and a collecting vessel that contained a small amount of PBS supplemented with 25 μg/ml heparin (Sigma Chemical, St. Louisville, MO) and 0.4 % BSA (Bovine Serum Albumin, Sigma). The aspiration took place under a constant vacuum pressure of < 60 mmHg at a flow rate of 27 to 29 ml/min. The needle and teflon tubing were intermittently rinsed with PBS (GIBCO, Life Technologies, Inc., Grand Island, NY), enriched with 0.4% BSA (Sigma), 1% (vol/vol) penicillin-streptomycin (GIBCO), and 25 μg/ml heparin (Sigma Chemical) to clear the tubing and needle. Aspiration was continued until all visible follicles (> 2 mm) were punctured. After completion of aspiration, the contents of the collecting bottle were rinsed into a modified all-in-one embryo collecting filter and search dish (Fujihira Industury Co. Ltd., Japan).

After the contents of the filter were rinsed several times with PBS, the oocytes were located with a 10x stereomicroscope and placed into in vitro maturation (IVM) washing medium.

Follicular fluid was collected from the largest follicle, but only if the largest follicle had a diameter of 10 mm and more. The same follicular aspiration procedures were followed to collect the follicular fluid, but the aspiration needle was connected to a 12 cc syringe (Fisher Scientific Co, Pittsburgh, PA). With the assistance of a second

operator, the targeted follicle was penetrated, the follicular fluid was removed and placed into a 14 ml collection tube (Starstedt, Newton, NC) and stored at -20°C until hormone assays were performed.

Oocyte Evaluation

Oocytes were classified as described by De Loos et al. (1989). Category 1 ooctyes had compact multi-layered cumulus cells and homogeneous ooplasm with the total cumulus-oocyte-complex (COC) light and transparent. Category 2 was oocytes that had compact multi-layered cumulus cells and homogeneous ooplasm with a coarse appearance and a darker zone around the periphery of the oocyte with the total COC complex slightly darker and less transparent. Category 3 oocytes that had less compact cumulus cells and irregular ooplasm containing dark clusters with the total COC complex darker than in category 1 and 2. Category 4 consisted of oocytes that had expanded cumulus cells, with cumulus cells scattered in dark clumps in a "jelly" matrix, irregular ooplasm containing dark clusters and with the total COC complex dark and irregular. Category 4 also included oocytes with no cumulus.

In vitro Maturation (IVM)

After collection, the oocytes were washed three times in washing medium, a stock solution of 25 mM HEPES-buffered tissue culture medium-199 (TCM-199, Gibco) that was supplemented with 10% (vol/vol) Fetal calf serum (FCS, Gibco). Washed oocytes were placed in 50 µl drops (5 to10 oocytes per drop) of IVM medium, a stock solution enriched with FSH (.01U/ml, Sigma Chemical) and 1 µg/ml 17-β Estradiol (E_4, Sigma Chemical). The oocytes collected from the early- and late group for each aspiration session were matured separately.

In vitro Fertilization and Sperm Preparation

After 24 h of incubation at 39°C and 5% CO_2, the expansion of the COC complex was recorded as an indicator of the percentage of maturation that occurred. The oocytes were then washed three times in Nunc wells (Thomas Scientific, Swedesboro, NJ) containing in vitro fertilizing (IVF) medium. After the washing procedures the oocytes were transferred into 50-µl drops (5 to 10 oocytes per drop) of IVF medium until sperm was added.

In vitro fertilization medium was prepared from a stock solution (Brackett-Oliphant medium; Brackett and Oliphant, 1975) containing 10 µg/ml heparin, (Sigma Chemical), 2.5 mM caffeine (Sigma Chemical), 0.5 % fatty acid free bovine serum albumin (BSA, Sigma Chemical), and fortified with 50 µl/ml gentamycin (Sigma Chemical).

The spermatozoa used for IVF were from a single ejaculate and semen quality was determined at the time of collection. The volume of sperm per individual straw was consistent for all straws prepared from the same ejaculate. One straw of semen was thawed in water at 37.5°C for 45 s, washed in Brackett-Oliphant medium containing 10 µg of heparin/ml, but without BSA or caffeine (Brackett and Oliphant, 1975), and followed by centrifugation at 500 x g for 5 min. After repeating the procedure for a second time the pellet that was obtained was re-suspended in the IVF medium, and the sperm numbers were adjusted to a concentration of 4 to 5 X 10^6 cells/ml. Fifty microliters of sperm was added to each 50 µl drop of IVF medium containing matured oocytes. The final concentration of sperm was approximately 2.5 x 10^5 sperm/ml drop (Chauhan et al., 1999).

In Vitro Culture (IVC)

After 16 h of co-incubation with spermatozoa, at 39°C and 5% CO_2, the oocytes were removed from the fertilization medium, washed (3x) and stripped from their cumulus cells in culture medium.

The in vitro culture medium for the first 6 d after IVF was modified synthetic oviductal fluid (Yoshioka et al., 1997) medium supplemented with 0.3% BSA. For the next 4 d, the medium was changed to 25 mM HEPES-buffered tissue culture medium-199 with 5% fetal bovine serum, and .01% BSA, fortified with gentamycin 50 µl/ml (Sigma Chemical). On d 2 (42 to 44 h) and 10 d after insemination. Seven days post-fertilization embryos were assesed for stage of development. For each embryo a development score was recorded corresponding to the stage of development as follows: 1= 1-cell, 2 =2-cell, 3 = 4-cell, 4 = 8-cell, 5 = 12-16 cell, 6 = morula, and 7 = blastocyts.

Hormonal Assays

Double antibody radioimmunoassay procedures were used to determine the concentrations of P_4, insulin, and IGF-I in serum (Bolt and Caldwell, 1992). The same procedures were followed to determine the concentrations of P_4, E_2, T and IGF-I in follicular fluid. However, serial dilutions of 1:100, 1:200, 1:400 and 1:800 were made of the follicular fluid samples prior to the beginning of the assays. Solid phase ^{125}I state radioimmunoassays kits (RIA; Coat-A-Count; Diagnostic Products (DPC; Los Angels, CA) were used for P4, E2, T and Insulin. Samples were assayed in duplicate, and intra-assay coefficients of variation were calculated.

IGF-I concentration in bovine serum and follicular fluid: Frozen samples of both follicular fluid and serum were thawed and 100 µl of each plus an extraction mixture made up of 87.5% ethanol (100%) and 12.5% of 2N NaCl were added to an microfuge tube. The tubes were vortexed, incubated for 1 h and centrifuged for 10 min at 9982 g. Five hundred microliters of the supernatant was removed and 200 µl of 0.855 M Tris Base were added to neutralize the samples. The samples were kept at -20°C for an hour and centrifuged at 3935 g for 30 min. The supernatant was poured off and kept at -80°C until assays were performed.

On d 1 of the assay, 100 µl of the tracer plus 100 µl of the first antibody (mouse anti IGF-I) were added to 40 µl of the supernatant and stored at -4ºC. After a 24 h incubation at –4ºC, the second antibody (goat anti-mouse) was added to each sample (100 µl) followed by a 72 h incubation period at -4ºC. After incubation, 1 ml of distilled PBS was added and the samples were centrifuged for 30 min at 2,200 g. Samples were then decanted and counted. All samples were assayed in duplicate and the unknown concentrations of IGF-I in each of sample were determined against a standard curve of 7 known concentrations ranging from .1ng/ml to 6.4 ng/ml of IGF-I (Weber et al., 1998; Kendrick et al., 1999). The with-in assay CV was 9.1% and 9.9% for IGF-I concentrations measured in serum and follicular fluid samples, respectively.

Progesterone, estrogen and testosterone concentrations in bovine follicular fluid: Follicular fluid samples were diluted to 1:100, 1:200 and 1:400 prior to the assays. One-hundred and 50 µl of diluted samples of E_2, T and P_4 were placed into a polypropylene antibody coated tubes (Diagnostic products, Los Angeles, CA) respectively, where-in ^{125}I labeled P_4, E_2 and T were competing for 3 h with the P_4, E_2 and T in the unknown sample (Kendrick et al., 1999). The follicular fluid P_4 with-in CV was 8.1% for E_2 it was 8.3% and for T it was 6.7%.

Progesterone concentrations in bovine serum samples: Serum samples (.1 ml) were analyzed using the Diagnostic Products Coat-A-Count Kits (Kendrick et al., 1999). The with-in assay CV was 7.3%.

Insulin concentrations in bovine serum samples: Two-hundred microliters sample of serum was placed into the antibody coated tubes provided by the Coat-A-Count kit from Diagnostic Products. After a incubation period of 24 h with ^{125}I labeled Insulin the unknown concentration of insulin in the samples were determined by comparing it against a standard curve of 6 known concentrations, ranged from 5 through 400 μIU/ml, of insulin. The with-in assay CV was 7.4%. The concentration of insulin in each sample was converted from μIU/ml to ng/ml by multiplying with a conversion factor of 0.0433 (Diagnostic Products).

Non-esterified fatty acid (NEFA) and glucose concentrations in bovine serum samples: Light absorbency was used to measure the concentration of non-esterified fatty acids (NEFA) and glucose in serum samples (McCutcheon and Bauman, 1986). The NEFA C kit, was purchased and modified for expected concentrations. (Wako Chemicals , Dallas, TX). The assay was modified to allow reduction in the sample volume (from 50 μl to 25 μl) and of the volume of reagents (to 15% of recommended volume). These changes substantially reduced assay costs but did not influence accuracy (McCutcheon and Bauman, 1986). The optical density of each sample was measured at 550 nm (Wako Chemicals). Serum glucose concentration was assayed in an enzymatic colorimeteric system using a Glucose kit (Sigma Diagnostics Inc. St. Louis, MO). The optical density was measured at 450 nm (Wako Chemicals).

Statistical Analysis

Data analysis was conducted using different general linear models (GLM) from the Statistical Analysis System (SAS® 1999). For data analysis parity 1 =1st lactation cattle, parity 2 = 2nd lactation and 3rd parity = 3rd and greater lactation. Each of the follicles from every cow aspirated was assigned to size categories according to its diameter measurement (mm). Categories started at 2 mm followed by a size category for each 1 mm increase in size up to 15 mm in diameter. All follicles larger than 15 mm were categorize into five additional categories up to 45 mm in diameter, accordingly. Small follicles were consider diameters of 2-4mm, medium follicles had a diameter of 5-9 mm, and large follicles were >9 mm. Each oocyte recovered received a quality score, based on a scale of either 1= poor, 2 = fair, 3 = good or 4 = excellent (De Loos et al 1989). The postpartum period was devided into 2 catogories for the oocytes recovered by TVFA procedures. Cows aspirated within the first 42 d postpartum (groups 1, 2 and 3) were the "early" category and cows aspirated later the "late" category. Oocyte development was recorded as: 1) for the number of oocytes that did not matured (lack of cumulus expansion, 2) umber of oocytes that matured but did not cleaved, and 3) for the number of oocytes that cleaved. Embryo development was analyzed in terms of oocytes that were recovered from each group in the winter compared with the oocytes that were recovered in fall for each stage of lactation (early vs, late) group.

Cow characteristics, BCS at various times relative to calving, BW and milk yield at aspiration were analyzed by using the models in appendix tables 16 to 21.

The indepented variables parity, days postpartum (DPP) the linear, quadratic and cubic components for the interaction between days postpartum and parity; hormone and

metabolite concentrations in serum and follicular fluid; and environmental variables (calving and aspiration season, maximum and minimum daily temperatures at calving and aspiration, and daily percipitation) were used to explain the variation in the total number of follicles and oocytes, follicle diameter and oocyte quality scores, CL presence and size. Specific variables that were used in the various models and are shown in tables 22 to 35 (Appendix). Differences in least squares means for significant factors in the various models were determined by PDIFF (SAS® 1999) In reduced models non-significant terms where eliminated. A Chi-square model including the variables category, season, and developmental score was used to determine the significance of season and category on the number of oocytes in the 3 developmental stages. A second analysis used GLM (SAS® 1999) to analyze developmental scores with a model that included season and category.

RESULTS

The means and standard errors for cow, environmental, ovarian, and endocrine variables are shown (Table 2).

Cow Variables

Body Condition Score Measurements: Body condition score (3.4 ± .02) was recorded for 78 cows at an average of 75 ± 4.2 d prior to parturition (Table 2). The pre- and postpartum body condition scores were affected by a quadratic pre- and postpartum days by parity interaction, and parity ($P < .01$; Appendix Table16). The BCS of older cattle (3^{rd} parity) was the lowest (Figure 1) of the three groups at 90 d prior to calving and declined the least amount over time, while the younger cattle (1^{st} parity) had the highest initial BCS at 90 d prior and showed a more drastic change in BCS over the time period. Both 1^{st} parity cattle and 2^{nd} parity cattle had a nadir in BCS at d 94 with a slight increase to d 100. However, older cattle (3^{rd} parity) had no increase in BCS. Overall, the three groups showed a decrease in BCS during the period prior to calving which continued to decrease until the approximated end of the data sampling period. The BCS means by parity are shown in Table 3.

Body condition score at calving was significantly affected by the following calving season ($P < .01$), BW ($P< .01$) and parity ($P< .01$; Appendix Table 17). The highest BCS (3.3 ± .06) was during summer compared to 2.9 ± .05 in fall and 2.6 ± .06 in winter (Table 4). The BCS recorded at aspiration was significantly ($P < .01$) affected by BW and milk yield (Appendix Table 18). Bodyweight had a positive association ($b = .0024$) with BCS, while milk yield had a negative association with BCS ($b = -.0112$).

The change in BCS from the day of calving to the day of aspiration was significantly affected by season of calving ($P < .01$), the change in BCS from pre-partum to calving ($P < .01$),and peak milk yield ($P < .01$; Appendix Table 19). The change BCS from pre-partum to calving ($b = -.723$) and peak milk yield ($b = -.015$), had negative impacts on the change in BCS from calving to aspiration. Table 5 shows the effect of calving season on the change in BCS from calving to aspiration. The greatest loss in BCS occurred with summer calving, while the least was with winter calving.

Bodyweight and Milk Yield: Calving season and parity had a significant ($P < .01$, Appendix Table 20) effect on BW. The average BW was the lowest for younger cattle and BW increased with age (Table 6). Body weight and parity were positively correlated ($r = .75$, $P < .001$). The BW was the lowest for cows that calved in winter (Table 7).

Milk yield was affected by the quadratic days postpartum by parity interaction ($P < .001$; Appendix Table 21). Milk yield peaked at 34 kg on 56 d postpartum for 1^{st} parity animals, while both, 2^{nd} parity cattle, and older cattle peaked on 46 d postpartum at 43 and 48 kg, respectively (Figure 2). Parity had a significant ($P < .001$) effect on milk yield at aspiration (Table 8).

Table 2. The means and standard errors for cow, environmental, ovarian and endocrine parameters obtained from analysis on data collected from Holstein cows.*

Cow Parameters	Unit	n	$\bar{x}$ ± STD
Days prior to calving		78	75 ± 6.90
Body Condition Score at Dry Off		78	3.4 ± 0.21
Body Condition Score at Parturition		78	3.0 ± 0.41
Maximum daily temperature at calving	°C	105	15.1 ± 10.34
Change in Body Condition from Dry Off to Parturition		78	0.35 ± 0.34
Change in Body Condition from Calving to Aspiration		78	0.32 ± 0.38
Maximum Daily Temperature at aspiration	°C	14	15.1 ± 7.84
Minimum Daily Temperature at aspiration	°C	14	0.9 ± 5.04
Precipitation at Calving	mm	105	13.1 ± 46.38
Body condition score at Aspiration		120	2.6 ± 0.28
Precipitation at Aspiration	mm	14	8.7 ± 19.78
Days Postpartum at Aspiration		120	47.3 ± 24.33
Bodyweight	kg	120	552.3 ± 74.24
Lactation Number		120	1.87 ± .9
Mean Milk Yield during week of Aspiration	kg/d	120	37.6 ± 8.9
Peak Milk Production	kg/d	120	43.4 ± 9.2

*Transvaginal follicular aspiration was performed on 120 Holstein cows.

Table 2 (cont.)

Ovarian and Endocrine Parameters	Unit	n	$\bar{x}$ ± STD
Diameter of corpus luteum present at Aspiration	mm	69	22.0 ± 5.9
Diameter of Largest Follicles at Aspiration	mm	120	14.3 ± 6.7
Number of follicles per cow:		120	11.04 ± 6.6
Size Category:			
Small	2 to 4 mm	120	6.8 ± 5.4
Medium	5 to 9 mm	103	3.5 ± 2.4
Large	> 9 mm	100	1.5 ± 0.72
Number oocytes per aspiration session:		102	5.3 ± 4.2
Grade 4		35	1.63 ± 1.10
Grade 3		47	1.59 ± 0.88
Grade.2		68	2.0 ± 1.67
Grade 1		76	3.3 ± 3.1
Metabolites & Hormone concentrations in Serum:			
Non-esterified fatty acids (NEFA)	μmol/ml	120	417 ± 315
Glucose	mg/dl	120	71.3 ± 15.2
Progesterone (P_4)	ng/ml	120	2.03 ± 2.4
Insulin-like growth factor I (IGF-I)	ng/ml	120	57.1 ± 18.0
Insulin	ng/ml	120	0.9 ± 0.34
Hormone concentrations in Follicular Fluid:			
Progesterone P_4	ng/ml	101	265 ± 510.8
Estrogen E_2	ng/ml	101	226 ± 308
Insulin-like growth factor IGF-I	ng/ml	99	65 ± 23.3
Testosterone (T)	ng/ml	101	2.7 ± 3.5
Metabolite & Hormone Ratios:			
E_2:P_4		101	3.8 ± 6.6
E_2:T		101	116 ± 163.2
P_4:T		101	416 ± 1538
Insulin:Glucose		120	0.01 ± 0.005
Insulin:IGF-I		120	0.018 ± 0.007
Glucose:NEFA		120	0.35 ± 0.77
IGF-I:NEFA		120	0.31 ± 0.74

BODY CONDITION SCORE
PRE- AND POSTPARTUM

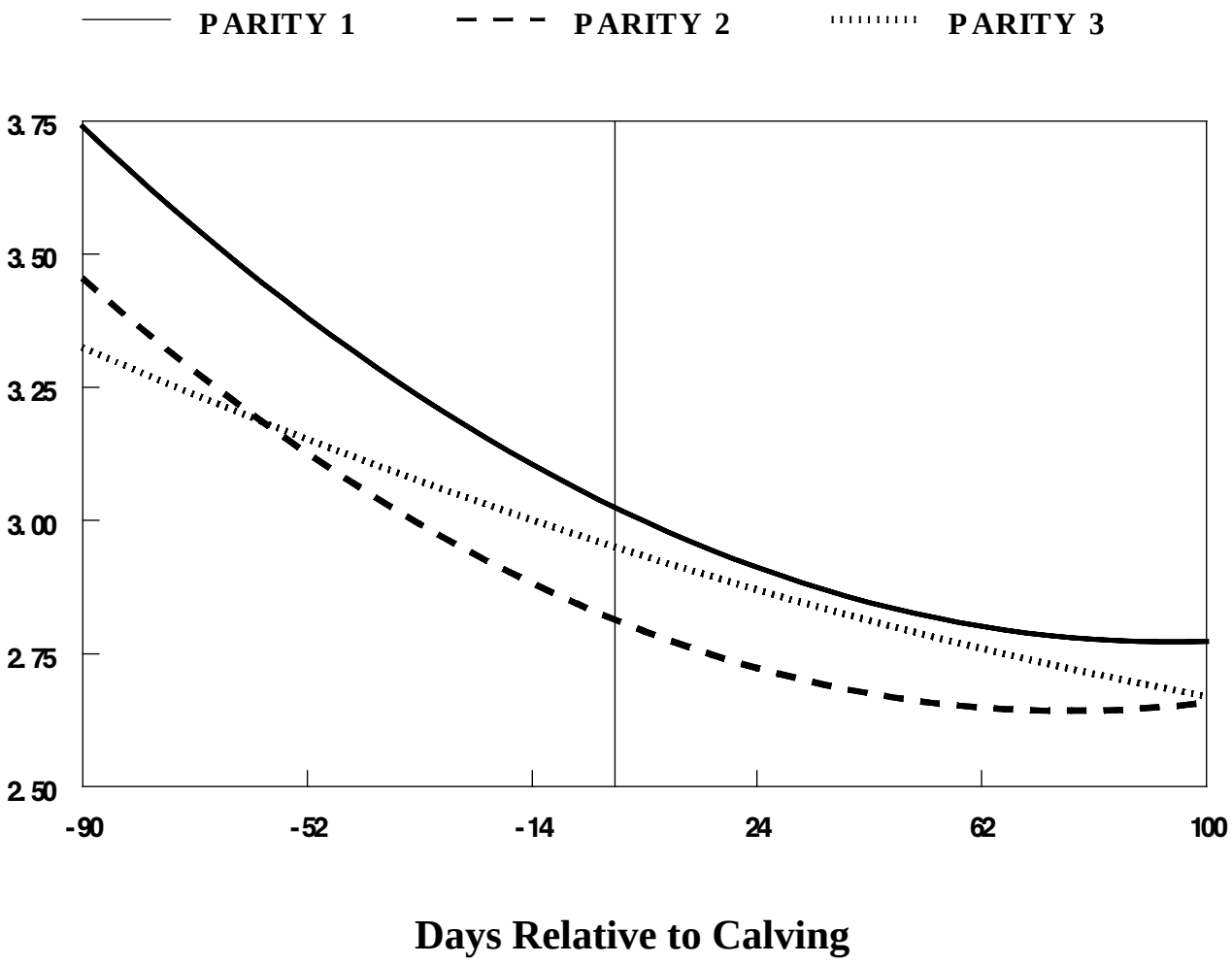


Figure 1. (Appendix Table 16) Changes in Prepartum and Postpartum Body Condition scores from d 90 prepartum to d 100 postpartum.

Table 3. Least-squares means (±SE) for pre- and postpartum body condition scores.

Parity	n	$\bar{x}$ ± SE
1st	189	2.95 ± .022 [a]
2nd	175	2.77 ± .03 [b]
3rd	85	2.87 ± .03 [c]

[a, b, c] Values in the same column with different superscript are different (P < . 01; Appendix Table 16).

Table 4. Least-squares means (±SE) for BCS at calving for the Holstein cows.

Season	n	$\bar{x}$ ± SE
Fall *	37	2.94 ± .05[a]
Winter **	27	2.63 ± .06[b]
Summer ***	55	3.29 ± .06[c]

[a, b, c] Values in the same column with different superscripts are different (P < .001; Appendix Table 17).

* 21st of September through 20th of December

** 21st of December through the 20th of March

*** 21st June through 20th of September

Table 5. Least-squares means (±SE) for change in BCS from calving to aspiration for different calving seasons.

Season	$\bar{x}$ ± SE
Fall *	-0.31 ± .05[a]
Winter **	-0.07 ± .08[b]
Summer ***	-0.53 ± .06[c]

[a, b, c] Values in the same column with different superscripts are different (P < .01; Appendix Table 19).
* 21st of September through 20th of December
**21st of December through the 20th of March
***21st June through 20th of September

Table 6. Least-squares means (±SE) for BW (kg) at aspiration for different parity groups.

Parity	n	$\bar{x}$ ± SE
1st	52	525 ± 13.3[a]
2nd	32	589 ± 14.6[b]
3rd	36	651 ± 13.0[c]

[a, b, c] Values in the same column with different superscripts are different (P < .0001, Appendix Table 20).

Table 7. Least-squares means (±SE) for BW (kg) at the day of aspiration for different calving seasons.

Season	$\bar{x}$ ± SE
Fall *	574 ± 7.6 [a,c,d]
Winter **	537 ± 6.6 [b]
Summer ***	587 ± 9.8 [c,d]
Spring****	656 [c, d]

[a, b, c,d] Values in the same column with different superscripts are different (P < .01, Appendix Table 20).
* 21st of September through 20th of December
**21st of December through the 20th of March
***21st June through 20th of September
****21st of March through the 20th of June

MILK YIELD

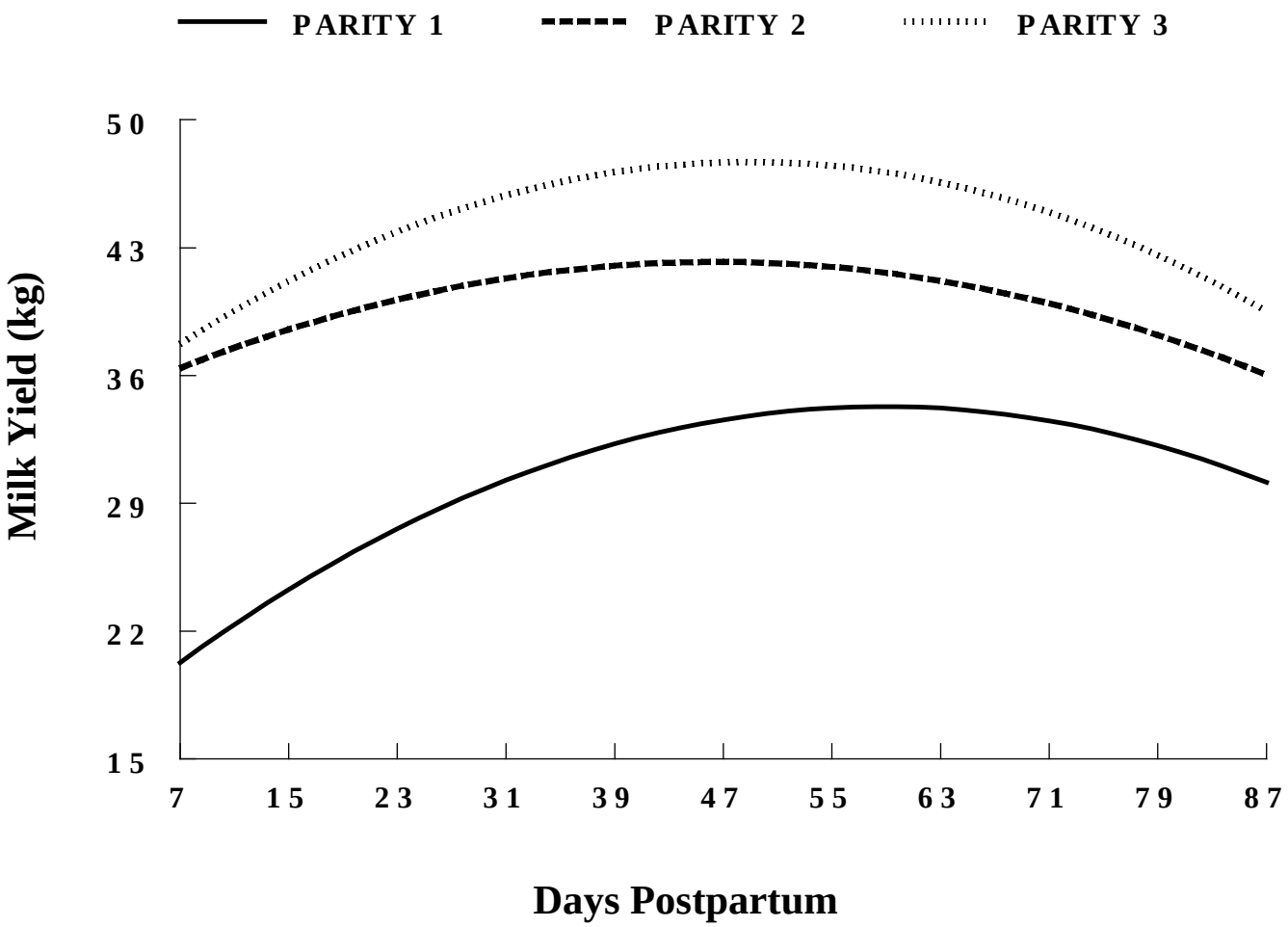


Figure 2.(Appendix Table 21) Average milk yield for parity groups during week of aspiration.

Table 8. Least-squares means (±SE) for milk yield at aspiration for parity groups.

Parity	$\bar{x}$ ± SE
1st	29.9 ± 1.9 [a]
2nd	39.7 ± 2.0 [b]
3rd	43.8 ± 1.8 [c]

[a, b, c] Values in the same column with different superscripts are different (P < .01; Appendix Table 21).

Table 9. Least-squares means (±SE) for average number of follicles for parity groups at each aspiration session.

Parity	$\bar{x}$ ± SE
1st	10.4 ± 1.0[a]
2nd	10.8 ± 1.3[a]
3rd	15.0 ± 1.2[b]

[a, b] Values in the same column with different superscripts are different (P < .01; Appendix Table 22).

Table 10. Least-squares means (±SE) for average number of oocytes for parity groups at each aspiration session.

Parity	$\bar{x}$ ± SE
1st	3.1 ± .74[a]
2nd	4.5 ± .94[a, b]
3rd	6.6 ± .82[b]

[a, b] Values in the same column with different superscripts are different (P < .001; Appendix Table 28).

Table 11. Least-squares means (±SE) for quality score for oocytes recovered after TVFA procedures from Holstein cows during different calving seasons.

Season	$\bar{x}$ ± SE
Fall	2.1 ± .08[a]
Winter	1.7 ± .11[b]
Summer	1.9 ± .07[b]

[a, b] Values in the same column with different superscripts are different (P < .05; Appendix Table 29).

Ovarian and Endocrine Parameters

Total number of follicles and follicle diameter: A total number of 1325 follicles was observed and aspirated through ultrasound-guided transvaginal follicular aspiration. The total number of follicles was affected by a linear days postpartum by parity interaction ($P < .001$; Appendix Table 22). Also, the main effect of parity was highly significant ($P < .0001$). The total number of follicles for 1st parity cattle, and 2nd parity cattle, increased linearly from d 7 to 87 (Figure 3) with the rate of increase greater for the 2nd parity cattle than first parity cattle. First parity cattle had an increase from 14 follicles at d 7 to 25 follicles at d 87, while 2nd parity cattle increased from 6 to 35 follicles on d 7 to d 87, respectively. The total number of follicles for 3rd parity cattle decreased in the same time period, from 24 follicles at d 7 to 6 follicles on d 87. The least squares means for the average number of follicles by parity at each aspiration are shown in Table 9, with older cattle having more follicles then the other two parity groups. In the same model the maximum daily temperature on the day of aspiration, milk yield, IGF-I concentrations in serum, T concentrations in follicular fluid and serum IGF-I to insulin ratio had significant effects on the total number of follicles observed per cow. Milk yield, maximum temperature at aspiration, IGF-I concentration and IGF-I:Insulin ratio had positive associations ($b = .199$, $b = .156$, $b = .136$ and $b = 286.0$) with total number of follicles, while the T concentrations in the follicular fluid had a negative association ($b = -.629$).

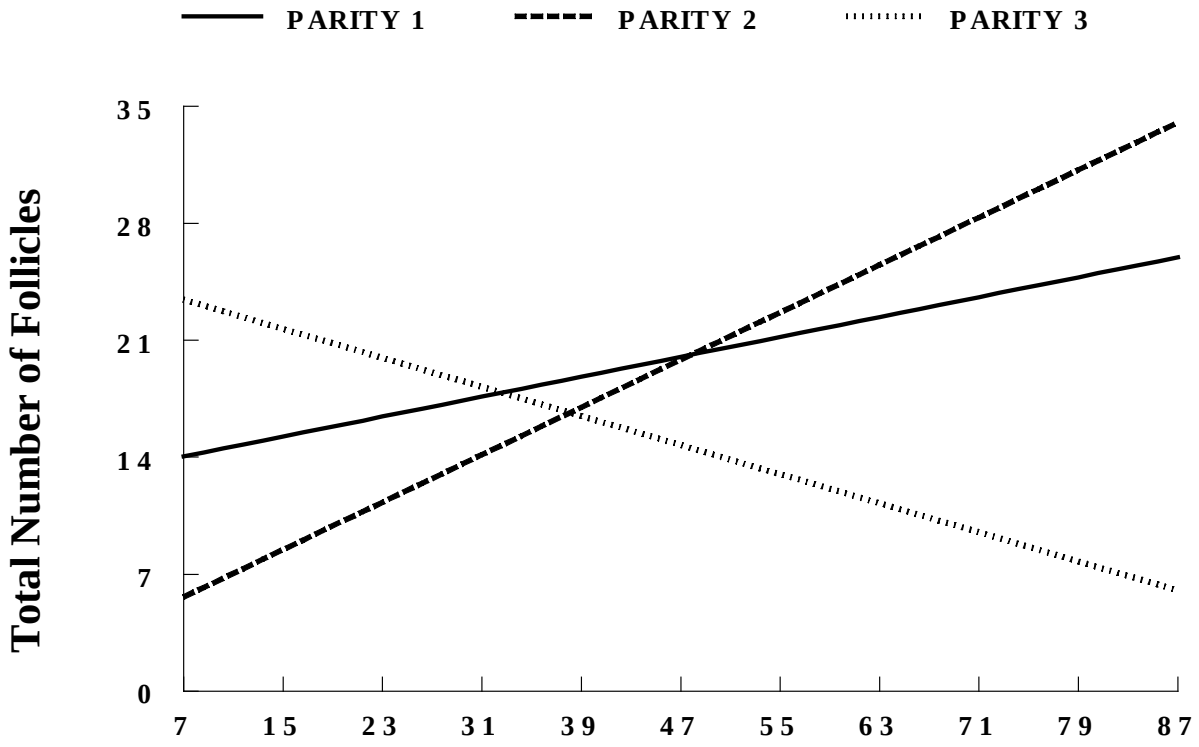
Additionally, in another model peak milk yield, serum P_4 concentrations and the ratio between P4 concentrations and T concentrations in follicular fluid had significant effects on the total number of follicles ($P < .001$; Appendix Table 23).

Average follicle diameter changed in cubic manner over days postpartum by parity interaction ($P < .001$, Appendix Table 24). Both 1st parity, and 2nd parity cattle had a steady increase in follicle diameter that peaked at approximately 6 mm (Figure 4), but at different days postpartum, d 38 and d 28, respectively. Thereafter, mean follicle diameter declined and reached a nadir of 4 mm at day 80 for 1st parity cattle and 3mm at d 76 for 2nd parity cattle. The mean follicle diameter for 3rd parity cattle was 4.5 mm at d 7 and continued to increase and peaked at 6 mm at d 87.

Additionally, in an expanded model, milk yield ($b = - .086$), presence of CL at aspiration ($b = .958$), the size of the largest follicle ($b = .230$), the wind chill temperature at calving ($b = - .048$), concentrations of glucose ($b = .04$) and insulin ($b = -3.188$) in serum at aspiration, ratio between IGF-I:Insulin ($b = 184.33$), Glucose:NEFA ($b = -7.97$), IFG-I:NEFA ($b = 8.810$), and the concentration progesterone in follicular fluid ($b = .0014$) had significant effects ($P < .01$; Appendix Table 25) on follicular diameter.

The largest follicle present at the day of aspiration was affected by the cubic components of days postpartum ($P < .01$; Appendix Table 26) and BW at aspiration ($P < .01$). The diameter increased rapidly from 6 mm on d 7, peaked at 15 mm on d 37, decreased slightly in diameter and reached a nadir at 14 mm on d 70 that was twice the initial size at d 7 (Figure 5). There was a significant correlation ($r = .24$, $P < .01$) between BW and the diameter of the largest follicle.

TOTAL NUMBER OF FOLLICLES



Days Postpartum

Figure 3. (Appendix Table 22). Linear relationship for average number of follicles for parity groups at each aspiration session.

FOLLICLE DIAMETER

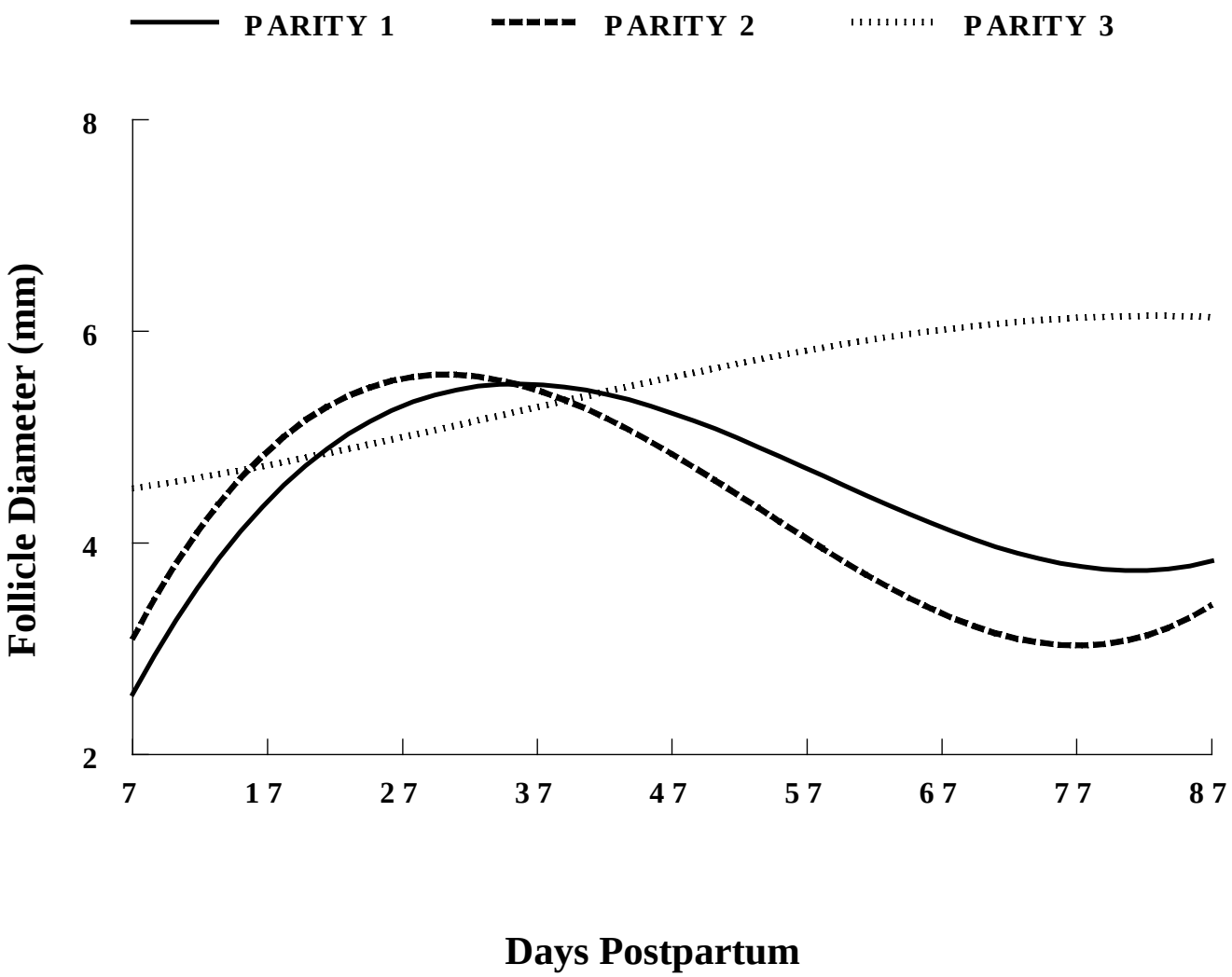


Figure 4. (Appendix Table 24) Changes in follicle diameter in a cubic manner for parity groups at each aspiration session.

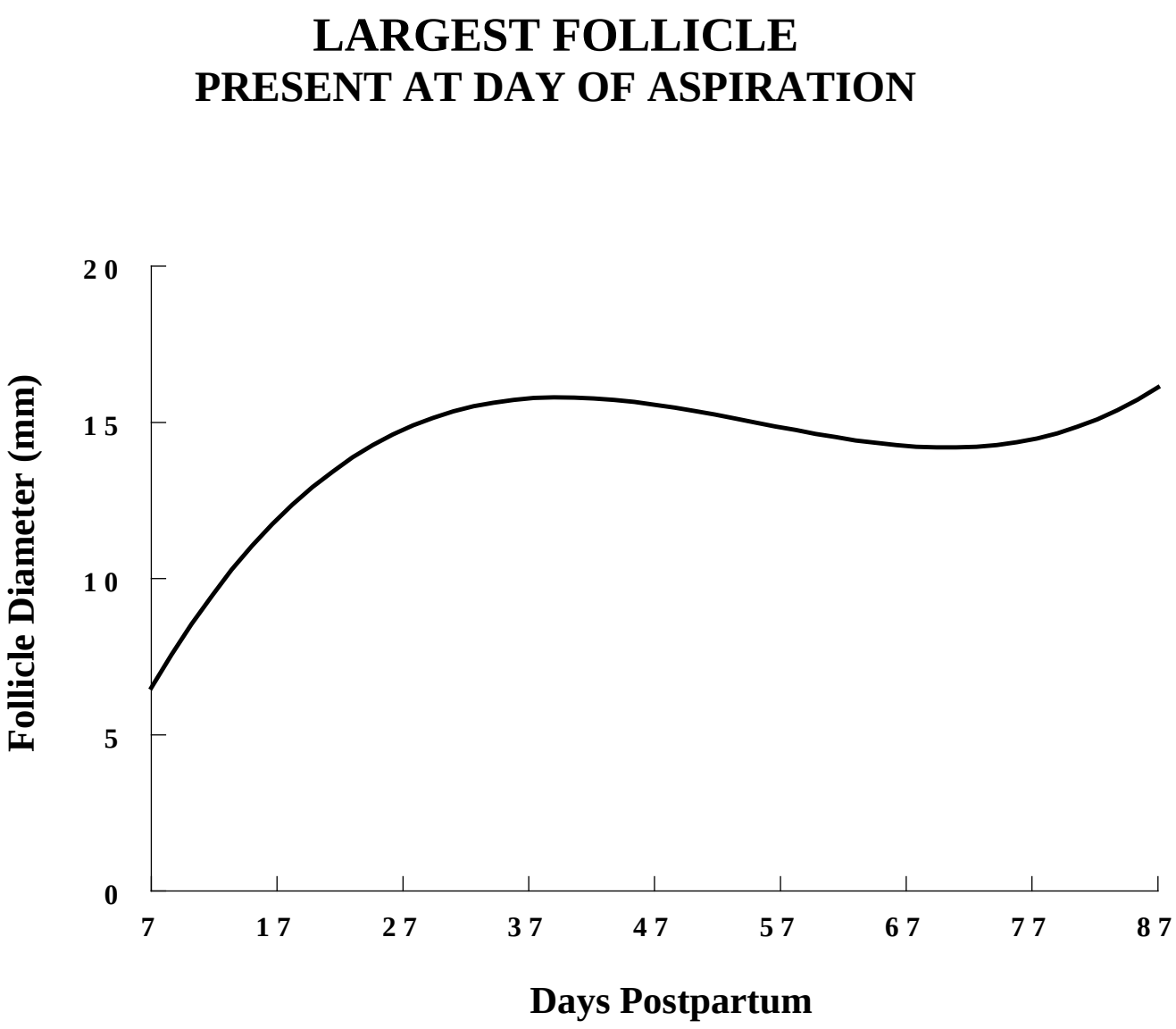


Figure 5. (Appendix Table 26) Changes in the diameter of the largest follicle recorded at day of aspiration.

The diameter of the CL that was present at aspiration was affected by the quadratic days postpartum by parity interaction ($P < .01$; Appendix Table 27). First parity cattle had a CL of 40 mm at d 7 that stayed relatively constant until approximately d 40, at which time it increased to almost twice the initial diameter at d 87. For 2nd parity cattle the initial CL diameter was 20 mm at d 7 and it increased more than 3 times the initial diameter by d 87 (Figure 6). Older cattle (3rd parity) that had a CL of 35 mm at d 7 that declined and reached a nadir of 20 mm on d 55 and then increase to 30 mm by d 87.

Total number of oocytes and oocyte quality: A total number of 527 oocytes was recovered with TVFA procedures. The days postpartum by parity interaction was significant ($P < .01$; Appendix Table 28) for the linear component and showed an increase in total number of oocytes for 1st, and 2nd parity cattle compared with 3rd parity cattle, which showed a decrease in the total number of oocytes recovered (Figure 7). The initial number of oocytes recovered were approximately 2 oocytes at d 7 from 1st parity cattle and this increased to 9 at d 87, while 2nd parity cattle initially had approximately 3 recovered oocytes at d 7 increasing to 10 recovered at d 87. For cattle from later 3rd parity the number of oocytes was highest (9) at d 7 and decreased to 5 recovered at day 87 (Figure 7). Average number of oocytes recovered for each parity group is shown in Table 10. There was no significant aspirator effect on the total number of oocytes recovered.

Oocyte quality score was affected by the quadratic days postpartum by parity interaction ($P < .01$), calving season ($P < .01$), the impact of the maximum daily temperature($P < .01$), daily precipitation on the day of aspiration ($P < .001$), the Glucose:NEFA ratio ($P < .01$) and the IGF-I:NEFA ratio ($P < .001$; Appendix Table 29).

Both 1st, and 2nd parity cattle showed and initial score of 1.4 at d 7 and the score increased curvelinearly but peaked differently for the two parities, 2.2 at 52 d and 2.0 at d 47, respectively. For both parity groups the quality score then decreased and was low at d 87. Cattle from the 3rd parity had an inverse response compared with earlier parities. The initial oocyte quality score was 1.6 at d 7 and it increased gradually to 2.1 at d 87 (Figure 8.) Overall, older cattle seemed to have higher quality oocytes towards the end of the 12 wk period compared to younger cattle that had better quality oocytes within the first 7-8 wk postpartum.

Summer or winter calving resulted in a low oocyte quality score while the oocyte quality score was highest in cows that calved during the fall (Table 11). The maximum temperature on the day of aspiration ($b = .018$) and the glucose:NEFA ratio ($b = 1.12$) had a positive association with oocyte quality score, while precipitation on the day of aspiration ($b = -.010$) and the IGF-I:NEFA ratio ($b = -1.29$) had a negative association with oocyte quality score.

Additionally, in an expanded model, oocyte quality score was significantly affected by BCS ($P < .05$; $b = -.42$) at aspiration, concentration of T in follicular fluid ($P < .0001$; $b = -.09$), ratios between E_2 and T concentrations in follicular fluid ($P < .05$; $b= -.0012$), concentration of IGF-I in serum ($P < .001$; $b= .01312$), the ratio between insulin

CORPUS LUTEUM SIZE AT ASPIRATION

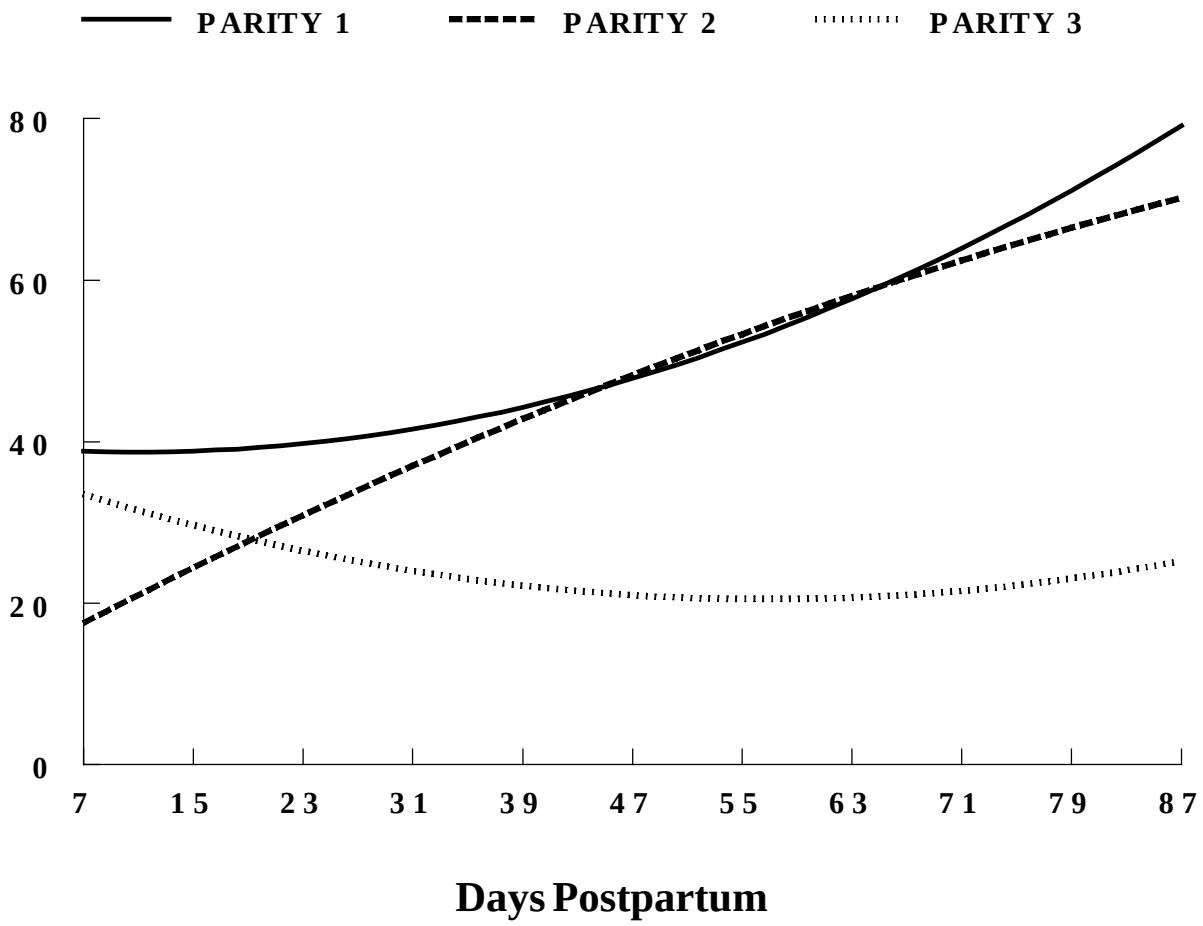


Figure 6. (Appendix Table 27) Cubic relationship for changes in corpus luteum size at aspiration by parity for lactating Holstein cows.

TOTAL NUMBER OF OOCYTES

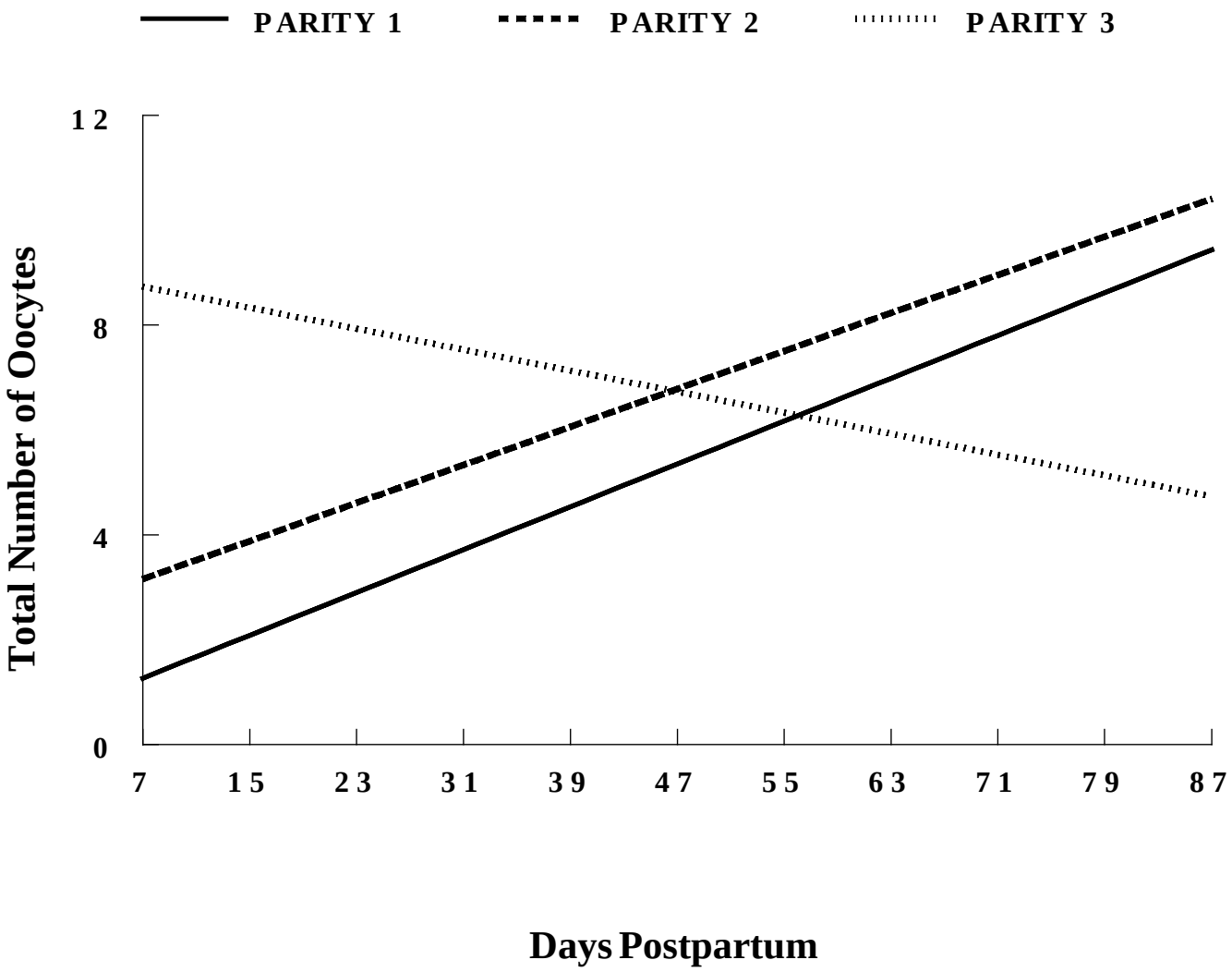


Figure 7. (Appendix Table 28) Linear relationship for average number of oocytes for parity groups at each aspiration session.

CHANGES IN OOCYTE QUALITY

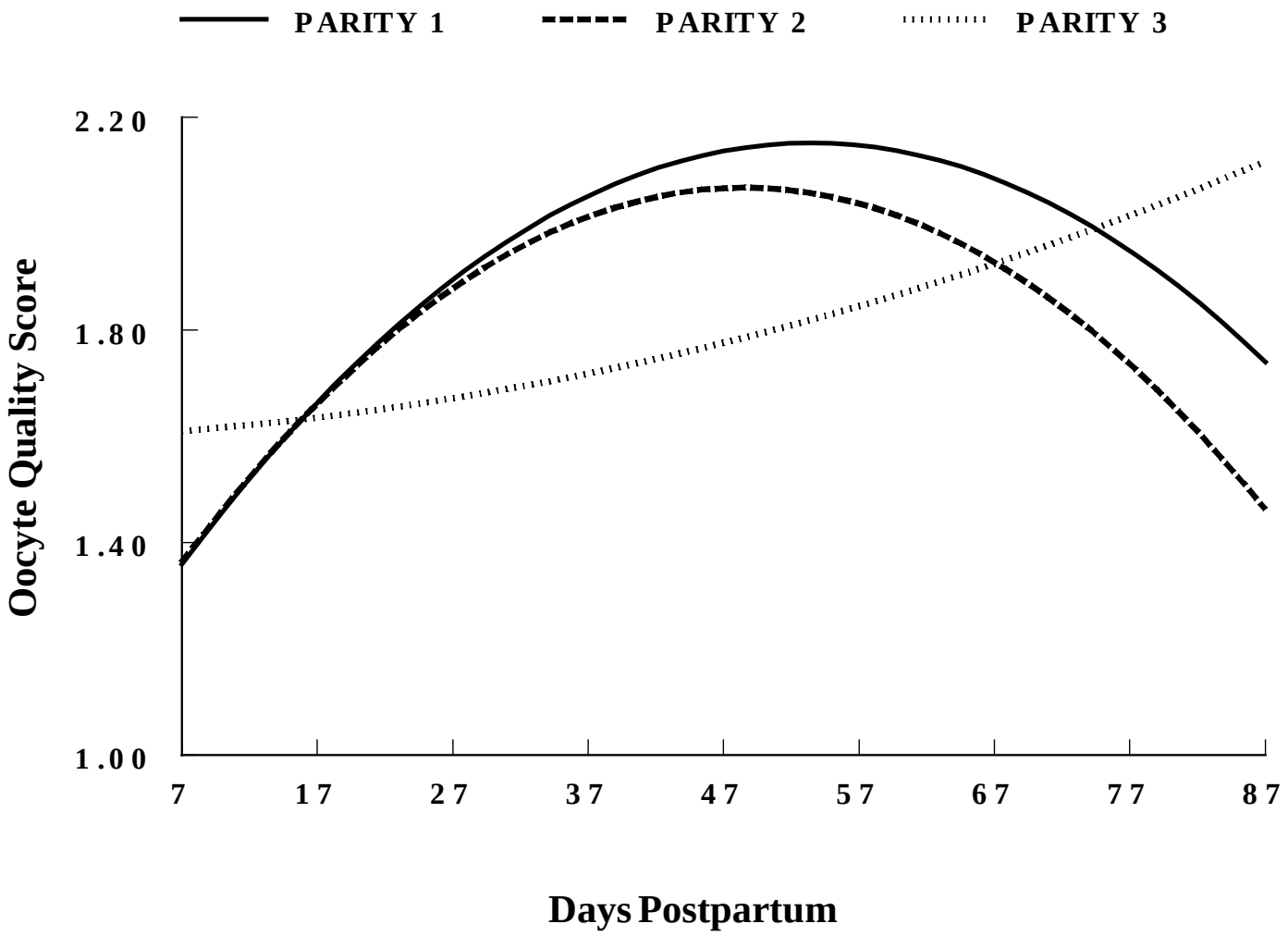


Figure 8. (Appendix Table 29) Changes in oocyte quality score for oocytes recovered after TVFA procedures from lactating Holstein cows of different ages. (4 = Excellent, 3 = Good, 2 = Fair, and
1 = Poor.

and glucose concentrations in serum ($P < .01$; b = 33.50), and the ratio between E2 and P4 concentrations in follicular fluid ($P < .05$; b= .0216; Appendix Table 30).

Hormone and metabolite concentrations in serum: Analysis of the metabolites and hormones in serum showed that P_4 concentrations were affected in a linear manner by the days postpartum by parity interaction ($P < .01$; Appendix Table 31). For 1st parity cattle there was no detectable concentrations of P_4 present in serum until after d 32 (> .1 ng/ml). This was followed by a linear increase to 3.3 ng/ml at d 87. For 2nd parity cattle concentrations of P_4 were detectable after the first 2 wk postpartum followed by a linear increase to 2.5 ng/ml at d 87. Cattle in 3rd parity had almost no change in P4 concentrations in serum from d 7 through d 87 (Figure 9).

Insulin concentrations were affected in a linear manner by days postpartum by parity interaction ($P < .01$; Appendix Table 32). A linear decrease was found for 1st parity cattle from d 7 though d 87 and it was almost parallel to the decrease for 2nd parity cattle; the concentration of serum insulin decreased from 1 ng/ml at d 7 to 0.44 ng/ml at d 87. Cattle from 3rd parity had a linear increase in insulin from 0.6 ng/ml to 1.3 ng/ml at d 87 (Figure 10). The insulin concentrations in serum were significantly different ($P < .01$) between 1st parity and older (3rd parity) cattle (Table 12).

Non-esterified fatty acid concentration in serum was affected by the cubic days postpartum by parity interaction ($P < .01$; Appendix Table 33). Non-esterified fatty acids concentrations peaked at 2900 μmol/ml for both the 1st and the 2nd parity cattle at 87 d postpartum.

Non-esterified fatty acids in 1st parity cattle had a nadir at 1284.7 μmol/ml at d 17 while, 2nd parity cattle had a nadir at 1119.3 μmol /ml at d 25 followed by a rapid increase as days postpartum increased. For older cattle (3rd parity) the NEFA concentration in serum declined after the initial concentration of 710 μmol/ml at d 7. The NEFA concentration decreased to 285.6 μmol/ml at d 87 (Figure 11). Least squares means for NEFA concentrations in serum for parity groups are showed in Table 13. An inverse relationship with insulin (Figure 10) was found for the days postpartum by parity interaction (Figure 11).

There were no significant differences found across days postpartum by parity interaction or calving season for either glucose or IGF-I concentrations in serum. Mean concentrations were 71.3 ± 1.4 mg/dl for glucose and 57.1 ± 1.6 ng/ml for IGF-I.

Hormone and metabolite concentrations in follicular fluid: There was a significant days postpartum (cubic) by parity interaction ($P < .05$; Appendix Table 34) for E_2 concentrations in follicular fluid. Second parity cattle had an initially rapid increase in E_2 concentration from 368.0 ng/ml at day 7 to almost 1212.7 ng/ml at d 27. Subsequently the concentration of E_2 increase more gradual to 1641.2 ng/ml at d 59, followed by another rapid increase to almost 2624.4 ng/ml at d 87. First parity cattle had a biphasic response almost the inverse to the response for 2nd and 3rd lactation cattle. The initial E_2 concentration of 1322.4 ng/ml at d 7 decreased and reached a nadir at 853.3 ng/ml on d 27, followed by an increase to approximate 2024.9 ng/ml at d 87.

SERUM PROGESTERONE CONCENTRATIONS

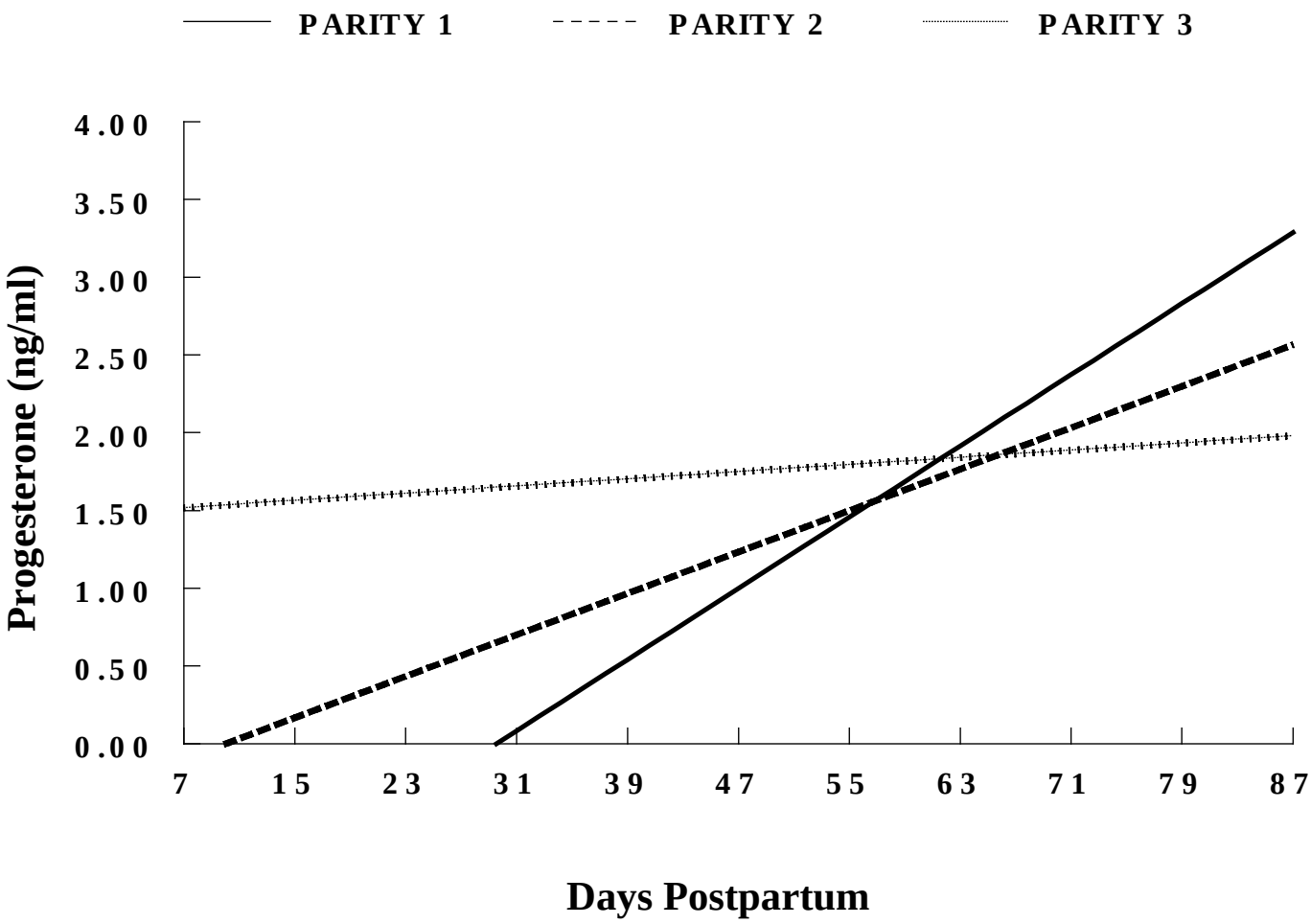


Figure 9.(Appendix Table 31) Serum Progesterone concentrations at aspiration by parity for lactating Holstein cows.

Table 12. Least-squares means (±SE) for serum insulin concentrations (ng/ml) at aspiration by parity for lactating Holstein cows from d 7 to 87 postpartum.

Parity	$\bar{x}$ ± SE
1st	1.1 ± .09[a]
2nd	1.0 ± .10[a, b]
3rd	1.0 ± .09 [b]

[a, b] Values in the same column with different superscripts are different (P < .05; Appendix Table 32).

Table 13. Least-squares means (±SE) for serum NEFA (μmol/ml) concentrations by parity for lactating Holstein cows from d 7 to 87 postpartum.

Parity	$\bar{x}$ ± SE
1st	515 ± 72[a]
2nd	443 ± 78[a, b]
3rd	386 ± 68[b]

[a, b] Values in the same column with different superscripts are different (P < .05, Appendix Table 33).

Table 14. Least-squares means (±SE) for IGF-I (ng/ml) concentrations[*] by parity for lactating Holstein cows from d 7 to 87 postpartum.

Parity	$\bar{x}$ ± SE
1st	78.8 ± 5.7[a]
2nd	62.1 ± 6.5[b]
3rd	53.0 ± 5.6[b]

[a, b] Values in the same column with different superscripts are different (P < .001, Appendix Table 35).

[*] Insulin-like growth factor-I concentrations in follicular fluid.

SERUM INSULIN CONCENTRATIONS

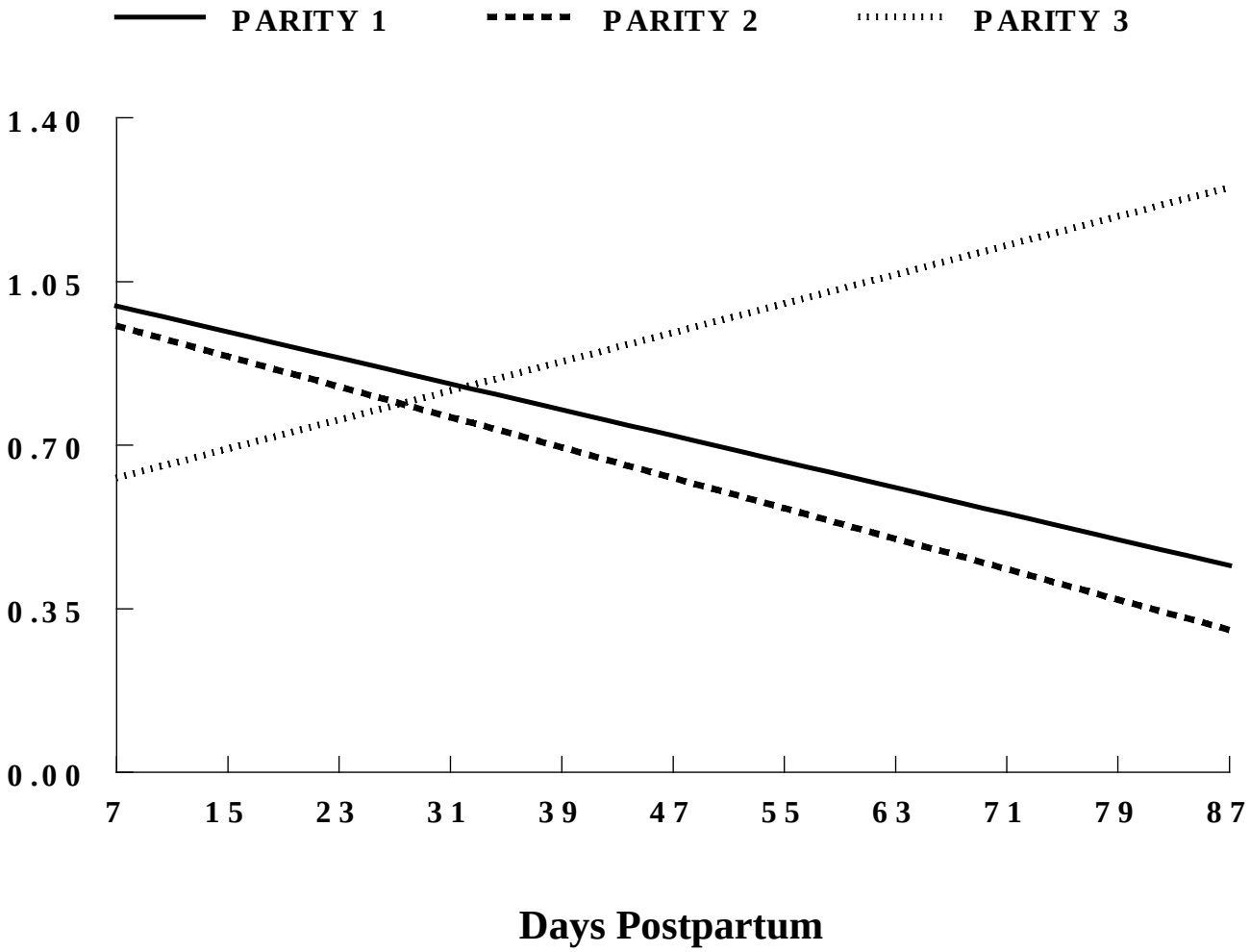


Figure 10. (Appendix Table 32) Serum insulin concentrations (ng/ml) at aspiration by parity for lactating Holstein cows.

SERUM NEFA CONCENTRATIONS

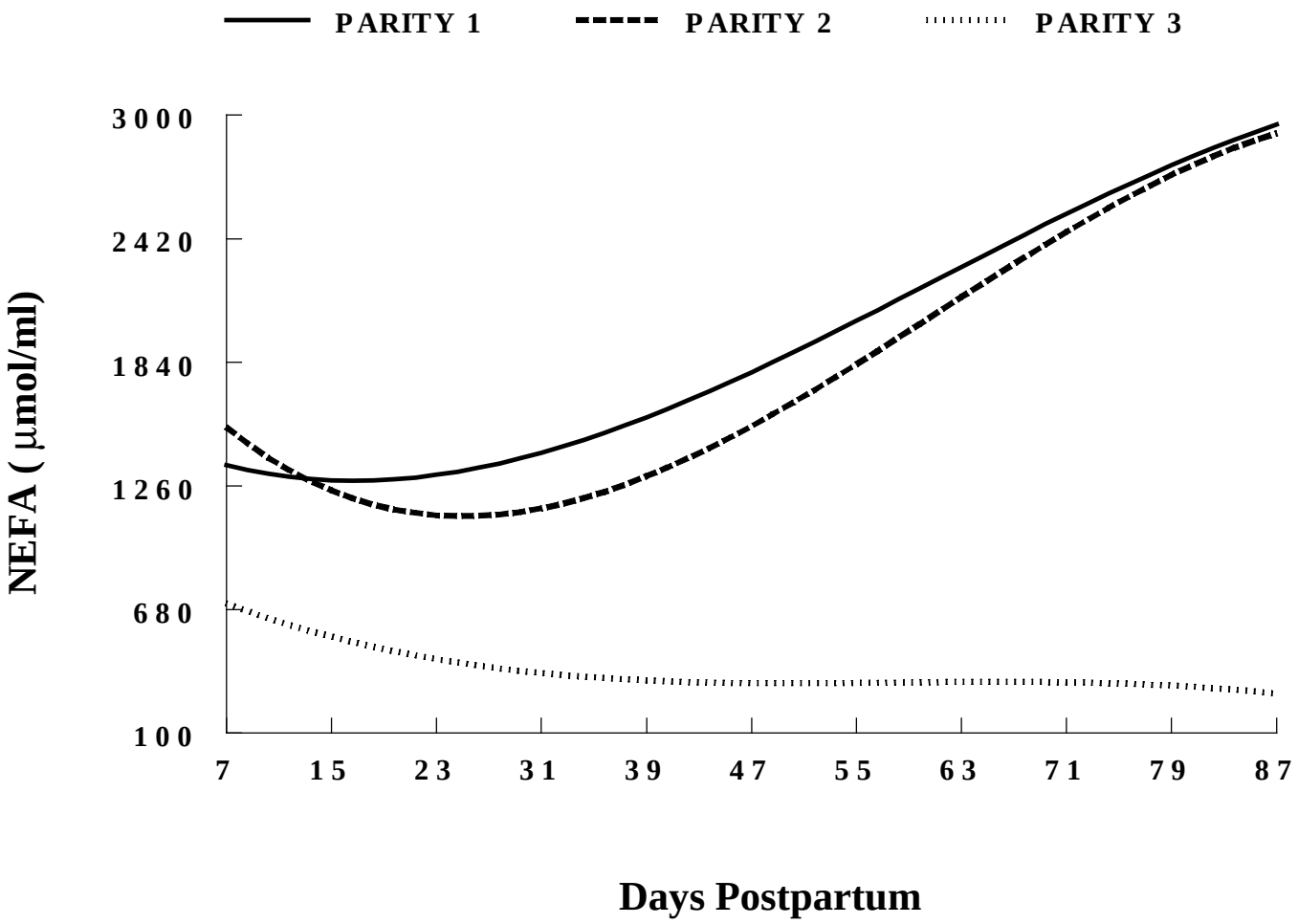


Figure 11. (Appendix Table 33) Serum NEFA concentrations at aspiration by parity for lactating Holstein cows.

ESTROGEN CONCENTRATIONS

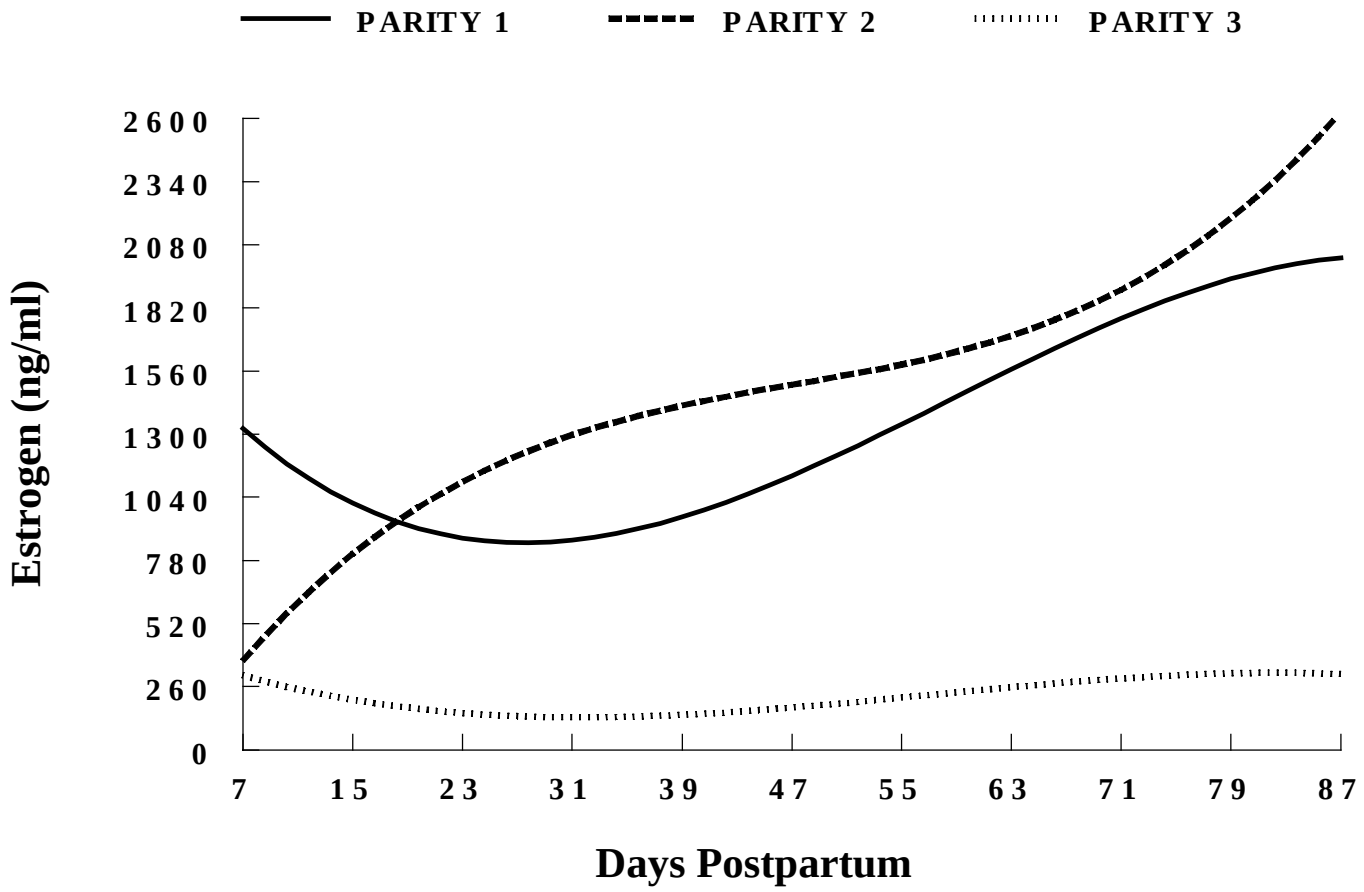


Figure 12. (Appendix Table 34) Follicular fluid estradiol concentrations at aspiration by parity for lactating Holstein cows.

Cattle from the 3rd parity had the least amount of change in E_2 concentration and the follicular fluid E_2 concentration were lower for 3rd parity in comparison with the other 2 parity groups (Figure 12). However, parity as a main effect had no significant affect on E_2 concentrations in follicular fluid. Follicular fluid concentrations of T (2.7 ± .3), P_4 (265 ± 51) and IGF-I (65 ± 2.3), were determined but were not significantly ($P < .001$) affected by the days postpartum by parity or calving season. However, IGF-I concentrations were significantly ($P < .001$; Appendix Table 35) affected by parity without the interaction by days postpartum. First lactation animals had the highest mean IGF-I concentrations in follicular fluid with older cattle being the lowest (Table 14).

Oocyte competence and development: The oocytes recovered by TVFA procedures from cows within the first 42 d postpartum (groups 1, 2 and 3) at each aspiration session were placed together in a category the "early group" and kept separately from the oocytes recovered from cows aspirated later, d 42 to 84, (groups 4, 5 and 6) named the "late group". The two groups of oocytes were than matured, fertilized and cultured in vitro, but kept in separate groups throughout each step. The overall development was poor and no oocytes developed past the initial cleavage to 2-cell stage. The total number of oocytes cultured in the two groups was 272 and 255, respectively. A Chi-square analysis showed that there was no significant difference in percentage of development between the two groups of oocytes. However, there was a significant difference between the percentages of development for oocytes recovered in winter vs. fall. Both groups had very high percentages of incompetent oocytes (75.3 % vs. 67.8 %). Only 21 % and 29 % matured with very low percentage cleavage (4.0 % vs. 3.1 %), respectively. For the oocytes recovered form the cows aspirated in early days postpartum grouping, during winter, only 11% matured with 0 % cleavage. In the fall 33.6% maturation took place and 10% cleaved. During the winter, 19% of the oocytes recovered from cows in later days postpartum grouping matured and only 1% cleaved compared to 40.7% maturation and 6% cleavage in the fall. The analysis of variance of development showed a lower ($P < .01$; Appendix Table 36) mean developmental score (1.16 ± .03) for winter than (1.53 ± .03) for fall aspirated oocytes (Table 15).

Table 15.(Appendix Table36) The competence[1] and development of oocytes recovered from Holstein cows with TVFA procedures, in Winter and Fall.

	Winter	Fall
Early Category:[*]		
Total Oocytes	n = 162	n = 110
Incompetent Oocytes %	88.3	56.4
Matured Oocytes %	11.7	33.6
Cleaved Oocytes %	0	10
Late Category:[**]		
Total Oocytes	n = 137	n = 118
Incompetent Oocytes %	80.3	53.4
Matured Oocytes %	19	40.7
Cleaved Oocytes %	1	6
Developmental Score:	Least-squares Means	
$\bar{x} \pm SE$	$1.16 \pm .03^a$	$1.53 \pm .03^b$

[a, b] Values in same row are significantly different ($P < .001$)

[1] difference between categories was not significant by χ^2,
but there was a significant ($P < .001$) difference between, fall and winter by χ^2

[*]Early category, cattle aspirated within the first 42 d postpartum (6 wk).
[**]Late category, cattle aspirated during wk 6 throughout wk 12.

DISCUSSION

Cow Measures: Body condition scores measured at various times pre- and postpartum (Figure 1) in this study were either at or below recommended BCS. Generally, cows that calve with BCS of about 3.25 to 3.75 perform best in terms of health, peak milk yield and fertility (Boisclair et al., 1986; Britt et al., 1992; Edmonson et al., 1989). Boisclair et al. (1986) reported a prepartum BCS of 3.5 that was similar to the 3.4 found in of our study at 90 d prepartum. However, they found a linear increase in BCS and BW with an increase in prepartum energy intake. Also, Gallo et al. (1996) reported a consistent BCS throughout the prepartum period that was higher than the current study that showed a decrease in BCS for each parity group over the prepartum period. The environment of the cows and the way in which they were managed might have contributed to conflicting results. Younger cattle were at a higher risk of losing BCS than older cattle (Figure 1). Grimmard et al. (1995) concluded that primiparous cows appeared much more sensitive to under-nutrition than multiparous cows. Younger cattle gave priority to utilizing energy for growth and body maintenance rather than milk production which becomes more exacerbated under stressful conditions (Ray et al., 1992).

Cows had higher BCS in summer compared to winter at calving. The lower BW and lower BCS for cows that calved in winter is most likely due to carry-over effects of summer, fall when they were in the last three months of their gestations. Cows that calved in summer were more expose to the effects of spring during gestation, suggesting that spring was more favorable than summer for maintaining BCS and BW. Most likely cows that calved in summer where exposed to more negative effects of heat stress than cows that calved in winter as indicated by the negative association of change in BCS from calving to aspiration with calving season. Moore et al. (1991) reported that late gestation heat stress had definite carry-over effects on postpartum productive and reproductive traits. Fuquay (1981) indicated that when average daily temperature exceeded 21°C milk yield decreased substantially, and there were negative effects on the duration and the intensity of estrus, conception rate, embryo survival, and days open. The impact of high daily temperatures became more severe when minimal daily temperatures failed to decrease well below 21°C. Elevated body temperature, one measure of heat stress in cattle, can reduce embryonic survival and milk yield. Araki et al. (1984) concluded that lactating cows were much more sensitive to the impact of the environment on reproductive and productive performance than dry cows. This might explain the difference of the effect of calving season on BCS at calving and aspiration.

The older cattle had a higher milk yield than younger cattle. Also, the positive correlation ($r = 0.8$) between milk yield during the week of aspiration and peak milk production indicated that older cattle had a higher peak milk yield. Pedron et al. (1993) found that the change in BCS in early lactation was related to peak milk production and postulated that this might explain the differences in mobilization of body reserves for cows of different parities. They reported a milk yield of 38 kg and an increase in BCS (after a initial decrease) at 10 wk postpartum for cows calving at a BCS of 3.0. Our results were similar with the BCS at calving of 3.0 and an increase at wk 11 postpartum.

The overall decrease in BCS in our study was approximately 1.0 point over all three the parity groups compared to 0.65, 0.62 and 0.5 reported by Gallo et al. (1996), Domecq et al. (1997b) and Pedron et al. (1993). According to Fonesca et al. (1983),

Villa-Godoy et al. (1988), and Spicer et al. (1990) high producing cows experience severe weight loss during the first 3 to 5 wk after calving. The adverse effect of this weight loss on developing follicles can be detrimental and lead to defective follicles and low levels of P_4 that are associated with low fertility (Butler and Smith, 1989). Oocyte quality score was affected by BCS at aspiration and our data showed that a decrease in BCS could lead to an almost 42% decrease in oocyte quality score. But, cows that consume sufficient dry matter during this period apparently have healthy follicles and maintain high fertility (Staples et al., 1990). A decrease in body condition score of more than 1.0 point during the first 5 wk after calving leads to lower fertility at first service (Fonesca et al. 1983; Butler and Smith, 1989).

Milk yield had a negative association with BCS and therefore, an increase in milk yield for the higher producers used in our study was related to a decrease in BCS at aspiration. Bauman and Currie (1980) stated that energy reserves of dairy cows supported > 30% of milk yield during the 1st month of lactation and that lactation utilization of body reserves occurred until milk yield decreased to < 80% of peak yield. Waltner et al., (1993) reported that a moderate loss of BCS was associated with greater milk yield, but high rates of BCS loss might have been associated with diminished milk yield rather than with potential yield. In contrast to the findings of Ray et al. (1992), and Kappel et al. (1984), milk yield was not affected by maximum temperature, wind chill factor or calving season, possibly because environmental measures were not extreme (Fuquay, 1981).

Ovarian and Oocyte Measures: The total number of follicles (Figure 3) and number of oocytes (Figure 7) recovered followed an almost similar trend postpartum for all three parity groups. The total number of oocytes was lower than total number of follicles observed and is most likely due to technical difficulties associated with ultrasound retrieval (Pieterse et al., 1991; Scott et al., 1994). The total number of follicles from d 7 to 87 for 1st parity animals did not increase at the same rate as for intermediate parity cattle and might reflect a higher rate of follicular development for 2nd parity cattle. Total number of follicles and number oocytes recovered from older cattle (3rd parity) was less than for younger cattle. Lonergan (1992) found that in general more oocytes were recovered from heifers than mature cows. Moreno et al. (1992) reported that the number of oocytes recovered was significantly greater in heifers than in cows. In contrast, Grimmard et al. (1995) concluded that parity had an effect on reproductive efficiency, suggesting that the process of follicular recruitment was slower and pulsatile LH secretion was lower in younger cattle comparing to older cattle.

There seems to be a relationship between follicle diameter (Figure 4), total number of follicles (Figure 3), and number of oocytes retrieved (Figure 7), even though the trend for follicle diameter was cubic, while linear trends were found for total number of follicles and oocytes. For 1st, and 2nd parity cattle the total number of follicles increased when follicle diameter decreased after 5 wk postpartum. For 3rd parity cattle the number of follicles decreased when there was an increase in follicle diameter. Dominquez et al. (1995) reported that the mean proportion of normal oocytes decreases with increasing follicular size, suggesting a normal process of atresia. However, for older cattle the amount of change in follicle diameter was not as large as the change in the number of follicles. The initial increase over the first 3 wk was most likely related to

endocrine (Figures 9, 10, and 12) and metabolite (Figure 11) changes and re-activation of ovarian activity within the first 3 wk postpartum.

Grimmard et al. (1995) reported that the number of small and medium sized follicles and the size of the largest follicle increased with time postpartum in mulitparous cows than in primiparous cows. Lucy et al. (1991) have shown a decrease in the number of smaller follicles and increase in the number of follicles as days postpartum increased. Hafez et al. (1987) reported that smaller follicles grow and move into larger classes of follicles (≥ 10mm) as time postpartum increases and they are not replenished during the first 25d postpartum. This might explain the increase in follicle diameter for the 1st, and 2nd and 3rd lactation cattle during the first 25 to 30 d followed by a decrease in overall diameter.

Pre- and postpartum energy levels have a significant influence on the size and the number of follicles. When low nutritional levels are combined with poor body condition, the growth of small follicles into larger follicles is suppressed, with a substantial decrease in the number of follicles present (Perry et al., 1991), and nutrition may be reflected in BCS changes seen by parity in the current study. The greater number of large follicles observed in adequately fed cattle when compared with under-nourish cattle suggests that the physiological mechanisms responsible for ovarian follicular growth and dominance may become functional at an earlier stage in adequately fed cows compared with underfed ones (Grimmard et al. 1995). In support, Kendrick et al. (1999) reported that a low energy diet reduced milk yield, BCS, IGF-I and E_2 concetrations in follicular fluid and serum P_4 concetrations, and had a negative impact on oocyte quality.

The size of the largest follicle at the day of aspiration (Figure 5) increased rapidly over increasing days postpartum and was significantly correlated ($r = .24$) with BW, but there was no parity effect. The size of the largest follicle was negatively correlated to the serum NEFA concentrations (Grimmard et al. 1995). In the current study the correlation ($r = -.03$, $P > .05$) between the diameter of the largest follicle and serum NEFA concentrations was is almost significant. Thatcher et al. (1992) reported that the dominant follicle controls the development of other follicles. Suppression of the size of subordinate follicles was associated with a decrease in E_2 and an increase in P_4 concentrations in follicular fluid (Badinga et al., 1992). Lewis et al. (1984) reported that the prepartum environment affected the average diameter of the largest follicle and had an indirect effect on the diameters of all other follicles present on the ovary. There was no correlation found between follicle diameter and follicular IGF-I concentration. In contrast, the concentration of IGF-I in follicular fluid was related positively to the size of the dominant follicle (Badinga et al., 1992). Reports have shown a relationship between bovine oocyte quality and follicle diameter (Britt et al. 1992; Lonergan et al. 1992).

Initially oocyte quality score (Figure 8) increased with the increasing number retrieved and then quality declined after the first 7 to 8 wk for 1st, and 2nd and 3rd lactation cattle, while oocyte numbers retrieved continued to increase. For older cattle the decreased numbers of follicles and oocytes were associated with an increase in oocyte quality score. Das et al. (1996) reported that the presence of a CL significantly reduced the recovery rate and quality of the oocytes collected. Zhang et al. (1991) recovered oocytes of greater quality from cows than heifers.

Endocrine and Metabolite Measures in Serum: When oocyte recovery and quality were related to the presence and diameter of a CL, and P_4 concentration, younger cattle had a

CL of greater size and had higher serum P_4 concentrations as lactation advance than older cattle. Even though the detectable concentration of serum P_4 was only observed at d 31 for 1st lactation cattle and after wk 2 for 2nd and 3rd, lactation cattle, the concentrations increased with the increasing CL diameter. The serum P_4 did not showed much variation for older cattle as the days postpartum progressed and CL diameter decreased, suggesting an earlier functional CL. The differences between P_4 concentrations measured in the blood and the detection of CL through rectal palpation or ultrasonography might be because while a CL is morphologically present, it might not produce a significant amount of P_4 (Gutiérrez et al., 1996). The functional status of the CL changes during the estrous cycle. At the beginning of the cycle the CL forms gradually and the production of P_4 increases progressively. Therefore, in some cases a CL might be physically present before the peripheral concentrations of P_4 reach the value of 1 ng/ml that is generally used as the criteria for an active CL (Hernández-Cerón et al.,1993). At the end of the estrous cycle there is a rapid decline in P_4 concentration, which is not accompanied by a significant decline in CL diameter. Therefore, the CL is physically present, but not functionally active (Gutiérrez et al., 1996).

The start of luteal activity was related to time when detectable concentrations of P_4 were observed in serum. The variation in start of luteal activity among the parity groups made it more difficult to estimate an average day postpartum when luteal activity began. De Vries and Veerkamp (2000) observed luteal activity at 29.7 d postpartum on average. However, the range of luteal activity was observed as early as d 10 postpartum to as late as d 97. Their data were supported other studies (Darwash et al., 1997), but were 9 to10 d later than data of Beam and Butler (1989) and Canfield et al. (1990). Serum P_4 concentrations reached 1 ng/ml at d 47 and d 39 for 1st, and 2nd and 3rd parity cattle, respectively, while the P_4 older cattle was above 1 ng/ml at d 7. Vizcarra et al. (1998) reported that a serum P_4 concentration of greater than 1 ng/ml for more than 1 wk indicated the start of luteal activity. Higher glucose concentrations were measured in cows where luteal activity had began compared with cows that had no luteal activity. Insulin concentrations measured were not different regardless if luteal activity had started or not.

In the current study oocyte quality was significantly influenced by the ratios of serum glucose to IGF-I and to serum NEFA concentrations, suggesting that oocyte quality for older cattle would be higher than for younger cattle based on a much lower serum NEFA and glucose concentrations found in older cattle. Harrison et al. (1990) found a negative correlation between NEFA concentrations in serum and glucose and observed that NEFA concentrations were the greatest at wk 1 postpartum and then gradually decreased over the next 11 wk thereafter. Vizcarra et al. (1998) implied that BCS at parturition and the level postpartum nutrition influence the occurrence of luteal activity and the concentrations of glucose, insulin, and NEFA in plasma. Cows had higher levels of glucose, insulin, and NEFA in their plasma when they calved in good body condition compared to cows calving in poor body condition. In support Butler (2000) reported that a negative EB delayes the time of first ovulation through inhibition of LH pulse frequency and low levels of blood glucose, insulin and IGF-I that collectively restrain E_2 production by dominant follicle.

The significant difference in NEFA concentrations measured between younger and older cattle in the current study may be related to the other differences observed

regarding total number of follicles (Figure 3), total number of oocytes (Figure 7), and oocyte quality score (Figure 8). Younger cattle had a higher increase in serum NEFA concentrations compared with older cattle that had a slight decrease in NEFA concentrations. The least amount of variation in BCS was for older cattle (>3rd lactation), indicating that they were less affected by early lactation effects than younger cattle. Changes in BCS were reflected in the serum NEFA concentrations that changes very little with advancing days postpartum in older cattle compared to younger cattle, suggesting that younger cattle were experiencing a greater negative EB balance than older cattle, and most likely explains the decrease in follicle diameter and oocyte quality even though their follicle population was greater than for older cattle.

Serum NEFA and serum IGF-I concentrations in serum were negatively correlated (r = -0.19). Plasma concentrations of NEFA and IGF-I are good indicators of adipose tissue mobilization for energy, and increases of NEFA in cows on low energy diets reflects the dietary induction of a negative energy balance. A low nadir of energy balance was correlated with a delay in the postpartum start of luteal activity (De Vries and Veerkamp, 2000). A decrease in energy levels caused a reduction in the IGF-I concentration, possibly the reason for a decrease in growth rate of the developing follicle. The daily growth rate and total growth of the dominant follicle were affected by the fluctuation in energy intake, both the rate and total growth were higher for cows on a high energy diet compared to cows on a low energy diet (Thatcher et al., 1992). In the current study the correlation (r = .05) was positive between the concentration IGF-I in serum and in follicular fluid. Ronge et al. (1988) reported the same finding and suggested that IGF-I derived from serum can be considered as a possible source of intraovarian of IGF-I.

There was a linear effect of the days postpartum by parity interaction on serum insulin concentrations and a main effect of parity. Serum insulin concentrations in the increased for older cattle, while, they decreased for younger cattle (Figure 10). Denbow et al. (1986) reported that there was a significant seasonal effect on plasma insulin concentrations. In contrast, Herbein et al. (1985) reported a quadratic effect of days in milk on plasma insulin concentrations, but they assessed insulin throughout the entire lactation. The decreasing insulin concentrations for 1st lactation cattle suggest a nutritional stress as energy restriction decreased insulin concentrations in heifers (Harrison and Randel, 1986). Plasma insulin were decreased in cows that had negative EB (Beam and Butler, 1998; Butler, 2000) and insulin is known to stimulate bovine follicular cells in vitro (Spicer et al., 1993). There was an inverse relationship between the serum insulin (Figure 10) and serum NEFA concentrations (Figure 11) across parity. Insulin is one of the primary regulators of blood glucose concentrations in non-ruminants and in ruminants (Exton et al. 1970). Insulin inhibits the rate of adipose tissue lipolysis and stimulates the rate of lipogenesis, the net effect of which is to direct NEFA to adipose tissue and retain it there (Rose et al., 1996).

We found no effects of either days postpartum or season on serum glucose. Herbein et al. (1985) reported a significant quadratic relationship between plasma glucose and days postpartum for a 305 d lactation, while Kappel et al. (1984) reported first lactation animals had higher glucose concentrations than cows in second or later lactations. In contrast, glucose concentrations they found were different between winter and summer calving seasons.

Endocrine and Metabolite Measures in Follicular Fluid: Younger cattle had a biphasic response for E_2 concentrations in follicular fluid (Figure 12) over days postpartum, while E_2 for older cattle showed no marked change, but was substantially lower than for younger cattle. Estradiol produced by ovarian follicles is the primary hormone stimulating estrous behavior in cattle (Hafez et al., 1987). Reduced IGF-I secretion caused by negative EB could alter ovarian follicular E_2 production, thereby suppressing expression of estrus (Spicer et al., 1990).

The ratio between P_4 and T concentrations in follicular fluid had an effect on the total number of follicles, while oocyte quality score was affected by T concentrations in follicular fluid and the ratios between E2 and P_4, and E_2 and T. Borromeo et al. (1996) found a close relationship between P_4, E_2 and T patterns in follicular fluid. Steriod hormone content in follicular fluid reflects the synthetic capacity of the granulosa cells and theca layers. Increased luteinization of a normal follicle after ovulation was indicated by a significant increase in P_4 and decrease in E_2 and T. Therefore, the ratio of E_2: P_4 in follicular fluid can be an indication of follicle health. The oocyte quality score was affected by the ratio between E_2:P_4 in follicular fluid (Borromeo et al., 1996, Beam and Butler 1998). Grimes et al. (1986) found a higher percentage of oocytes matured when the E_2:P_4 was less than one, compared to a more positive ratio greater than one. Ahmad et al. (1995) reported that prolonged high concentrations of E_2 were associated with persistent follicles and may be a direct cause to low fertility. Beam and Butler (1998) suggested that steriodogenesis was decreased by non-ovulatory dominant follicles cells, thereby preventing final follicular maturation.

There was a significant difference in IGF-I concentrations in follicular fluid by parity, younger cattle had the highest IGF-I concentration compared with the two older parity groups, suggesting the results of Echterncamp et al. (1990). In postpartum dairy cows the levels of IGF-I were 40-50% higher during the first 2 wk in cows in which the the dominant follicle would ovulate as compared to levels in cows with non-ovulatory follicles (Beam and Butler, 1998).

There was no relationship between E_2 and IGF-I concentrations in follicular fluid. However there was a significant ($r = -.21$) negative correlation between IGF-I concentrations in follicular fluid and serum NEFA concentrations. In contrast, Echternkamp et al. (1990) and Beam and Butler (1998) reported that there was a positive correlation between IGF concentrations and E_2 concentration in follicular fluid. Our findings support Spicer et al. (1993) who showed that the IGF-I concentrations were higher in follicular fluid of large estrogen-active follicles than in the follicular fluid from small to medium estrogen-inactive follicles. Hammond et al. (1988) provided evidence that IGF-I concentrations in follicular fluid increase with follicular size and that the concentrations of IGF-I in follicular fluid were positively correlated to P_4 concentrations across individual follicles. However, there was no correlation between P_4 and IGF-I concentrations in the current study.

Oocyte Competence and Development: Overall development was low with no further development passed the initial cleavage to 2-cell stage. The percentage of development was higher for oocytes recovered during winter vs. fall. In contrast, similar studies (Chauhan et al., 1999; Snijders et al., 1999; Konishi et al., 1996; Shioya et al., 1988) have shown much higher cleavage and development rates, especially for grade 4 and 3 oocytes. Poor development may be related to the very high percentage of grade 1 or

incompedent oocytes retrieved compared to the percentages for grades 4, 3, and 2. Snijders et al. (1999) reported that there was no difference in cleavage rates and blastocyst formation between grade 4 and 3 oocytes, while the cleavage and subsequent development for grade 2 and grade 1 oocytes were substantially lower. Bovine oocytes failed to mature or had a low maturation rate when cultured in the absence of cumulus cells (Kobayashi et al., 1994). The frequency of fertilization of denuded oocytes was significantly less than after maturation of cumulus cell-enclosed oocytes (Ball et al., 1983). Even though individual oocyte quality scores in the current study could not be assigned to a follicle size, the percentage of smaller follicles observed and aspirated was higher than the percentage medium and large follicles aspirated. Therefore, the largest number of oocytes recovered was from the smaller follicles. Small follicles contain mostly oocytes with fully expanded or no cumulus layers and a very heterogeneous ooplasm (Blondin et al., 1996).

De Loos et al. (1989) reported that oocytes of poor quality have a significantly diminished ability to mature and develop under in vitro conditions. Konishi et al. (1996) reported that oocytes needed cumulus cells for proper nuclear and cytoplasmic maturation that is essential for further development. Cleavage and blastocyst formation rate were higher for oocytes recovered from cows with BCS between 3.3 to 4.0 compared with cows with a BCS between 2.5 and 1.5 (Snijders et al. (1999). They postulated that fertility was influenced by oocyte quality. Bandiga et al. (1985) reported that fertility decreased with age and that it was probably related to lactational stress and subsequent problems associated with calving multiparous females.

Summary: This study demonstrated that conditions related to early lactation have a negative effect on oocyte quality and endocrine measures of dairy cattle and that animals of different ages are preferentially affected. Body condition score of dairy cattle were affected by stage of lactation, parity, environment, especially calving season, and the effect was reflected in the fluctuations of metabolites and hormones in the blood and follicular fluid. Increased serum NEFA concentrations with simultaneous decreases in serum insulin concentrations for younger cattle implied a more negative EB status than for older cattle. Younger cattle had higher E_2 and IGF-I concentrations in follicular fluid associated with a higher number of total follicles and number of oocytes, compared to older cattle. However, reproductive performance of younger cattle, as determined by oocyte quality, seemed to be reduced and less favorable than for older cattle. Cattle in 4^{th} and greater parity showed very little change in BCS and hormone and metabolite measures during early lactation, with no apparent decrease in oocyte quality, despite the aging effect on follicle numbers. The changes in follicular dynamic patterns and oocyte quality in younger and mature cattle may be an indication of the impact of early lactational performance on subsequent fertility.

LITERATURE CITED

Adashi, E.Y., C.E. Resnick, A.J. D'Ercole, M.E. Svoboda, and J.J. van Wyk. 1985. Insulin-like growth factors as intraovarian regulators of granulosa cell growth and function. Endocr. Rev. 6:400-420.

Ahmad, N., F.N. Schrick, R.L. Butcher, and E.K. Inskeep. 1995. Effect of persistent follicles on early embryonic losses in beef cows. Biol Reprod. 52:1129-1135.

Araki, C.T., R.M. Nakamura, L.W.G. Kam, and N. Clarke. 1983. Effect of lactation on diurnal temperature patterns of dairy cattle in hot environments. J. Dairy Sci. 67:1752-1760.

Ball, G.D., M.L. Leibfried, R.W. Lenz, R.L. Ax, B.D. Bavister, and N.L. First. 1983. Factors affecting successful in vitro fertilization of bovine follicular oocytes. Biol Reprod. 28:717-725.

Badinga, L., R.J. Collier, W.W. Thatcher, and C.J. Wilcox. 1985. Effects of climatic and management factors on conception rate of diary cattle in subtropical environment. J. Dairy Sci. 68:78-85.

Badinga, L., M.A. Driancourt, J.D. Savio, D. Wolfenson, M. Drost, R.L. De La Soata, and W.W. Thatcher. 1992. Endocrine and ovarian responses associated with the first–wave dominant follicle in cattle. Biol. Reprod. 47:871-883.

Bauman, D. E., and W. B. Currie. 1980. Partitioning of nutrient during pregnancy and lactation:a review of mechanics involving homeostasis and homeorhesis. J. Dairy Sci. 63:1514-1529.

Beam, S.W., and W.R. Butler. 1997. Energy balance and ovarian follicle development prior to the first ovulation postpartum in dairy cows receiving three levels of dietary fat. Biol Reprod. 56:133-142.

Beam, S.W., and W.R. Butler. 1998. Energy balance, metabolic hormones, and early postpartum follicular development in dairy cows fed prilled lipid. J. Dairy Sci 81:121-131.

Blondin, P., and M.A. Sirad. 1995. Oocyte and follicular morphology as determining characteristics for development competence in bovine oocytes. Mol Reprod. Dev. 41:54-62.

Blondin, P., K. Coenen, L.A. Guibault, and M.A. Sirad. 1996. Superovulation can reduce the developmental competence of bovine embryos. Theriogenology 46:1191-1203.

Boisclair, Y., D.G. Grieve, J.B. Stone, O.B. Allen, and G.K. MacLeod. 1986. Effect of prepartum energy, body condition, and sodium bicarbonate on production of cows in early lactation. J. Dairy Sci. 69:2636-2647.

Bolt, D. J., and D.W. Caldwell. 1992. Inhibin-like activity in plasma from ovariectomized ewes and serum albumin: pitfalls for radioimmunoassay. Domest. Anim. Endocrinol. 9:57-69.

Boni, R.S., Roviello, and L. Zicarelli. 1996. Repeatd ovum pick-up in Italian Mediterranean buffalo cows. Theriogenology 46:899-909.

Borromeo, V. S. Bramani, A. Berrini, G. Sironi, M. Finazzi, F. Cremonesi, and C. Secchi. 1996. Growth hormone but not prolactin concentrations in the fluid of bovine ovarian cysts are related to the cystic stage of luteinization. Theriogenology 46:481-489.

Brackett, B.G. and G. Oliphant. 1975. Capacitation of rabbit spermatozoa in vitro. Biol Reprod.12:260-274.
Britt J.H. 1992. Impacts of early postpartum metabolism on follicular development and Fertility. Bov Proc. 24:39-43.
Butler, W .R., and R.D. Smith. 1989. Interrelationships between energy balance and postpartum reproductive function in dairy cattle. J. Dairy Sci. 72:767-783.
Butler, W.R., 2000. Nutritional effects on resumption of ovarian cyclicity and conception rate in postpartum dairy cows. Anim. Sci. in press.
Canfield, R.W. and W. R. Butler. 1990. Energy balance and pulsatile luteinizing hormone secretion in early postpartum dairy cows. Dom. Anim. Endocrinol. 7:323-330.
Chauhan, M.S., S. Nadir, T.L. Bailey, A.W. Pryor, S.P. Butler, D.R. Notter, W.H. Velander, and F.C. Gwazdauskas. 1998. Bovine follicular dynamics, oocyte recovery, and development of oocytes microinjected with green fluorescent protein construct. J. Dairy Sci. 82:918-926.
Das, G. K., G.C.Jain, V.S. Sloanki, and V.N. Tripathi. 1996. Efficacy of various collection methods for oocyte retrieval in Buffalo. Theriogenology 46:1403-1411.
Darwash, A.O., G.E. Lamming, and J.A. Wooliams. 1997. The phenotype association between interval to postpartum ovulation and traditional measures of fertility in dairy cattle. J. Anim. Sci. 65:9-16.
De Loos, F., C. Van Vliet, P. van Maurik, and Th. A.M. Kruip. 1989. Morphology of immature Bovine oocytes. Gamete Res.24:197-204.
Denbow, C.J., K.S. Perera, F.C. Gwazdauskas, R.M. Akers, R.E. Pearson, and M.L. McGilliard. 1986. Effect of season and stage of lactation on plasma insulin and glucose injection in Holstein cattle. J. Dairy Sci. 211-216.
De Vries, M.J., and R.F. Veerkamp. 2000. Energy Balance of Dairy Cattle in Relation to Milk Production. J. Dairy Sci. 83:62-69.
Domecq, M.M. 1995. Effects of body condition, reproductive status and breed on follicular population and oocyte quality in cows. Theriogenology. 43:1405-1418.
Domecq, J.J., A.L. Skidmore, J.W. Lloyd, and J.B. Kaneene. 1997b. Relationship between body condition score and conception at first artificial insemination in a large diary herd of high yielding Holstein cows. J. Anim. Sci. 80:113-120.
Dominquez, M.M., 1995. Effects of body condition, reproductive status and breed on follicular population and oocyte quality in cows. Theriogenology 43:1405-1418.
Echternkamp, S.E., L.J. Spicer, K.E. Gregory, S. F. Canning, and J.M. Hammond. 1990. Concentrations of insulin-like growth factor-I in blood and ovarian fluid of cattle selected for twins. Biol. Reprod. 43:8-14
Edmonson, A. J., L.J. Lean, L. D. Weaver, T. Farver, and G. Webster. 1989. A body condition scoring chart for Holstein dairy cows. J. Dairy Sci. 72:68-78.
Eisemann, J.H., A.C. Hammond, D.E. Bauman, P.J. Reynolds, S.N. McCutcheon, H.F. Tyrell, G.L. Haaland. 1986. Effect of bovine growth factor hormone administration on metabolism of growing Hereford heifers: protein and lipid metabolism and plasma concentrations of metabolites and hormones. J. Nutr. 116:2504-2515.

Exton, J.H., L.E. Mallett, L.S. Jefferson, E.H.A. Wong, N. Friedman, T.B. Miller, and C.R. Park. 1970. The hormonal control of hapatic gluconeogenesis. Rec. Progr. Horm. Res. 26:411-418.

Fonesca , F.A., J.H. Britt., B.T. McDaniel, J.C. Wilk, and A.H. Rakes. 1983. Reproductive traits of Holsteins and Jerseys. Effects of age, milk yield and clinical abnormalities on involution of cervix and uterus, ovulation, estrous cycles, detection of estrus, conception rate and days open. J. Dairy Sci. 66:1128-1147.

Fuquay, J.W., 1981. Heat stress as it affects animal production. J. Animal Sci. 52:164-174.

Gallo, L., P. Carnier, M. Cassandro, R.Mantovani, L. Bailoni, B. Contiero, and G. Bittante. 1995. Change in body condition score of Holstein cows as affected by parity and mature equivalent milk yield. J. Dairy Sci. 79:1009-1015.

Gibbons, J.R., R.L. Krisher, E.G. Faber, R.E. Pearson, and F.C.Gwazdauskas. 1994. Effects of once versus twice-weekly transvaginal follicular aspiration on oocyte recovery and embryo development. Theriogenology 42:405-419.

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

Ginther, O.J., K.Kot, L.J. Kulick, and M.C. Wiltbank. 1997. Sampling follicular fluid without altering follicular status in cattle: oestradiol concentrations early in a follicular wave. J. Reprod. Fertil. 109:181-186.

Grimmard, B., P. Humblot, A.A. Ponter, J.P. Mialot, D. Sauvant, and M. thibier. 1995. Influence of postpartum energy restriction on energy status, plasma LH and estradiol secretion and follicular development in suckled beef cows. J. Reprod. Fertil. 104:173-179.

Grimes, R.W. and J.J. Ireland. 1986. Relationship of macroscopic appearance of the surface of bovine ovarian follicles concentrations of steriods in follicular fluid, and maturation of oocytes in vitro. Biol. Reprod. 35:725-732.

Gutiérrez, A.C., L.Zarco, C.S. Galina, I.Rubio, and H. Basurto. 1996. Predictive value of palpation per rectum for detection of the CL in Zebu cattle as evaluated by progesterone concentrations and ultrasonography. Theriogenology 46:471-479.

Harrison, L. M., and R.D. Randel. 1986. Influence of insulin and energy intake on ovulation rate, luteinizing hormone and progesterone in beef heifers. J. Dairy Sci. 63:1228-1235.

Harrison, R.O., S.P. Ford J.W. Young, A.J. Conley, and A.E. .Freeman. 1990. Increased milk production versus reproductive and energy status of high-producing dairy cows. J.Dairy Sci. 73:2749-2758.

Hafez, E.S.E., 1987. Folliculogenesis, Egg Maturation and Ovualtion, In: Reproduction in Farm Animals. 5th ed. Lea & Febiger, Philadelphia, PA. p 130.

Hammond, J.M., C.J. Hsu, J.M. Klindt, L. Ainsworth, B.R. Downey, and B.K. Tsang. 1988. Gonadotropins increase concentrations of immunoreactive insulin-like growth factor I in porcine follicular fluid in vivo. Biol. Reprod. 38:304-308.

Herbein, J.H., R.J. Aiello, L.I. Eckler, R.E. Pearson, and R.M. Akers. 1985. Glugacon, insulin, growth hormone, and glucose concentrations in blood plasma of lactating cows. J. Dairy Sci. 68:320-325.

Hernández-Cerón, J., L. Zarco, and V. Lima-Tamayo. 1993. Incidence of delayed ovulation in Holstein heifers and its effects on fertility and early luteal function. Theriogenology 40:1073-1081.

Kappel, L.C., R.H. Ingraham, E.B. Morgan, L. Zeringue, D. Wilson, D.K. Babcock. 1984. Relationship between fertility and blood glucose and cholesterol concentrations in Holstein cows. Am. J. Vet Res. 45:2607-2612.

Kendrick, K.W., T.L. Bailey, A.S. Garst, A.W. Pryor, A. Ahmadzadeh, R.M. Akers, W.E. Eyestone, R.E. Pearson, and F.C. Gwazdauskas. 1999. Effects of energy balance on hormones, ovarian activity, and recovered oocytes in lactating Holstein cows using transvaginal follicualr aspiration. J. Dairy Sci. 82:1731-1740.

Kobayashi, K., S. Yamashita, and H. Hoshi. 1994. Influence of epidermal growth factor and transformiong growth factor-α on in -vitro maturation of cumulus cell-enclosed bovine oocytes in a defined medium. J. Reprod. Fertil. 100:439-446.

Konishi, M., Y. Aoagi, T. Takedomi, H. Itakura, T. Itoh, and S. Yazawa. 1996. Presence of granulosa cells during oocyte maturation improved in vitro development of IVM-IVF bovine oocytes that were collected by ultrasound-guided trnasvaginal aspiration. Theriogenology 45:573-581.

Loefler, S. H., M. J. De Vries, and Y. H. Schukken. 1999. The effects of time of disease occurrence , Milk yield, and body condition on fertility of dairy cows. J. Dairy Sci. 82:2589-2604.

Lonergan, P. 1992. Studies on the in vitro maturation, fertilization and culture of bovine follicular oocyte. Ph.D Thesis, National University of Ireland, Dublin

Lewis, G.S., W.W. Thatcher, E.L. Bliss, M. Drost, and R.J. Collier. 1984. Effects of heat stress during pregnancy on postpartum reproductive changes in Holstein cows. J. Anim.Sci. 174-186.

Lucy, M.C., C.R. Staples, F.M. Michael, and W.W. Thatcher. 1991. Energy balance and Size and number of ovarian follicles detected by ultrasonography in early postpartum cows. J. Dairy Sci. 74:473-482.

McCutcheon, S. N. and D. E. Bauman. 1986. Effect of chronic growth hormone treatment on responses to epinephrine and thyrotropin-releasing hormone in lactating cows. J. Dairy Sci. 69:44-51.

Moore, R.K., B.W. Kennedy, L.R. Schaeffer, and J.E. Moxley. 1991. Relationship between age and body weight at calving and production in first lactation Ayrshires and Holsteins. J. Dairy Sci. 74:269-278.

Moore, R.B., J. W. Fuquay, and W.J. Drapala. 1992. Effects of late gestation heat stress on postpartum milk production and reproduction in dairy cattle. J. Dairy Sci. 75:1877-1882.

Moreno, J.F., C.J. Turczynski, G. Flores-Foxworth, D.C. Kraemer. 1992. Influence of parity of the donor and presence of a CL on quantity and quality of bovine oocytes from ovarian follicles aspirated post-mortem. Biol. Reprod. 48 (Suppl. 1):169. (Abstr.).

Nebel, R.L., and M.L. McGilliard. 1993. Interactions of high milk yield and reproductive performance in dairy cows. J. Dairy Sci. 76:3257-3268.

Pedron, O.F. Cheli, E. Senatore, D, Baroli, and R. Rizzi. 1993. Effect of body condition score at calving on performance, some blood parameters, and milk fatty J.Dairy Sci. 76:2528-2535.

Perry, R.C., L.R. Corah, R.C. Cochran, W.E. Beal, J.S. Stevenson, J.E.Minton, D.D. Simms, and J.R. Brethour. 1991. Influence of dietary energy on follicular development, serum gonadotropins, and first postpartum ovulation in suckled beef cows. J. Anim. Sci 69:3762-3773.

Pieterse, M.C., P.L. Vos Th A.M. Kruip, Y.A. Wurth, Th H. van Beneden. 1991. Transvaginal ultrasound guided follicular aspiration of bovine oocytes. Theriogenology. 44:915-923.

Ray, D.E., T.J. Halbach, and D.V. Armstrong. 1992. Season and lactation number affects on milk production and reproduction of dairy cattle in Arizona. J. Dairy Sci. 75:2976-2983.

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

Rose, M.T., Y. Obara, H. Fuse, F. Itoh, A. Ozawa, Y. Takahashi, K. Hodate, and S. Ohashi. 1996. Effect of growth hormone -releasing factor on the response to insulin of cows during early and late lactation. J. Dairy Sci. 79:1734-1745.

Roy, S.K., and G.S.Greenwald. 1991. In vitro effects of epidermal growth factor, insulin-like growth factor-I, fibroblast growth factor, and follicle-stimulating hormone on hamster follicular deoxyribonucleiec acid synthesis and steriodogenesis. Biol Reprod. 44:889-896.

SAS® Institute, INC. 1999. SAS User's Guide: Statistics. Cary, NC, USA. Edition 6.12.

Savio, J.D., L. Keenan, M.P. Boland, N. Hynes, and J.F. Roche. 1988. Pattern of growth of dominant follicles during the oestrous cycle of heifers. J. Reprod. Fertil. 83:663-671.

Scott, C.A., L. Robertson, R.T. de Moura, C. Paterson, and J.S. Boyd. 1994. Technical aspects of transvagional ultrasound-guided follicular aspiration in cows. Vet. Rec. 134:440-443.

Shioya, Y., M. Kuwayama, M. Fukushima, S. Iwassaki, and A. Hanada. 1988. In vitro fertilization and cleavage capability of bovine follicular oocytes classified by cumulus cells and matured in vitro. Theriogenology. 30:488-499.

Smith, L.C., M. Olivera-Angel, N.P. Groome, B. Bhatia, and C.A. Price. 1996. Oocyte quality in small antral follicles in the presence or absence of a large dominant follicle in cattle. J. Reprod. Fertil. 106:193-199.

Snijders, S.E.M., P. Dillon, D.O'Callaghan, and M.P.Boland. 2000. Effect of genetic merit, milk yield, body condition and lactation number on in vitro oocyte development in dairy cows. Theriogenology 53:981-989.

Spicer, L.J., W.B. Tucker, and G.D. Adams. 1990. Insulin-like growth factor-I in dairy cows: Relationship among energy balance, body condition, ovarian activity, and estrous behavior. J. Dairy Sci. 73:929-937.

Spicer, L.J., E. Alpizar, S.E. Echternkamp. 1993. Effects of insulin, insulin-like growth factor-I, and gonadotropins on granulosa cell proliferation, progesterone production, estradiol production, and (or) insulin-like growth factor-I production in vitro. J. Anim. Sci. 71:1232-1241.

Staples, C.R., W.W. Thatcher, and J. H. Clark. 1990. Relationship between ovarian activity and energy status during the early postpartum period of high producing dairy cows. J. Dairy Sci. 73:938-947.

Thatcher , W.W. , J. Savio, C.R. Staples, M.C. Lucy, and R.L. de La Sota. Nutritional and hormonal factors influencing follicle development in cattle. 1992. Bov. Proc. 24:44-50.

Villa-Goday, A., T.L. Huges, R.S. Emery, L.T. Chapin, and R.L. Fogwell. 1988. Association between energy balance and luteal function in lactating dairy cows. J. Dairy Sci. 73:2759-2765.

Vizcarra, J.A., R.P. Wetteman, J.C. Spitzer, and D.G. Morrison. 1998. Body condition at partirution and postpartum weight gain influence luteal activity and concentration of glucose, insulin and non-esterified fatty acids in plasma of primiparous beef cows. J. Anim. Sci. 76:927-936.

Waltner, S. S., J.P. McNamara, and J.K. Hillers. 1993. Rlaetionship of body condition score to production variables in high producing Holstein dairy cattle. J. Dairy Sci. 76:3410-3419.

Wandji, S.A., J.J.Eppig, and J.E. Fortune. 1996. FSH and Growth factors affect the growth and endocrine function in vitro of granulosa cells of bovine preantral follicles. Theriogenology 45:817-832.

Weber, M. S., P.L. Boyle, B.A. Corl, E. A. Wong, F. C. Gwazdauskas, and R. M. Akers. 1998. Expression of ovine insulin-like growth factor (IGF-I) stimultes alveolar bud development in mammary glands of transgenic mice. Endocrine 8:251-259.

Wildman, E.E., G.M. Jones, P.E. Wagner, R.L. Boma, H.F. Troutt, and T.N. Lesch. 1982. A dairy cow body condition scoring system and its relationship to selected prodution characteristics. J. Dairy Sci. 65:495-501.

Yoshioka, K., A.M. Othman, T. Taniguchi, H. Yamanaka, and K. Sekikawa. 1997. Differentila patterns of blastulation in bovine morulae cultured in synthetic oviduct fluid medium containing FCS or BSA. Theriogenology 48:997-1006.

Zhang, L., R.S. Denniston, T.D. Bunch, and R.A. Godke. 1991. Cows vs. heifers for the production of in vitro fertilized (IVF) embryos. J. Anim. Sci. 69 (Suppl. 1) 49.

APPENDIX A

Table 16. Analysis of variance for factors affecting pre- and postpartum body condition scores (BCS).

Variable	df	Mean Squares
Parity	2	.71 **
*Days X Parity	3	5.54 **
Days^2 X Parity	3	.54 **
Error	448	.10

** P < .01
* Days relative to calving
Days^2 relative to calving (quadratic)

Table 17. Analysis of variance for factors affecting BCS at calving.

Variable	df	Mean Squares
Calving Season	2	2.10**
BW***	1	.74**
Days Pre-partum	1	.06
Parity	2	.23*
Error	71	.07

* P < .05
** P < .01
****Bodyweight (kg)

Table 18. Analysis of variance for factors affecting BCS at aspiration for Holstein cows.

Variable	df	Mean Squares
Calving Season	2	.067
BW*	1	.778**
Days Pre-partum	1	.078
Parity	2	.117
Milk Yield	1	.330**
Maximum Temperature***	1	.018
Wind chill factor***	1	.061
Precipitation	1	.027
DPP****	1	.011
DPP X Parity	2	.062
DPP^2 X Parity	3	.039
Error	62	.046

** P < .01,
*Bodyweight (kg)
****Days Postpartum
***At the day of calving

Table 19. Analysis of variance for factors affecting the change in BCS from calving to aspiration for Holstein cows.

Variable	df	Mean Squares
Calving Season	2	.52**
BW*	1	.15
Parity	1	.04
Change in BCS***	1	1.89**
Peak milk yield	1	.50**
DPP****	1	.20
DPP X Parity	2	.05
DPP^2 X Parity	3	.09
Error	68	.06

** P < .01,
*Bodyweight (kg) at aspiration
****Days Postpartum
***From days pre-partum to calving

Table 20. Analysis of variance for factors affecting the change in BW at aspiration for Holstein cows.

Variable	df	Mean Squares
Calving Season	3	18713.2**
Parity	2	160249.4**
DPP*	1	3175.1
Error	113	2116.8

** P < .01,
*Days Postpartum

Table 21. Analysis of variance for factors affecting the milk yield at aspiration for Holstein cows.

Variable	df	Mean Squares
Calving Season	3	40.07
Parity	2	198.40**
DPP* X Parity	3	265.90**
DPP^2 X Parity	3	259.38**
Error	108	39.28

** P < .01
*Days Postpartum
DPP^2 = days postpartum (quadratic)

Table 22. Analysis of variance for factors affecting the total number of follicles aspirated at each aspiration session.

Variable	df	Mean Squares
Maximum Temperature at aspiration	1	108.3*
Days Pre-partum	1	30.8
Parity	2	297.9**
Milk yield	1	96.9*
Insulin-like growth factor (IGF-I)****	1	154.3**
Testosterone	1	118.8*
Estrogen	1	43.5
IGF-I:Insulin ratio****	1	129.5*
IGF-I:NEFA ratio****	1	8.0
Glucose:NEFA ratio****	1	7.7
DPP***	1	68.6
DPP X Parity	2	225.8**
Error	66	20.7

** P < .01
* P < .05
***Days Postpartum
**** concentrations in serum
NEFA = non-esterified fatty acids
IGF-I = insulin-like growth factor I

Table 23. Analysis of variance for additional factors affecting the total number of follicles aspirated at each aspiration session.

Variable	df	Mean Squares
Maximum Temperature at aspiration	1	100.4**
Days Pre-partum	1	18.1
Body condition score at calving	1	19.7
Body condition score at dry	1	22.0
Parity	2	60.6**
Glucose:NEFA ratio****	1	1.8
Insulin****	1	12.7
Progesterone‡	1	7.8
Peak milk yield	1	59.0**
Progesterone****	1	323.5**
Estrogen‡	1	10.7
Progesterone: Testosterone ratio‡	1	31.2*
DPP*** X Parity	3	27.3*
Error	48	6.3

** P < .01
* P < .05
***DPP
**** concentrations in serum
‡ concentrations in follicular fluid
NEFA = non-esterified fatty acids

Table 24. Analysis of variance for factors affecting follicle diameter at day of aspiration.

Variable	df	Mean Squares
Parity	2	36.9
DPP[***]	1	185.9[**]
DPP^2	1	148.1[**]
DPP^3	1	119.2[**]
DPP X Parity	2	58.5[*]
DPP^2 X Parity	2	61.4[*]
DPP^3 X Parity	2	55.4[*]
Error	1382	16.6

[**] P < .01

[*] P < .05

[***]Days Postpartum

DDP^2 = days postpartum (quadratic)

DDP^3 = days postpartum (cubic)

Table 25. Analysis of variance for additional factors affecting follicle diameter at aspiration.

Variable	df	Mean Squares
Milk Yield	1	121.8*
Presence of Corpus Luteum	1	93.0*
Calving Season	3	47.9
Wind chill factor at calving	1	104.8*
Insulin****	1	102.3*
Progesterone‡	1	180.3**
Estrogen‡	1	45.3
Size of largest follicle	1	844.2**
Glucose****	1	151.5*
NEFA****	1	28.2
Estrogen:Progesterone ratio‡	1	44.4
IGF-I****	1	18.9
IGF-I‡	1	31.0
Insulin:IGF-I ratio****	1	149.5*
Glucose:NEFA ratio****	1	170.8**
IGF-I:NEFA ratio****	1	183.0**
Parity	2	4.5
DPP***	1	138.4*
DPP^2	1	116.1*
DPP^3	1	91.1*
Error	663	23.6

** P < .01
* P < .05
***Days Postpartum
DDP^2 = days pospartum (quadratic)
DDP^3 = days postpartum (cubic)
**** concentrations in serum
‡ concentration in follicular fluid
NEFA = non-esterified fatty acids
IGF-I = insulin-like growth factor I

Table 26. Analysis of variance for factors affecting the diameter of the largest follicle present at aspiration.

Variable	df	Mean Squares
Body weight	1	282.1**
DPP***	1	342.7**
DPP^2	1	283.6**
DPP^3	1	249.4*
Error	115	38.9

** P < .01
* P < .05
***Days Postpartum
DDP^2 = days pospartum (quadratic)
DDP^3 = days postpartum (cubic)

Table 27. Analysis of variance for factors affecting size of the corpus luteum at aspiration.

Variable	df	Mean Squares
Parity	2	95.8*
DPP***	1	99.7
DPP X Parity	2	85.0
DPP^2 X Parity	3	102.4*
Error	60	29.4

* P < .05
***Days Postpartum
DDP^2 = days pospartum (quadratic)

Table 28. Analysis of variance for factors affecting total number of oocytes per cow at each aspiration session.

Variable	df	Mean Squares
Technician	2	39.8
Glucose****	2	19.1
Progesterone‡	1	21.5
Parity	1	105.6**
DPP***	1	8.2
DPP X Parity	2	46.9*
Error	75	13.8

** P < .01
* P < .05
‡ concentrations in follicular fluid
**** concentrations in serum
***Days Postpartum

Table 29. Analysis of variance for factors affecting oocyte quality at each aspiration session.

Variable	df	Mean Squares
Maximum temperature at aspiration	1	8.5**
Precipitation at aspiration	1	16.9**
Calving Season	2	5.1**
Parity	2	1.3
DPP*** X Parity	3	3.6*
DPP^2 X Parity	3	3.6*
Glucose:NEFA ratio****	1	9.5**
IGF-I:NEFA ratio****	1	12.6**
Error	506	1.0

** P < .01
* P < .05
**** concentrations in serum
NEFA = non-esterified fatty acids
***Days Postpartum
DDP^2 = days pospartum (quadratic)
IGF-I = insulin-like growth factor I

Table 30. Analysis of variance fo additional factors affecting oocyte quality at each aspiration session.

Variable	df	Mean Squares
Body condition Score at aspiration	1	3.73*
Milk Yield	1	.34
Progesterone‡	1	.53
IGF-I****	1	13.31**
Testosterone‡	1	18.66**
Estrogen:Progesterone ratio‡	1	3.89*
Estrogen:Testosterone ratio‡	1	5.73*
Insulin:Glucose****	1	6.35**
Glucose:NEFA ratio****	1	14.22**
IGF-I:NEFA ratio****	1	19.09**
Parity	2	.77
DPP***	1	8.65**
DPP X Parity	2	1.70
DPP^2 X Parity	3	3.77**
Error	417	.94

** P < .01
* P < .05
***Days Postpartum
DDP^2 = days pospartum (quadratic)
**** concentrations in serum
‡ concentration in follicular fluid
NEFA = non-esterified fatty acids
IGF-I = insulin-like growth factor

Table 31. Analysis of variance for factors affecting serum Progesterone concentrations

Variable	df	Mean Squares
Parity	2	18.5*
Calving Season	3	1.4
DPP***	1	64.5**
DPP X Parity	2	18.3*
Error	111	4.6

* P < .05
** P < .01
***Days Postpartum

Table 32. Analysis of variance for factors affecting serum Insulin concentrations from samples collected at aspiration.

Variable	df	Mean Squares
Parity	2	.502**
Calving Season	3	.230
DPP***	1	.376*
DPP X Parity	2	.364*
Error	111	.094

* P < .05
** P < .01
***Days Postpartum

Table 33. Analysis of variance for factors affecting serum non-esterified fatty acid (NEFA) concentrations in serum collected at day of aspiration.

Variable	df	Mean Squares
Parity	2	270833.6*
Calving Season	3	142112.5
DPP***	1	1372691.4**
DPP X Parity	2	175916.4*
DPP^2 X Parity	3	361770.7*
DPP^3 X Parity	3	260753.1**
Error	105	56331.0**

* P < .05
** P < .01
***Days Postpartum
DDP^2 = days pospartum (quadratic)
DDP^3 = days postpartum (cubic)

Table 34. Analysis of variance for factors affecting Estrogen concentrations in follicular fluid collected from the largest follicle present at day of aspiration.

Variable	df	Mean Squares
Parity	2	210229.0
Calving Season	3	59223.0
DPP***	1	51537.5
DPP X Parity	2	278303.9
DPP^2 X Parity	3	224225.1*
DPP^3 X Parity	3	244715.4*
Error	86	89174.1

* P < .05
***Days Postpartum
DDP^2 = days pospartum (quadratic)
DDP^3 = days postpartum (cubic)

Table 35. Analysis of variance for factors affecting IGF-I[1] concentrations in follicular fluid collected from the largest follicle present at day of aspiration.

Variable	df	Mean Squares
Parity	2	6015.78**
Calving Season	3	324.03
DPP***	1	861.89
Error	92	375.62

** P < .01
***Days Postpartum
[1]Insulin-like growth factor I

Table 36. Analysis of variance of developmental scores for winter and fall aspirated oocytes.

Variable	df	Mean Squares
Season[1]	1	17.1**
Category[2]	1	.26
Error	524	.26

**P < .001
[1]Winter and fall aspirated oocytes
[2]"early " and "late" category

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Ahmadzadeh, A., M.A. Barnes, F.C. Gwazdauskas, and **A.H. Walters**. Dopamine Antagonist affects Cortisol secretion in lactating Dairy Cows. 2000. American Dairy Science Association Annual Meeting.

Momcilovic D., Archbald L.F., **A.H. Walters**, T. Tran, D.Kelbert, C.Risco, W.W. Thatcher. 1998. Reproductive performance of lactating cows treated with gonadotrophin-releasing hormone (GnRH) and/or prostaglandin F2a (PGF2a) for synchronization of estrus and ovulation. Theriogenoloy 50:1131-1139.