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Dietary manipulation of *Bos indicus* × heifers during gestation affects the prepubertal reproductive development of their bull calves

T.M. Sullivan, G.C. Micke, R.M. Greer, V.E.A. Perry*

School of Veterinary Science, The University of Queensland, St Lucia, QLD 4072, Australia

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ABSTRACT

We determined the influence of nutritional protein and energy during the first and second trimesters of pregnancy in composite beef heifers on prepubertal reproductive parameters of their male calves. At artificial insemination, heifers were stratified by weight within each composite genotype into 4 treatment groups: High/High (HH = 250% crude protein (CP) and 243% metabolisable energy (ME) for first and 229% CP and 228% ME for second trimester of pregnancy), High/Low (HL = 250% CP and 243% ME for first trimester and 63% CP and 176% ME for second trimester) Low/High (LowH = 75% CP and 199% ME for first trimester and 229% CP and 228% ME for second trimester) or Low/Low (LL = 75% CP and 199% ME for first trimester and 63% CP and 176% ME for second trimester). At 5 months of age, male calves were castrated, and gonadotrophins and testosterone (pre- and post-GnRH challenge), IGF-I and leptin were measured along with testicular parameters.

Lower maternal dietary protein and energy levels during gestation were associated with increased prepubertal FSH concentrations ($P = 0.03$) and paired testicular volume ($P = 0.04$) in male offspring. Serum LH ($P < 0.001$) and FSH concentrations ($P = 0.04$) were correlated with seminiferous tubule diameter. Testosterone concentrations were positively correlated with testis measures: paired testicular weight ($P < 0.001$), volume ($P = 0.03$) and seminiferous tubule diameter ($P < 0.001$). Although leptin concentrations were associated with prepubertal age ($P = 0.04$) and body weight ($P = 0.006$), they were not associated with any of the measures of reproductive development, but insulin-like growth factor-I was associated with prepubertal FSH ($P = 0.005$). In conclusion, prepubertal reproductive development of bulls may be affected by prenatal nutrition during early and mid gestation.

1. Introduction

Age at puberty is a major determinant of beef production efficiency as it enables breeding at a younger age thus reducing generation intervals, and increasing genetic gain and lifetime productivity (Yilmaz et al., 2006). Age at puberty may be affected by under nutrition at critical stages of development during fetal life as males undernourished in utero have reduced germ cell multiplication, Sertoli cell proliferation and development of the hypothalamic–pituitary axis (Da Silva et al., 2001; Bielli et al., 2002). Prepubertal gonadotrophins luteinising hormone (LH) and follicle stimulating hormone (FSH), scrotal circumferences (SC), paired testicular weights (PTW) and size, and peripubertal concentrations of the metabolic hormones insulin-like growth factor (IGF-I) and leptin have been reported to indicate sexual development and predict age at puberty (Moura and Erickson, 1997; Aravindakshan et al., 2000; Brito et al., 2007a,b).

As the testicle matures, it also changes shape increasing in the ratio of length to width (Chandolia et al., 1997) and increasing seminiferous tubule diameters (Evans et al.,

* Corresponding author. Tel.: +61 7 46712417; fax: +61 7 46712781.
E-mail address: v.perry@uq.edu.au (V.E.A. Perry).

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1996). There are close associations of testis parameters taken at 5, 12 and 24 months of age (Coulter and Keller, 1982; Moura and Erickson, 1997).

Bulls have an early transient rise in gonadotrophin secretion between 8 and 20 weeks characterizing the prepubertal period and stimulating a gradual increase in testosterone during this period. After 20 weeks, this is followed by a marked increase in testosterone in the pubertal period, associated with later stages of spermatogenesis and testis growth (Barth et al., 2008). The prepubertal concentrations of FSH are correlated with testis diameter and related to number of Sertoli cells per testis (Moura and Erickson, 1997). Prepubertal increases in the metabolic hormones leptin and IGF-I (Brito et al., 2007a) are also associated with rising SC (Thomas et al., 2002), testosterone (Renaville et al., 1996) and gonadotrophin concentrations (Wang and Hardy, 2004).

Prenatal nutrition affects age at puberty, testicular weight (Bielli et al., 2002), seminferous tubule diameter (Kotsampasi et al., 2009), prepubertal testosterone (Da Silva et al., 2001) and LH and FSH response to GnRH (Rae et al., 2002b; Kotsampasi et al., 2009) in rams. Similarly, in male rats, prenatal nutrition affects pubertal LH and testosterone in male rats (Zambrano et al., 2005). In the bovine, genetic differences also exist in age at puberty, SC, testicular volumes (Chase et al., 1997), testis size (Lunstra et al., 1988), prepubertal testosterone (Thomas et al., 2002) and LH (Bagu et al., 2006).

There has been limited study on the impact of prenatal nutrition, particularly the level of dietary protein, on prepubertal reproductive development of male calves. We tested the hypothesis that, in male calves, development of the testis and hypothalamic–pituitary axis and the associated synthesis of gonadotrophins would be affected by maternal dietary intake, genotype, and would correlate with IGF and leptin.

2. Materials and methods

2.1. Animals, management and treatments

One hundred and twenty composite beef breed heifers (mean age 23 months, range 21.6–24 months) were held on feedlot premises located on an extensive grazing property in south west Queensland, Australia (28°52′S, 150°33′E). Heifers were individually stall fed throughout the experiment, and acclimatized for 45 days to the new environment and management practices, before being synchronized for timed artificial insemination (AI). Oestrus synchronization was achieved using a combination of intravaginal progesterone-releasing devices (EAZI-BREED™ CIDR® cattle device, Pfizer Animal Health, Australia, 1.9 g progesterone), 1 mg oestradiol benzoate i.m. (Ciderol®, Genetics Australia, Bacchus Marsh, Australia) and 25 mg prostaglandin i.m. (Lutalyse®, Pfizer Animal Health, Australia). Heifers were artificially inseminated with semen from the same Senepol bull on the same day. Thirty five percent of the heifers were BeefX genotype (1/2 Senepol, 1/4 Brahman, 1/8 Charolais, 1/8 Red Angus) and 65% were CBX (1/2 Senepol, 1/4 Brahman, 1/4 Charolais).

Heifers were initially divided into two treatment groups stratified by body weight and genotype. For the first trimester of gestation (0–93 days post AI), the groups were fed either a high (H) or low (L) protein diet (Table 1). During the second trimester (93–180 days post AI) half the animals in each treatment group changed to the alternate group, giving rise to four treatment groups: high/high (HH), high/low (HL), low/high (LowH), low/low (LL). During the third trimester (181 days post AI to term) all heifers were fed on a standard diet (Table 1).

Of the 120 animals, 2 were removed for temperament, 41 because they were found to be not pregnant at day 39 post AI and 6 because they aborted. This left a total of 71 animals distributed across treatment groups at calving: HH = 16, HL = 19, LowH = 17 and LL = 19.

Feed rations consisted of cottonseed meal (*Gossypium* spp.), cracked sorghum seed (*Sorghum* spp.), Bambatsi hay (*Panicum coloratum*), or barley straw (*Hordeum* spp.), lime and a vitamin and mineral premix. Water was provided ad libitum. The nutritional content was measured using a combination of wet chemistry and near infrared spectrophotometry (CASCO Agritech, Toowoomba, Queensland, Australia). Samples of cottonseed were analyzed for free gossypol by AOCS official method Ba 7-58 (AOCS, 1985). Dietary requirements were calculated according to NRC dietary recommendations for pregnant replacement heifers (Table 1 (NRC, 1996)). Post calving dams grazed native pastures and calves were weaned at 6 months of age.

2.2. Testicular measures

At 5 months of age, the male calves (HH = 7, HL = 9, LowH = 9, LL = 8; CBX = 23, BeefX = 10) were weighed and castrated. Testes were removed through 10 cm long cranioventral incisions in the scrotal sac, 2–3 cm either side of the median groove. The tunica was incised longitudinally to expose the testis and the testis was freed by separation of the tunica from the proper ligament at the tail of the epididymis. The deferent duct, testicular artery and pampiniform plexus were transected 5 cm ventral to the testis. Left and right testes were used for testis measures. Testis length was measured from the head to the tail of the epididymis using Vernier callipers. Testis width was measured horizontally across the widest section of the body of the epididymis. Two width measures were taken at right angles to each other in craniocaudal and mediolateral dimensions. Weight of each testis was recorded and PTW was calculated. Paired testicular volume was calculated as the sum of volume of the right and the left testicle. Testicles were considered paraboloids, and the equation for calculating the volume was $v = \pi r^2 h$, where r = (width A + width B)/4 and h = length (Chase et al., 1997).

Testicles were sectioned in a mid sagittal plane and 10 mm square sections of testicular parenchyma were taken from the central region of each testicle and fixed in 10% buffered formalin. Testis samples were embedded in paraffin wax, sectioned (7 μm thick) and stained with hematoxylin–eosin. Average seminiferous tubule diameter of each testicle was determined from 50 randomly selected tubular cross sections with a visibly round cir-