

EFFECTS OF PROTEIN SUPPLEMENTATION AND SOURCE IN COW/CALF
SYSTEMS AND HEIFER DEVELOPMENT

by

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A DISSERTATION

Presented to the Faculty of
The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Doctor of Philosophy

Major: Animal Science

Under the Supervision of Professors Richard N. Funston and Andrea S. Cupp

Lincoln, Nebraska

August, 2007

UMI Number: 3271935

PREVIEW

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EFFECTS OF PROTEIN SUPPLEMENTATION AND SOURCE IN COW/CALF SYSTEMS AND HEIFER DEVELOPMENT

Jeremy L. Martin, Ph.D.

University of Nebraska, 2007

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Four experiments evaluated protein supplementation in cow/calf systems and heifer development diets. In the first experiment, cows grazed dormant Sandhills range and received either supplement or no supplement in late gestation and were fed hay or grazed subirrigated meadow after calving. Heifers from supplemented cows were heavier from weaning through three years of age, had greater pregnancy rates, and more calved during the initial 21 days of calving.

A subsequent experiment evaluated effects of protein supplementation of cows grazing dormant range or corn crop residue on cow and progeny performance. Cows that grazed corn stalks were heavier and had greater BCS before weaning and the subsequent breeding season, and had higher pregnancy rates than cows that grazed winter range. Cow BW and BCS prior to calving and breeding, and calf weaning BW was greater for calves from supplemented cows that grazed range. Final BW of steer progeny was heavier if dams grazed corn residue. Dam supplementation increased the proportion of steers grading USDA Choice. Heifer pre-breeding BW was greater for heifers from supplemented cows that grazed range than unsupplemented cows.

Additional experiments compared protein source during heifer development on reproductive performance. Heifers in the first experiment were fed dried distillers grains (DDG) as an energy source during development. The control supplement contained similar crude protein, energy, and lipid but differed in protein degradability. Growth,

BCS, and age at puberty were similar between treatments. Heifers fed DDG had greater conception and pregnancy rates.

A final experiment evaluated the effects of feeding soybeans or DDG on reproductive characteristics of heifers. Pregnancy rate was unaffected by supplement, but heifers fed soybeans had greater dominant follicle diameter. Vascular Endothelial Growth Factor (VEGF) mRNA expression of the angiogenic isoform VEGF120 and the anti-angiogenic isoform VEGF164b were greater in granulosa cells from heifers fed DDG compared to heifers fed soybeans, expression of the dominant angiogenic isoform VEGF164 was not affected by treatment.

In summary, these experiments support a fetal programming effect of protein supplementation in cow/calf systems, increased fertility in heifers supplemented with DDG, and differential VEGF gene expression due to supplement.

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INTRODUCTION

Beef cow-calf production systems are historically break-even businesses (Fuez and Umberger, 2003). Fuez and Umberger (2003) also noted that high-cost producers spent an average of \$586 annually to maintain a bred female, whereas low-cost producers spent \$320. Purchased feeds accounted for \$107 of the noted difference, and high-cost producers averaged three times as much investment in purchased feed. Higher purchased feed investment led to lower net returns and greater profit variability due to increased price risk associated with larger operating investments. However, minimizing feed input per unit of output is not economically feasible if reproduction is not maintained (Davis et al., 1994). In order to be profitable and low-risk, a beef cow-calf system needs to have optimal reproductive performance and feed inputs.

The mature cow is the basic production unit of the beef industry. Nebraska producers maintain an inventory of approximately 2 million beef cows. At a modest 15% replacement rate, this represents almost 300,000 replacement heifers developed annually. The overwhelming majority of these heifers are fed a forage-based diet containing supplemental protein and energy. Traditional heifer development programs utilize substantial energy, cereal grains, and capital investment to support non-producing animals, and generally involve placing weaned heifers into a feedlot or drylot situation and then providing them with a ration formulated so that the heifers weigh 65% or more of projected mature weight at the time of breeding. This type of heifer development evolved because producers wished to achieve maximum pregnancy rates in the first breeding season, and dormant forages alone do not contain the nutrients necessary to support this level of performance.

Intensive heifer development systems may attain maximum pregnancy rates, but not necessarily optimum performance in terms of profit or sustainability. Developing heifers in this manner requires significant use of fossil fuels and cereal grains, and high capital investment in equipment and facilities. The energy demands for this system are high because of the requirement for fuel to harvest the feed and then deliver it to the cattle. Cereal grains are often used as a major source of energy in the diet, detracting from the sustainability of this system due to growing demand for cereal grains for human food and ethanol production.

Recent research efforts in Nebraska are focused on developing a systems approach and mentality towards heifer development, where profit and sustainability are the indicators of success. Heifers have been developed to lighter than previously recommended target weights with no adverse affects on reproduction or production. Furthermore, effects of prenatal environment on future reproductive success of beef heifers have demonstrated that heifer development begins before birth.

Critical Events in Heifer Development

Traditional recommendations advocate substantial energy inputs for replacement heifer development because pregnancy rates in heifers are dependent upon the number displaying estrus early in the breeding season (Short and Bellows, 1971). Byerley et al. (1987) reported heifers should experience two or three estrous cycles before the onset of the breeding season because fertility of the first estrus is lower than subsequent estrous cycles. Therefore, time of puberty is an important factor in determining pregnancy rates in heifers (Patterson et al., 1992).

Achievement of puberty involves a number of metabolic and endocrine considerations that act in concert, but the preovulatory luteinizing hormone (LH) surge is often considered the key factor (Schillo et al., 1992). Recent research investigating the induction of precocious puberty in beef heifers beginning at 102 d of age revealed LH pulse frequency increased with age in heifers fed to gain either 1.27 or 0.85 kg/d (Gasser et al., 2006a). However, LH pulse frequency was greater by 190 d of age in heifers fed to gain more rapidly, and preceded attainment of puberty. Concomitant with increased LH pulse frequency, attainment of puberty in heifers is marked by increasing dominant follicle diameter and decreased sensitivity to estradiol negative feedback on LH secretion (Melvin et al., 1999; Rodrigues et al., 2002; Gasser et al., 2006b). As heifers near puberty, estradiol receptor populations in the medial basal hypothalamus decline, reducing negative feedback of estradiol on GnRH, and thus LH, secretion (Day et al., 1987). In summary, increased LH pulse frequency supports increased dominant follicle diameter, which promotes greater estradiol secretion in prepubertal heifers. When combined with decreased sensitivity of the hypothalamus to estradiol, these events lead to the preovulatory LH surge, ovulation, and establishment of estrous cycles.

FACTORS IN HEIFER DEVELOPMENT AND REPRODUCTION

Genetics

Cundiff et al. (1986) reported distinct between-breed differences in age and weight at puberty, as well as in subsequent reproductive performance. In general, faster-gaining breed groups of larger mature size reach puberty at later ages than do slower-

gaining breed groups of smaller mature size (Cundiff et al., 1986; Thallman et al., 1999).

Physiologically, differences in age at puberty seem to be driven by compositional differences between diverse genotypes (Martin et al., 1992).

Comparison of tissue growth and distribution in Hereford, Charolais, and Simmental growing heifers revealed differences in body composition between the breeds of differing biological type (Buckley et al., 1990). Regardless of breed, fat-free tissue percentage of empty body weight decreased from 97% at 2 d of age to 66% at 14 months of age. Across all ages, Hereford heifers were significantly fatter, with less body protein than Simmental or Charolais heifers. Further evaluation of these animals, using open-circuit calorimetry revealed that Hereford heifers had lower fasting heat production (FHP)/kg^{0.75} than either other breed at 14 months of age (Baker et al., 1991). Across breeds, those with increased body protein, decreased body fat, and increased maintenance energy requirements tend to reach puberty later than heifers with lower maintenance energy requirements and greater genetic predisposition to accumulate adipose tissue (Cundiff et al., 1986; Martin et al., 1992; Thallman et al., 1999).

Nutrition

Energy/Growth Rate

The strong influence of nutritional management on heifers was demonstrated by Gasser et al. (2006d). Early weaning in combination with feeding a 60% corn high-energy diet from 126 to 402 d of age induced precocious puberty in 67% of heifers. In a related study, (Gasser et al., 2006c) early weaning and feeding high-concentrate diets to heifers beginning at 99 d of age potentiated precocious puberty due to hastened reduction

in sensitivity of the hypothalamus to estradiol negative feedback. Heifers fed high-concentrate diets only from 126 to 196 d or 196 to 402 d of age had lower rates of precocious puberty (47%), but still higher than heifers fed 30% corn diets from 126 to 402 d of gestation. Numerous studies have been performed to determine how energy inputs, defined in numerous ways, affect the success of heifer development programs.

Studies of sexual maturity in numerous species provide evidence for the importance of diet during development and suggest developmental nutrition can partially control physiological changes necessary for puberty (Frisch, 1984). Energy balance or plane of nutrition influences reproductive performance in heifers and cows (Short and Adams, 1988; Butler and Smith, 1989; Swanson, 1989; Randel, 1990; Robinson, 1990). Numerous studies have reported inverse correlations between postweaning growth rate and age at puberty (Short and Bellows, 1971; Arije and Wiltbank, 1974; Wiltbank et al, 1985).

Age at puberty was increased by 64 d in heifers fed to gain 0.3 kg/d compared to heifers fed to gain 0.9 kg/d during development (Bergfeld et al., 1994). Along with delayed puberty, heifers fed for lower rate of gain had reduced dominant follicle diameter and lower circulating concentrations of estradiol. Rates of follicle growth and atresia were similar but low gain heifers had reduced duration of follicular waves. Clearly, level of nutrition during development can influence pubertal development in heifers fed for divergent gains, but pregnancy rate and future productivity were not measured in this study.

Freetly and Cundiff (1998) reported accelerated rates of gain during the postweaning period, over a diverse group of breeds, did not result in increased production

efficiency in the cows. Specifically, heifers were raised on 15.8 or 12.6 Mcal ME per day from weaning resulting in average daily gains of approximately 0.66 and 0.44 kg/d averaged across sire groups (Freetly and Cundiff, 1997). Age at puberty and pregnancy rate was similar between heifers on high and low energy intake. Nutritional treatment did not influence age at first calving or subsequent milk production (Freetly and Cundiff, 1998).

Increased postweaning gain was positively related to age at puberty when heifers grazing stockpiled fescue pastures were supplemented with 3.68 or 2.99 kg/d ground corn. The higher energy diet resulted in 9.6% more heifers becoming pubertal prior to the breeding season (Buskirk et al., 1995). Pregnancy and calving rates, however, were not affected by postweaning rate of gain. Further research from the same group determined that heifers fed to gain 1.2 kg/d during development had reduced milk production compared to heifers fed to gain 0.7 kg/d, presumably due to fat deposition in mammary tissue of the faster-gaining heifers (Buskirk et al., 1996).

In another study that utilized heifers developed on dormant native pasture with supplement, heifers fed 0.9 kg/d of 40% CP soybean meal, reached puberty at the same age as heifers fed 2.7 kg/d of 20% CP supplement containing greater energy (Marston et al., 1995). However, a lower percentage of the heifers reached puberty before or during breeding, so pregnancy rate was only 67% for heifers supplemented with soybean meal compared to 94% for heifers supplemented with the 20% CP supplement. These studies indicate pubertal development may be enhanced by increasing dietary energy between weaning and breeding, but pregnancy rates or production efficiency do not appear to be enhanced unless achievement of puberty is the limiting factor.

Other researchers have focused on timing of gain during heifer development to limit input costs by creating periods of compensatory growth or allowing producers to limit supplementation to critical periods of heifer development. Delaying heifer gain until 47 or 56 d prior to the breeding season did not negatively influence reproductive performance, but reduced the amount of feed needed (Lynch et al., 1997). In one year of this study, puberty was delayed in heifers fed to achieve lower early gains, but first-service conception rate tended to be improved in these same heifers. In a similar study, Freetly et al. (2001) compared heifers fed 263 or 238 kcal ME(BW)^{0.75} for 205 d with heifers fed 157 kcal ME(BW)^{0.75} for 83 d followed by 277 kcal ME(BW)^{0.75} for the next 157 d. Total energy intake was reduced in the delayed gain heifers, but calving rate, age at calving, postpartum interval, and second year pregnancy rate were not affected. Therefore, total energy intake, and possibly heifer development costs, may be reduced by limiting heifer gain early in the postweaning period followed by accelerated gains before the breeding season.

System of Development

Limited research has been performed to determine whether inherent differences in development systems affect reproductive efficiency or future productivity of heifers. By design, these studies are confounded by differences in total energy intake. Grings et al. (1998) determined that heifers grazed on pasture regrowth for the initial 56 d of development achieved similar reproductive performance as those developed entirely in drylot. Heifers grazing pasture gained less during the initial 56 d but were placed in a drylot following the grazing period and achieved similar pre-breeding weight as those developed entirely in drylot. In a similar study, heifers developed in drylot reached

puberty 29 d earlier than their contemporaries fed protein supplement on pasture (Marston et al., 1995), despite similar growth rates. However, this did not result in improved pregnancy rate or reduced age at first calving. Collectively, these two studies provide evidence that heifer development systems can influence reproductive performance, but do not provide evidence of effects independent of energy intake and/or growth rate.

In a more recent study, Ciccoli et al. (2005) compared heifers grazing pasture supplemented with 42% CP over the winter to heifers in a drylot fed high-starch diets for either 30 or 60 d and heifers self-fed low starch diets on pasture. Pre-breeding weight (270 kg) and ADG (0.51 kg/d) of heifers supplemented only with 42% CP pellets on pasture were substantially lower than traditional recommendations. As a result, pasture-developed heifers were older at puberty. However, pregnancy rates were similar across groups. The same study compared heifers developed on pasture and supplemented with energy for 60 d prior to breeding to heifers receiving only 0.9 kg/d 42% CP pellets. When pubertal development was limited by winter ADG, supplementation improved pregnancy rates. However, if heifers achieved moderate (0.51 kg/d) winter gains, pregnancy rates were not improved by supplementation.

Target Weight

Heifer development should be achieved at low cost in order to manage financial risk and prevent over-investment in a non-productive female. Previous studies (Patterson et al., 1992) indicated that puberty occurs at a genetically predetermined size, and only when heifers reach their target weight can high pregnancy rates be obtained.

Recommended guidelines generally have been 60 to 66% of mature BW in beef heifers

(Patterson et al., 1992). This development program requires substantial resources because an accelerated rate of gain is needed to reach the target weight. Wiltbank et al. (1985) fed heifers to pre-breeding target weights of 272 or 318 kg and exposed heifers to bulls for a 90-d breeding season. Heifers fed to weigh 272 kg prior to breeding had 16% lower first-calf pregnancy rate than heifers that weighed 318 kg at the initiation of the first breeding season (66 vs. 82% pregnancy rate for 272 and 318 kg heifers, respectively). Freetly et al. (2001) reported limit-feeding heifers may decrease first-calf survival, but when heifers met a minimal BW prior to mating, calf growth potential was not effected. Development programs that allow heifers to conceive early as yearlings at the lowest cost possible are needed.

Feeding replacement heifers to traditional target weights increases development costs relative to more extensive heifer development (Funston and Deutscher, 2004; Clark et al., 2005; Martin et al., 2007). Funston and Deutscher (2004) reported similar pregnancy rates from the initial through fourth breeding season for heifers developed to reach either 53 or 58% of mature weight prior to breeding as yearlings, proving heifers developed to only 53% of mature weight could achieve similar initial pregnancy rates and retention compared to heifers developed to 58% of mature weight. Further research using the same herd proved pre-breeding weights as low as 51% of mature weight (RLX) was more cost effective than development to 57% of mature weight (INT) when lighter heifers were allowed 60 d to become pregnant (Martin et al., 2007). Extending the breeding season by 15-d for lighter heifers resulted in first-calf conception rates being similar between systems when a 45- and 60-d breeding season was used for the INT and RLX systems, respectively. Retrospective analysis considering only RLX heifers bred within

the first 45 days of the breeding season, based on days pregnant at pregnancy diagnosis, revealed similar 45-d pregnancy rates for INT (89.8%) and RLX (77.9%) systems. During the extended 15 d breeding period (from 45 to 60 d) for the RLX heifers, an additional 9.3% of heifers became pregnant.

Interestingly, further characterization of non-bred heifers within each system revealed that 78.9% (14 of 17) of open RLX heifers (after a 60-d breeding season) but only 45% (5 of 11) of open INT heifers (after a 45-d breeding season) were pre-pubertal prior to start of the breeding season. This lends support to the hypothesis that one of the major determinants to a heifer's ability to conceive during her first breeding season is the age at which she attains puberty, especially in relation to the onset of the breeding season but indicates feeding heifers to meet traditional recommendations is unnecessary for successful development. In fact, feeding heifers to traditional target weights increases development costs per pregnant 2-yr-old cow (Clark et al, 2005; Martin et al., 2007).

Heifers calving early during their first calving season have higher lifetime calf production than those calving late and are more likely to become pregnant again as two-year-olds and calve early in the season as three-year olds (Lesmeister et al., 1973). However, there was no difference in second-calf conception rates between cows developed to 51 or 57% of mature weight as prior to breeding as yearlings (Martin et al., 2007). This indicates lighter heifers that became pregnant during the 15-d extension during the first breeding season rebred with similar efficiency as those bred within the initial 45 days of the breeding season. Therefore, proportion of heifers retained as pregnant 2-yr olds was similar between systems. Clearly, heifers may be developed to

lighter-than-traditional target weights without negative effects on profitability or future productivity.

Protein

The metabolizable protein (MP) system separates nitrogen (N) requirements of the cow from that of the rumen microbial population (NRC, 1996). Degradable intake protein (DIP) is used by rumen microflora for growth and reproduction and contributes to N supply for the host animal only after microbial passage from the rumen and subsequent digestion of microbial crude protein (MCP). Undegradable intake protein (UIP) is not available in the rumen, and is instead enzymatically digested in the lower digestive tract. The total MP supplied to the animal is the combination of UIP and MCP, the ratio of which varies in response to the type of dietary protein supplied. Above requirements, MP may be recycled and contribute to DIP supply. However, DIP intake above requirements does not contribute to MP balance, and is simply excreted.

Supplemental DIP can influence reproduction in cattle. However, it is likely that DIP primarily contributes positively to performance through impacts on energy balance. Forage digestibility and energy released from forage is dependent on adequate DIP supply. It is unlikely that DIP supplement would supply a specific metabolite or produce an endocrine response because amino acid profiles of MCP are similar across diets (Martin et al., 1996; Korhonen et al., 2002).

Another possibility is that excess DIP could limit reproductive performance due to decreases in uterine pH during the early luteal phase. Predictably, excess DIP can increase BUN, and uterine pH is inversely related to blood urea nitrogen (BUN; Elrod et al., 1993). Furthermore, Elrod et al. (1993) found that on day seven of the estrous cycle,

uterine pH was lower in cows supplemented with protein above requirements than in cows receiving a protein-balanced supplement. Increasing the proportion of DIP supplied by urea to 40% or greater in prepartum supplements tends to reduce pregnancy rates (Koster et al., 2002). This may be due to interference with embryo transport in the first 5 d following fertilization, as reported in ewes fed 200% of NRC requirements for protein (Berardinelli et al., 2001).

Excess undegradable protein, especially when DIP is adequate, can also increase BUN and therefore reduce uterine pH. Miner et al. (1990) and Alderton et al. (2000) noted increased BUN in cows supplemented with UIP, but high UIP supplement did not increase BUN in other cases (Kane et al., 1998; Strauch et al., 2001). Uterine pH 7 d after estrus was reduced to the same extent by feeding diets with excess UIP (19.8% CP, 55.1% degradable) as excess DIP (20.4% CP, 75.4% degradable) to lactating Holstein cows (Elrod et al., 1993). However, Kane et al. (2002) did not produce differences in uterine pH in primiparous beef cows fed 36% CP supplements consisting of 108 or 165 g/d UIP or a 46% CP supplement that supplied 335 g/d UIP.

Supplementation of prepubertal heifers with 421 g/d UIP, primarily from blood meal, increased weight at puberty compared to control heifers fed 231 g/d UIP and age at puberty compared to heifers fed monensin (Lalman et al., 1993). In the same study, fewer high-UIP supplemented heifers were detected in estrus during the first 21 d of the breeding season but pregnancy rates were similar. Kane and coworkers (2004) fed postpubertal heifers 321, 216, or 115 g/d of UIP, mainly from feather meal and fishmeal. High-UIP heifers had increased follicular fluid IGFBP-2 and IGFBP-4 on d 12 to 14 of the estrous cycle, along with lower basal serum FSH compared to low and mid-UIP

heifers. Furthermore, FSH area under the curve was reduced for heifers fed 321 g/d supplemental UIP compared to heifers fed 115 g/d supplemental UIP, indicating potential negative effects on reproduction from UIP supplementation above predicted requirements.

Recently, research has focused on the metabolic effects of protein supplement, particularly supplement of UIP. Insulin, various metabolites, and more recently IGF-1, have been the primary compounds researched. Supplementation of UIP in diets with equal DIP increases insulin concentration and decreases plasma growth hormone (Sletmoen-Olson et al., 2000) in late gestation, but does not affect insulin concentration postpartum (Alderton et al., 2000; Kane et al., 2002) or in pubertal, yearling heifers (Kane et al., 2004). Decreased insulin-like growth factor binding protein-2 (IGFBP-2) in cows fed adequate DIP plus UIP may increase IGF-1 activity and explain why these cows maintained BCS when cows supplemented with DIP lost condition. Furthermore, increased IGFBP-3 in cows supplemented with DIP and not UIP may have decreased IGF-1 bioavailability and therefore exacerbated their weight loss (Alderton et al., 2000). Strauch and coworkers (2001) observed a transient increase in IGF-1 at calving in UIP-supplemented heifers, but this did not translate into improved pregnancy rate. Kane et al. (2004) were unable to identify differences in IGF-1, but follicular fluid IGFBP-2 and IGFBP-4 were greater in heifers fed high levels of UIP. Since decreases in IGFBP-2 and IGFBP-4 usually enhance follicular growth, increased expression of these IGFBPs from diets high in UIP could be detrimental to follicular development. Interestingly, heifers fed diets high in UIP (321 g/hd/d) had lower basal FSH secretion. Previous work from the same group had indicated 300 g of UIP per day could enhance basal FSH secretion

(Hawkins et al., 1999), or 335 g UIP did not influence FSH secretion, but increased GnRH-induced LH release (Kane et al., 2002). In summary, UIP supplement may have metabolic effects beyond improving N or amino acid balance, but endocrine and metabolic hormone response to UIP supplementation has been variable and probably interacts with body fat and (or) energy balance.

Embryonic Mortality

Embryonic mortality refers to losses that take place from fertilization until the period of differentiation, at approximately 42 d of gestation (Committee on Reproductive Nomenclature, 1972). Embryo loss is the major cause of reproductive failure in cattle and represents significant economic loss to the industry (Dunne et al., 2000). Numerous studies report a fertilization rate of 80-90% in beef heifers (Henricks et al., 1971; Diskin and Sreenan, 1980; and Roche et al., 1981). However, these studies also report that at 42 days following insemination, embryo survival rate was only 60% of initial. Furthermore, Roche et al (1981) demonstrated that embryo loss occurs primarily in the first 16 d following breeding. Dunne et al (2000) found embryo survival rates decreased to d 14 with no further reduction. These studies clearly demonstrate that embryo loss occurs primarily in the first 14 d following insemination.

Research in suckled beef cows, which may represent a similar nutritional demand as growth in heifers, indicates a positive influence of concentrate supplementation three weeks prior to and three weeks following breeding. In this study, all cows were restricted at 70% of requirements from calving until three weeks prior to breeding. Subsequently, cows were either maintained on the restricted diet or placed on a supplemented diet. On d 21, the supplemented group had a greater pregnancy rate (100% vs. 20%) compared to

the unsupplemented group (Khiredine et al., 1998). This indicates a positive effect of supplemental nutrition on embryo survival. Similarly, Short and Bellows (1971) reported heifers that gained $0.23 \text{ kg}\cdot\text{d}^{-1}$ throughout the winter experienced a 10% increase in early embryonic mortality and loss of pregnancy compared to heifers fed to gain 0.45 and $0.68 \text{ kg}\cdot\text{d}^{-1}$. Supplementation may be more beneficial in heifers that are nutritionally challenged or on a low plane of nutrition (Funston, 2004). Therefore, heifers developed at low to moderate gains may benefit from targeted supplementation around the time of breeding.

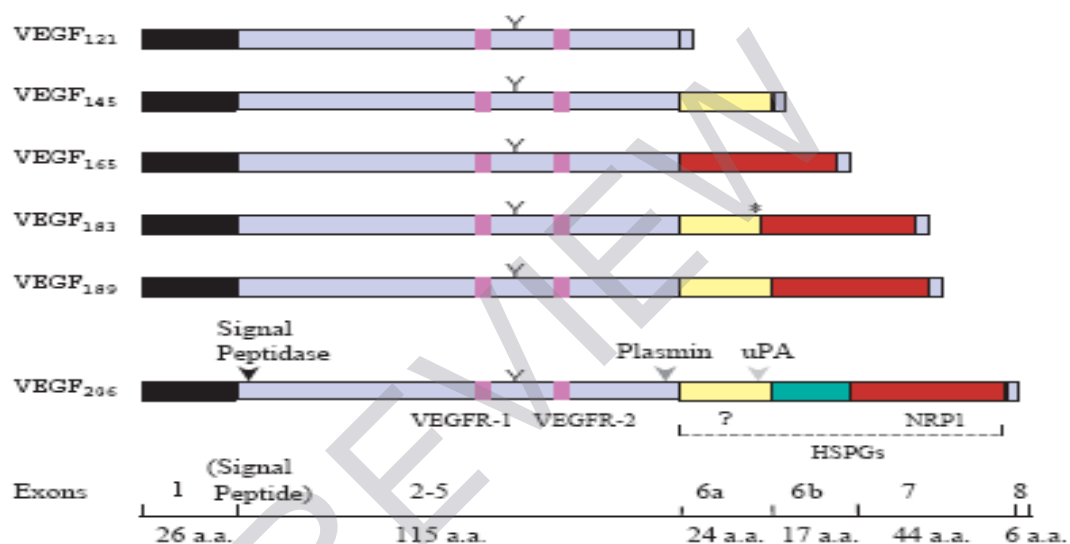
Effects of VEGF on Follicle Development

VEGFA Isoforms

Alternative splicing of the *VEGFA* gene produces at least six isoforms of the VEGFA protein in the human (Robinson and Stringer, 2001), as illustrated in Figure 1. The human *VEGFA* isoforms are the products of eight exons divided by seven introns (Ferrara et al., 2003). The murine *VEGFA* gene produces proteins one amino acid shorter than their human counterparts and is functionally similar with regards to the production of VEGFA120, VEGFA164, and VEGFA188 (Shima et al., 1996).

The most prevalent murine and human VEGFA isoforms are VEGFA120/121, VEGFA164/165, and VEGFA188/189 (Ferrara and Davis-Smyth, 1997). The single most abundant isoform is VEGFA164/165 (Ferrara et al., 2003). Chemically, VEGFA isoforms are homodimers consisting of monomers attracted by hydrophobic interactions and stabilized by Cys-Cys disulfide bonding (Potgens et al., 1994). Both VEGFA164 and VEGFA188 are basic glycoproteins that have heparin-binding domains. Conversely,

VEGFA120 is weakly acidic and does not contain a heparin-binding domain (Ferrara and Davis-Smyth, 1997). The mobility of VEGFA isoforms is dependent on size and the ability to bind heparin. The smaller of the common isoforms, VEGFA120, is freely diffusible and is not associated with the extracellular matrix. However, VEGFA188 is primarily bound to the extracellular matrix, and VEGFA164 is balanced between the free and bound state (Ferrara, 2004; Park et al., 1993; Houck et al., 1992).



Human VEGFA isoforms (adapted from Robinson and Stringer, 2001).

VEGF Anti-Angiogenic Isoforms

In addition to angiogenic VEGFA proteins, the VEGFA gene produces anti-angiogenic isoforms via substitution of exon 8a with an alternate terminal amino acid sequence produced by differential downstream mRNA splicing. A ninth exon, exon 8b, responsible for the production of anti-angiogenic isoforms is present (Figure 2; Bates et al, 2002). Exon 8b, previously called exon 9, of the *VEGFA* gene is highly conserved across species, with human and bovine sharing 90% homology (Figure 3; Bates et al.,