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Introduction

The specific biological mechanisms that control pubertal development, testicular growth and onset of sperm production in the beef bull have not been well defined. It is known that two protein hormones (the gonadotropins, LH and FSH) produced by the pituitary stimulate testicular growth, cause an increase in numbers of testicular receptors for LH and FSH, and stimulate production of steroid hormones by the testes. Researchers have shown that the patterns of LH and FSH secretion diverge during the peripubertal period in several species, including the bull. While LH increases during the peripubertal period of development in the bull, FSH exhibits little change during this period. Inhibin, a protein hormone produced by the testes, has been shown to selectively inhibit FSH secretion in other species. Thus, inhibin may explain the divergence in peripubertal patterns of LH and FSH secretion in the bull and may be an important regulatory factor in bovine testicular development.

In other species, immunization of males against endogenous inhibin has been shown to influence secretion of FSH and testicular function. Structurally, the inhibin molecule in various species is composed of two protein chains termed the alpha and the beta chains. Each protein chain is composed of numerous amino acids, but the sequence of amino acids in the alpha chain contributes to inhibin's unique hormonal properties and species specificity. Thus, immunization against inhibin is usually performed by utilizing the alpha chain to induce immunological specificity against inhibin. Immunoneutralization of endogenous inhibin has been shown to cause increased secretion of FSH in rats, and similar immunization in rams has resulted in increased FSH, LH, testis size, daily sperm output and epididymal sperm reserves. Although inhibin is present in peripheral blood of bulls, the role of inhibin in regulation of gonadal growth and function in bulls is unknown. Therefore, the objective of the present experiment was to determine the importance of inhibin in regulation of secretion of FSH, LH and testosterone, testicular growth, and/or sperm production in young beef bulls.

Procedures

Animals, Treatments and Samples—Beginning at 14 wk of age (3.5 ± 0.1 mo of age and 215 ± 9 lb body weight; mean ± SE), 20 Angus x Hereford-Brahman bulls (n = 10 per treatment group) were actively immunized against the first 26 amino acids of bovine inhibin alpha (bINH-Immum bulls) conjugated to a carrier protein, human alpha globulin (HAG), or were immunized against HAG alone (control bulls). The primary immunization was followed by booster immunizations given at 28, 30, and 34 wk of age. Body weight and scrotal circumference (an excellent indicator of paired testicular mass) were measured at the beginning of the experiment and 10 days after each immunization. A single jugular blood sample (10 ml) was collected from each bull 10 days after each booster for determination of bINH antibody titer and for hormone assays. Ten days after the last booster (at ~36 wk of age), blood was sampled at 1-hr intervals for 8 hr to quantify serum concentrations of FSH, LH, and testosterone. Bulls were castrated at 9 mo (36 wk) of age, and testicular daily sperm production was determined via homogenization.

Testicular Sperm Production—Three subsamples of testicular tissue per bull (1-2 gram subsample from the proximal, middle, and distal portions of the left testis from each bull) were obtained, and subsamples were thoroughly homogenized. The average number of homogenization-resistant spermatids per gram of testicular tissue was determined for each bull from hemacytometer counts using a microscope. To determine testicular daily sperm production (DSP), the number of homogenization-resistant spermatids per gram of testicular tissue was divided by 5.32 day (species-specific constant for calculation of daily sperm production in bulls).

Inhibin Antibody Titers—To determine the amount of anti-inhibin antibodies (i.e., bINH-antibody titer) that had been induced in each bull, blood serum was diluted 1:4000 and bINH-antibody titers were determined in all serum samples collected 10 days after each immunization. All titers were determined in one assay and the intrassay coefficient of variation (CV) was 3.7%.

Gonadotropin and Steroid Hormone Assays—Serum samples were assayed for testosterone, LH and FSH via validated laboratory radioimmunoassays. The intraassay CV was 4.4% for FSH and 4.2% for LH. Extraction efficiency for testosterone was 94%; intraassay and interassay CVs for testosterone were 3.8% and 7.6%, respectively.

Results & Discussion

Both body weight and scrotal circumference increased continuously (P<0.01) between 14 and 34 wk of age in both treatment groups of bulls. However, body weight and scrotal circumference of treated bulls did not differ (P>0.20) from control bulls throughout the experiment (Martin et al., 1991). Substantial anti-inhibin titers were established in bINH-Immum bulls, and serum diluted 1:4000 from bINH-Immum bulls bound 38 ± 4%, 52 ± 5%, and 53 ± 4% radioiodinated bINH (mean ± SE of 10 bulls per group) 10 days after boosters given at 28, 30, and 34 wk of age, respectively, while binding was less than 2% in control bulls. For the single blood samples taken 10 days after the first and second boosters, serum concentrations of FSH and testosterone were similar (P>0.20), but concentrations of LH were decreased (P<0.05) in bINH-Immum compared with control bulls. However, for the blood samples obtained at hourly intervals for 8 hr (i.e., at 9 mo of age), serum concentrations of FSH were increased (P<0.05) substantially and serum concentrations of LH were decreased (P<0.001) markedly in bINH-Immum bulls compared with control bulls (Figure 1). Despite this reduction in serum LH, concentrations of serum testosterone also were increased (P<0.05) in bINH-Immum bulls at 9 mo of age (Figure 2). While testis size (scrotal circumference) did not differ between bINH-Immum bulls and control bulls at 9 mo of age (Martin et al., 1991), daily sperm production per gram testicular tissue was dramatically increased (P<0.05; Figure 2) in bINH-Immum bulls compared with control bulls.
The threefold increase in serum FSH present at 9 mo of age in bulls immunized against inhibin, compared to control bulls, supports the concept that inhibin functions to suppress FSH in the bovine. Other research has indicated that castration of bulls causes a two- to four-fold increase in serum FSH (MacDonald et al., 1991). Although castration removes other potential FSH-regulatory factors that originate from the testis, such as steroids, our data indicate that inhibin may have a potent negative feedback effect of secretion of FSH in bulls.

Other researchers have reported that both intratesticular and blood concentrations of testosterone increase around 4-5 mo of age in bulls. High intratesticular concentrations of testosterone promote Sertoli cell differentiation, leading to differentiation of germ cells into spermatogonia and establishment and maintenance of spermatogenesis. In our study, the increased secretion of FSH could have increased testicular sensitivity to LH, since FSH increases numbers of FSH and LH receptors in testes. Despite the lower concentrations of LH, testosterone concentration was greater in blNH-immun bulls than in controls. We speculate that exposure of the testes to high concentrations of FSH may have markedly increased the sensitivity of the testes in blNH-immun bulls to circulating gonadotropins. Therefore, increased sensitivity of the testes to gonadotropins may explain the increased secretion of testosterone, despite decreased secretion of LH, in blNH-immun bulls.

Our most significant finding was that total daily sperm production and sperm production per gram of testicular tissue increased approximately two-fold following immunization against inhibin. This increase in sperm production coincided with increased FSH and testosterone concentrations in blNH-immun bulls, suggesting that both hormones may enhance testicular sperm production in bulls. In bulls, testosterone is an important regulator of testicular maturation. Alternatively, inhibin has been reported to have local inhibitory effects on testicular function, and removal of inhibin via immunoneutralization may have released testicular sperm production from the local inhibitory actions of inhibin. Others have reported that inhibin decreases numbers of spermatogonia when injected into the testis of mice or hamsters. In support of this hypothesis for a localized effect of inhibin, unilateral intratesticular injections of inhibin have been shown to decrease numbers of spermatogonia in the inhibin-injected, but not in the contralateral testis, of mice and hamsters. Therefore, increased testosterone secretion and enhanced sperm production in blNH-immun bulls may be due, in part, to removal of local inhibitory actions of inhibin on the testes.

The mechanism(s) by which inhibin immunoneutralization selectively increased spermatid density in the testis of neopubertal beef bulls is unknown. It was surprising that total daily sperm production and sperm production per gram of testicular tissue was increased without significant changes in testis size (scrotal circumference). The lack of change in scrotal circumference, despite a twofold increase in spermatids in blNH-immun bulls, may indicate that Sertoli cell function was altered and resulted in increased production of spermatids within the seminiferous tubules. In support of our findings, Schanbacher (1991) recently reported that sperm density per gram of testis, but not testicular size, tended to be increased at 13 mo of age in beef bulls that had been immunized against porcine inhibin alpha. It is possible that immunoneutralization of inhibin in young beef bulls may result in an acceleration of pubertal development and earlier onset of sperm production. Further studies are needed to determine if the increased sperm production induced by inhibin immunoneutralization continues to be maintained postpubertally in more mature bulls.

In summary, immunoneutralization of inhibin in young beef bulls increased serum concentrations of FSH and testosterone, decreased serum concentrations of LH, dramatically enhanced sperm production per gram of testis and total daily sperm production, and did not alter testis size or body weight. We conclude that inhibin plays an important role in the regulation of secretion of gonadotropins and in regulating testicular development and sperm production in young beef bulls.

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References
Figure 1 – Effect of active immunization against inhibin during development on serum concentrations of FSH and LH in control and bINH-Immun bulls at 9 mo (36 wk) of age. Blood was sampled at 1-hr intervals for 8 hr at 9 mo of age. Values shown are means over the 8-hr sampling period ± SE for 10 bulls per treatment group.

Figure 2 – Effect of active immunization against inhibin during development on serum concentrations of testosterone and on daily sperm production per gram of testis for control and bINH-Immun bulls at 9 mo (36 wk) of age. Daily sperm production per gram testicular tissue was calculated by dividing the number of homogenization-resistant spermatids per gram by a constant of 5.32 days. Values shown are means ± SE for 10 bulls/treatment group.