Serum samples and BW were obtained from 2-yr-old beef heifers, pregnant with either single (SF, n = 12) or twin (TF, n = 7) fetuses, at 7-d intervals from d 190 of gestation until calving. Serum insulin-like growth factor I (IGF-I) concentrations of SF heifers gradually declined from d 190 (69.9 ± 1.0 ng/ml) to d 263 (55.6 ± .8 ng/ml), then exhibited a slight increase by d 277 (63.4 ± 1.1 ng/ml). Serum IGF-I concentrations of TF heifers essentially paralleled, yet were lower (P < .05) than, concentrations in SF heifers for all days tested except d 197 and 205. The SF heifers pregnant with heifer fetuses (n = 6) had higher IGF-I concentrations (P < .1) than heifers pregnant with bull fetuses (n = 6) for all days tested except d 214 and 235. Instantaneous absolute growth rate (IGR) of SF heifers declined from 1.485 kg/d at d 190 to .257 kg/d by d 277. Rate of decline in IGR of TF heifers was much greater (P < .0001). Correlations between serum IGF-I concentrations and IGR for SF and TF heifers were .79 (P < .001) and .59 (P < .05), respectively. These data suggest that number and sex of fetus influence maternal concentrations of IGF-I and that the combined growth rate of the dam and conceptus during gestation is related to serum IGF-I concentration.

(Key Words: Pregnancy, Growth, IGF-I, Twinning, Beef Cattle.)

Materials and Methods

Donor cows were superovulated and bred artificially and the resultant embryos were collected nonsurgically (Elsden et al., 1976) 7 d postbreeding. Acceptable embryos were bisected into demi-embryos (Williams et al., 1984) and transferred nonsurgically into estrous-synchronized yearling beef heifers, each heifer receiving either one or two demi-embryos. Following pregnancy determination by rectal palpation, heifers pregnant with a single embryo were paired either by identical demi-embryo or full-sib embryo, resulting in 12 heifer pairs. Of the 12 pairs, eight were pregnant with identical concentrations and growth of the dam during pregnancy have not been investigated. Objectives of this study were to determine serum profiles of IGF-I throughout the third trimester of gestation (when most fetal growth occurs) in beef heifers pregnant with either single or twin fetuses and to determine the relationships between serum IGF-I concentration and gestational growth, measured by instantaneous absolute growth rate, of the dams.

Introduction

Insulin-like growth factor I (IGF-I) is a mitogenic peptide that has been found in the serum of several mammalian species including cattle (Bala and Bhaumick, 1979; Honegger and Humbel, 1986). Biologically, IGF-I mediates the anabolic actions of growth hormone in skeletal tissues (Daughaday et al., 1972) and mimics the effects of insulin in the muscle cell and adipocyte (Zapf et al., 1984).

Profiles of IGF-I during gestation have been reported for mice (D’Ercole and Underwood, 1980), rats (Sheppard and Bala, 1986) and humans (Furlanetto et al., 1978; Wilson et al., 1982). The relationships between serum IGF-I concentrations and growth of the dam during pregnancy have not been investigated. Objectives of this study were to determine serum profiles of IGF-I throughout the third trimester of gestation (when most fetal growth occurs) in beef heifers pregnant with either single or twin fetuses and to determine the relationships between serum IGF-I concentration and gestational growth, measured by instantaneous absolute growth rate, of the dams.
twins and four were pregnant with full-sibs. Of the heifers that received two demi-embryos, seven were confirmed pregnant with twins.

On d 190 of gestation, one heifer from each single pregnancy pair was assigned to an adequate protein diet and the other to a restricted protein diet (approximately 100% and 60%, respectively, of NRC, 1984) to assess the effects of prepartum nutrition on neonatal calf parameters (Carstens et al., 1987; Holland et al., 1987b). Heifers pregnant with twins had ad libitum access to the adequate protein diet. In this communication, only data collected from heifers pregnant with single (n = 12) and twin (n = 7) fetuses that received an adequate protein ration are reported.

On a DM basis, the adequate protein diet consisted of 93% corn silage and 7% soybean meal. Dry matter, CP and ME intakes for heifers pregnant with single and twin fetuses were 6.18, .62 and 14.63 vs 6.74 kg/d, .67 kg/d and 15.94 Mcal/d, respectively. Intakes were calculated based on pen averages. All heifers received an injection of 1,500,000 IU vitamin A, 225,000 IU vitamin D and 15 IU vitamin E on d 190. A mineral supplement consisting of trace mineralized salt, biophos (21% P, 15 to 17% Ca) and limestone was available ad libitum. Crude protein of the diets was determined by micro-Kjeldahl procedures (AOAC, 1975) and ME values were calculated from NRC (1984).

Blood samples were obtained via jugular venipuncture immediately prior to daily feeding, at 7-d intervals from d 190 of gestation until d 277 for heifers pregnant with a single fetus and d 270 for heifers pregnant with twin fetuses. Serum was harvested by centrifugation and stored at -20°C until assay. Body weights were measured on the days blood samples were collected. Body condition scores, as described by Richards et al. (1986), were determined at 1-mo intervals and at calving.

Serum IGF-I concentrations were determined by the RIA technique for IGF-I in bovine serum as described by Holland et al. (1987a). Briefly, serum was acidified in .1 M glycine-glycine HCl for 24 h at 37°C and diluted 40-fold with assay buffer. Standards5 and acidified-diluted serum samples were preincubated with a final tube dilution of 1:8,000 anti-IGF-I6 for 72 h at 4°C. Ten thousand cpm [125I]IGF-I then were added and tubes were incubated an additional 16 h at 4°C. Separation of bound from free [125I]IGF-I was accomplished by addition of pre-precipitated sheep anti-rabbit gamma globulin-normal rabbit serum gamma globulin complexes. Tubes then were incubated 4 h at 4°C, centrifuged at 1,850 x g, decanted and counted. Intraassay variation (determined by sampling a single serum pool 10 times) and interassay variation of three separate assays were 3.03% and 2.83%, respectively.

Growth curves were mathematically represented by fitting second-order polynomial equations to the data (Finney, 1978). The use of polynomial equations permitted statistical estimation of instantaneous absolute growth rates (Brody, 1945). In second-order polynomial equations, the first derivative with respect to time (dy/dx, where y = live weight and x = time) may be used to estimate instantaneous absolute growth rate. (i.e., growth rate at any finite time point on the growth curve).

The effects of type of pregnancy and sex of fetus on serum IGF-I concentration by day of gestation were tested for significance by ANOVA (SAS, 1982). The effects of type of pregnancy and sex of fetus on instantaneous absolute growth rate were analyzed using simple linear regression (SAS, 1982). Differences in regression lines were tested for significance using dummy variable regression techniques (Weisburg, 1980). The dummy variable regression technique was used also to test differences between regressions of IGF-I concentrations on instantaneous absolute growth rates for heifers pregnant with single or twin fetuses.

Results and Discussion

Average serum IGF-I concentrations of heifers pregnant with single (SF) or twin (TF) fetuses are shown in Figure 1. Both SF and TF heifers showed a slight increase in IGF-I from d 190 to d 197 (69.9 ± 1.0 to 75.0 ± 1.1 and 57.1 ± 1.3 to 69.8 ± 1.9 ng/ml, respectively) of gestation. After a plateau of 14 d, serum IGF-I of SF heifers gradually declined to the lowest concentration of 55.6 ± 1.0 ng/ml at d 263. Serum IGF-I then increased to 65.6 ± .9 ng/ml by d 270 and declined nonsignificantly to 63.4 ± .1 ng/ml by d 277.

Serum IGF-I concentrations of TF heifers essentially paralleled, yet were lower (P < .05)
than, concentrations exhibited by SF heifers, with differences being significant at all days tested except d 197 and 205. Concentrations of IGF-I plateaued between d 197 and 205 (69.8 ± 1.9 and 68.9 ± 1.3 ng/ml, respectively), declined slightly by d 214 then plummeted to 25.0 ± 2.3 ng/ml at d 221. After a recovery period, IGF-I gradually declined to 37.6 ± .8 ng/ml by d 263 and increased to a final concentration of 46.9 ± .8 ng/ml at d 270.

Whereas both SF and TF heifers experienced a decrease in serum IGF-I concentrations at d 221 of gestation, no explanations are apparent for the dramatic decline seen in the TF group, though an environmental change occurred. Between d 214 and 221, the mean environmental temperature was approximately −23°C, considerably lower than at any other time during gestation. Maintenance requirements of the heifers would increase during this period of environmental stress in order to maintain body temperature. Because IGF-I mimics effects of insulin, such as stimulating lipogenesis and glycogen synthesis (Zapf et al., 1978; Poggi et al., 1979), a decline in circulating IGF-I concentrations may enhance lipolysis and glucose metabolism to maintain body temperature. Furthermore, the more dramatic decrease in IGF-I for TF heifers may be due to the greater metabolic stress placed on their system by the two fetuses. Additional studies, however, are required to substantiate any environmental temperature effects on circulating IGF-I concentrations.

No data are available in the literature dealing with serum IGF-I profiles during gestation of domestic farm animal species. However, serum profiles of the SF and TF heifers were similar to gestational profiles reported for IGF-I in mice (D’Ercole and Underwood, 1980) and rats (Sheppard and Bala, 1986). In these species, IGF-I concentrations also decline during the third trimester. These results contrast with data reported for the human (Furlanetto et al., 1978; Bala et al., 1981) in which serum IGF-I concentrations are elevated throughout the last stage of pregnancy. This discrepancy between serum IGF-I profiles suggests a possible difference in gestational requirements or biological activity of IGF-I between these animal species and the human.

Quadratic equations representing growth of SF (y = −121.56 + 4.08x − .0069x²; r² = .95) and TF (y = −239.06 + 5.45x − .0104x²; r² = .78) heifers during the third trimester of gestation are given in Figure 2. It is imperative to note that growth of the heifers not only includes growth of maternal tissues but growth of the conceptus as well. The BW at which growth rate for TF heifers was equal to zero was 474.9 kg and corresponded to d 262 of gestation. From this point of gestation to d 270, BW of TF heifers declined slightly. This decline also was evident in actual BW of the heifers. It is difficult to ascertain if this decline was a physiological response to pregnancy. It is possible, however, that gut crowding due to the presence of the...
twin conceptus (mean birth weight of 27.3 kg per calf) depressed feed intake. As a result, this decline in BW may have been due to decreased gut fill and not to loss of empty body mass. At this advanced stage of gestation it also is possible that substantial amounts of maternal tissues were being mobilized to maintain the twin conceptus. Thus, a loss in maternal BW may be observed because at this stage of gestation the fetus is not rapidly increasing in mass (Eley et al., 1978; Anthony et al., 1986). Compositional studies have shown a decrease in BW during the latter portion of gestation in ewes pregnant with multiple fetuses (Robinson et al., 1978). The BW decline was mainly attributed to a loss in body fat with minimal redistribution of body protein. Similarly, monotocous beef cows lose total body solids accompanied by increased maternal hydration throughout gestation (Degen and Young, 1980). Body condition scores obtained from TF heifers on d 190 and 249 and at calving (6.0, 6.0 and 5.1, respectively) suggested a loss (P < .01) in total body fat during the last 25 d of gestation. Based on condition score declines, the BW decline of TF heifers may have been due to loss of empty body mass exemplified by mobilization of carcass fat deposits. However, because compositional studies were not performed in the current trial, it is not possible to substantiate whether the loss in BW was due to gut crowding, fat mobilization or a combination of these factors.

As shown in Figure 2, a BW at which growth rate was equal to zero was never reached by SF heifers, probably due to the smaller metabolic drain imposed on the maternal system by the single conceptus (mean birth weight of 34.0 kg). By extrapolating the growth curve past d 277 and assuming the same rate of quadratic growth, a zero growth rate would be reached by d 295 at a weight of 481.6 kg. Although this may not be physiologically significant, it suggests that heifers pregnant with single fetuses have greater ability to withstand the metabolic stresses of pregnancy than do heifers pregnant with twins. As a result, SF heifers would be capable of expressing a greater proportion of their growth potential during gestation.

Instantaneous absolute growth rates (IGR) for SF and TF heifers are given in Figure 3. During the initial phase of the third trimester, IGR was similar for both SF and TF heifers (1.45 and 1.49 kg/d, respectively). However, as gestation progressed, IGR of TF heifers decreased at a much greater rate (P < .0001), culminating with negative growth rates by d 263 and 270.

Instantaneous absolute growth rate was chosen as the measure of growth to relate to serum IGF-I concentration because it accurately defines growth of an individual at any finite point along the growth curve. Serum IGF-I concentration was regressed on IGR of SF (y = 56.76 + 12.29x; r² = .63) and TF (y = 41.59 + 14.28x; r² = .35) heifers to establish the relationship between IGF-I and growth (Figure 4). A simple correlation across stages of gestation (r = .79; P < .001) existed for SF heifers. The relationship was not so strong within TF heifers (r = .59; P < .05). The erratic gestational profile of IGF-I in TF heifers undoubtedly contributed to the lower correlation between IGF-I and IGR. As a result, the data for TF heifers also were analyzed excluding the extremely low IGF-I value obtained on d 221. Subsequent regression resulted in a correlation (r = .86; P < .001) with no significant change in slope.

It is not clear whether maternal serum IGF-I concentrations are influenced by fetal IGF-I production. It has been demonstrated that [125I]IGF-I does not cross the placental barrier of rats, dogs or sheep (Underwood et al., 1979; D’Ercole et al., 1980). Although there is debate over the homology of placentation between sheep and cattle, it seems likely that fetally derived IGF-I does not circulate in the maternal bovine system. As a result, maternal IGF-I
concentrations probably reflect growth of maternal tissues and not growth of the conceptus. The conceptus, however, may alter maternal serum IGF-I concentrations. This is evident when the relationships between IGF-I and IGR of the SF and TF heifers are examined (Figure 4). For example, the amount of IGF-I associated with 1 kg/d growth in SF heifers was 69.1 ng/ml, whereas only 55.9 ng/ml were present in TF heifers. As noted previously, IGF-I exerts a negative effect on the lipolytic pathway in the adipocyte (Zapf et al., 1984). Thus, lower IGF-I concentrations in the TF group would allow increased lipolysis to maintain the twin conceptus. However, in order for the TF group to obtain an equal amount of growth with 20% lower IGF-I concentrations there must have been an increase in tissue sensitivity to IGF-I, possibly via increased receptor numbers or affinity. It thus appears that the number of fetuses within the gravid uterus influences maternal IGF-I concentrations throughout the third trimester of gestation.

Another observation that suggests the fetus influences maternal serum concentration of IGF-I is the gestational IGF-I profiles of the SF heifers based on sex of the fetus. Throughout the third trimester, SF heifers pregnant with heifer calves (SFH, n = 6) had higher serum IGF-I concentrations (P < .1) than females pregnant with bull calves (SFB, n = 6) for all days tested except on d 214 and 235 (Figure 5). There was no difference between TF heifers pregnant with heifer calves (n = 2) or bull calves (n = 5), probably due to the small sample size of the heifer calf subclass. The IGR for SF heifers pregnant with bull or heifer fetuses is shown in Figure 6. The IGR of the SFH and SFB heifers were similar at d 190 (1.45 and 1.49 kg/d, respectively). However, as gestation progressed, IGR of SFB heifers declined at a
much faster rate ($P < .0001$). This same pattern of IGR decline was evident for TF heifers pregnant with bull calves as opposed to those pregnant with heifers. Although there was no difference in actual birth weight between heifer and bull calves from SF dams (33.8 to 34.2 kg, respectively), there was a trend ($P = .15$) for bull calves to be heavier when birth weight was adjusted for gestation length (32.9 vs 35.1 kg). This difference in birth weight in part may explain the differing IGF-I concentrations and IGR of the dams as related to the amount of fetally induced stress placed on the maternal system. However, because there was no difference in birth weight between sexes, the discrepancy between IGF-I concentrations and IGR of the SFH and SFB heifers may reflect a differential maternal adaptation to the fetoplacental unit based on sex of fetus. It thus appears that not only the number of fetuses, but that the sex of the fetus as well, influences maternal serum IGF-I concentrations throughout the third trimester of gestation.

Although total serum somatomedin activity has been correlated to weight gain in yearling bulls (Lund-Larsen et al., 1977), the present study is the first to demonstrate a strong positive relationship between serum IGF-I concentration and growth in beef heifers. Throughout the third trimester of gestation in beef heifers, maternal serum IGF-I declines. This may be due to pregnancy itself or simply may reflect the postinfectionary phase of growth when BW is increasing at a decreasing rate in heifers of this age. Further investigations with mature cows are required to verify that pregnancy per se influences serum IGF-I concentrations. The effects of endocrine, metabolic and environmental factors that may be associated with these phenomena are speculative due to lack of research in this area. Interactions between these parameters and gestational growth merit further investigation so that the pregnancy process and growth in general may be understood more completely.

Literature Cited


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