The effect of Vitamin A treatment on testicular development in young peri-pubertal bulls

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The establishment of the final number of Sertoli cells in the bull calf testicle around puberty is one of the key determining factors of the animal's adult fertility. Vitamin A administration did not have any significant effect in testicular development in peri-pubertal bull calves.

Summary

Fourteen Angus and Shorthorn bull calves between 11 and 12 months of age were assigned randomly to one of two treatments: 1) Intramuscular injection of 1 million International Units (IU) of vitamin A (Vit A) or 2) no treatment (Control). Scrotal circumference (SC) was measured in all bull calves at the time of the treatment application and 11 days later at castration. Testes and epididymis were dissected and weighed, and samples of parenchyma were collected from each testicle. Samples were fixed in formaldehyde, embedded in paraffin and cut. Slides were deparaffinized with successive washes of xylene and alcohol. After antigen retrieval and blocking with 10 percent normal goat serum, sections were incubated sequentially with mouse monoclonal antibody to androgen receptor and goat-antirabbit (IgG CF633) fluorescent stain. Samples were examined under a fluorescent microscope and the images captured with a digital camera and processed using Image-Pro Plus software. Effects of treatment on SC, testicular weight, testicular parenchyma weight and epididymis weight, number of Sertoli cells, germ cell, and ratio of germ cells to Sertoli cells within the seminiferous tubule (ST) were analyzed using the ANOVA procedure of SAS. No differences were observed among treatments for SC (1.63 ± 0.26 vs. 2.17 ± 0.17), testicular weight (228.169 ± 17.002 vs. 221.792 ± 24.983), testicular parenchyma weight (grams [g]) (141.02 ± 11.82 vs. 131.46 ± 14.49), epididymis weight (g) (12.01 ± 1.10 vs. 9.43 ± 1.01), number of Sertoli cells/ST (45.56 ± 3.14 vs. 48.04 ± 2.18), number of germ cells/ST (217.29 ± 26.83 vs. 156.04 ± 16.75) and the ratio of germ cells/Sertoli cell (5.03 ± 0.93 vs. 3.30 ± 0.43) for vitamin A and control, respectively. These results suggest that vitamin A has no positive effect on fertility when administered in peri-pubertal bulls.

Introduction

More than 95 percent of our beef herds rely on natural service as the main breeding system. Very little information is available about interventions administered during the peri-pubertal period in the bull and their ramifications on adult fertility.

Sperm production in the bull is influenced early in life through the establishment of the definite number of Sertoli cells in the testicle, which nourish developing sperm before puberty, when they stop replicating (Sharpe et al., 2003). Bulls with a larger Sertoli cell population have greater daily sperm production (DSP), testicular weight and SC, compared with bulls having fewer Sertoli cells (Berndston et al., 1987).

Recent findings showed that the proportion of Sertoli cell numbers was higher in bulls that produced good-quality semen with higher numbers of viable spermatozoa after thawing in comparison with bulls that had lower proportions of Sertoli cells (Rajak et al., 2016). Sertoli cell proliferation has a specific window of time that extends from the fetal stage at midgestation up to six to 10 weeks in the newborn calf (Moura et al, 1997).

Recent findings suggest that influencing the final number of Sertoli cells in the bull testis is possible once differentiated (Zakhidov et al., 2015). Several factors have been described as having an effect on Sertoli cell replication, such as thyroid hormone (T3), testosterone (T), follicle-stimulating hormone (FSH), insulin growth factor (IGF-I) and vitamin A through its active compounds retinoic acid (RA) and retinol (RE) (Lucas et al., 2014).

Retinoic acid also has been reported to interact with transforming growth factor beta (TGF-β) in various other tissues, not limited to the testis, affecting cell proliferation and differentiation (Cupp et al., 1999).

Retinoic acid receptors RARα and RARαhave been discovered in rat Sertoli cell nucleus (Livera et al., 2002), and the fundamental role of RA in spermatogenesis has been known for several years, where animals deficient in vitamin A are sterile with a complete halt in spermatogenesis. This was demon-

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strated in genetically engineered mice lacking RA receptors, showing a delay in Sertoli cell differentiation, progressive spermatogenic degeneration and infertility (Nicholls et al., 2013). Retinoic acid in the rat had a detrimental effect in the fetal testis and a beneficial effect on the neonatal rat testis (Livera et al., 2000).

Most of the studies performed on Sertoli cell proliferation and differentiation have been done on species such as rats, sheep, humans and pigs but very little information is available on the effects exerted by vitamin A on Sertoli cell populations in the bull.

Experimental Procedures

Fourteen Aberdeen Angus and Shorthorn bull calves (28 7± 3.3 days, 683 ± 29 pounds) from the North Dakota State University Beef Unit were used in the study. Ten days prior to castration, bulls were assigned randomly to one of two treatments; 1) intramuscular injection of 1 million IU of vitamin A (Vitamin AD Injectable, Durvet Laboratories, Blue Springs, Mo.; Vit A); or 2) no treatment (Control).

Testicle scrotal circumference (SC) was measured in each bull at the time of treatment administration and also at the time of castration 11 days later. All bulls were castrated using the open method with a scalpel following application of epidural and local anesthesia. Once the testicles were removed, the epididymis was dissected and detached from the testicles. Testicles and epididymis were weighed separately.

The tunica albuginea was dissected from each testicle and the testicular parenchyma was weighed. Samples for histological examination (4 by 4 millimeters [mm]) from one region of each testis parenchyma were taken, fixed in a 10 percent formaldehyde solution, embedded in paraffin and cut into sections 5 micrometers (µm) thick using a microtome (Leica Biosystems Inc., Buffalo Grove, Ill.).

Samples were submerged in sodium citrate buffer and placed in an antigen retriever (2100 Retriever, Aptum Biologics, UK) and incubated sequentially using mouse monoclonal antibody to androgen receptor (ab9474, abcam, Cambridge, Mass.) at 4 C overnight with agitation, and then stained with goat-antirabbit IgG CF633 fluorescent stain.

For each tissue section, images were taken at 100 times magnification using a Zeiss Imager M2 epifluorescence microscope equipped with Zeiss piezo automated stage and AxioCam HRm camera (Carl Zeiss International, Jena, Germany). Image analysis (Image-Pro Plus, Media Cybernetics Inc., Bethesda, Md.) was performed for images of five randomly chosen fields. Within each image, four to six seminiferous tubules were selected randomly for Sertoli and germ cell individual cell counts using the Image-Pro Plus image analysis software (Media Cybernetics Inc., Rockville, Md.).

The ANOVA procedure of SAS (SAS Inst. Inc., Cary, N.C.) was used to analyze differences in scrotal circumference, testicular weight, testicular parenchyma weight and epididymis weight between treatments. The number of Sertoli cells and germ cells per seminiferous tubule (ST) were counted and means calculated for each testicle.

The ratio of germ cells to Sertoli cells was calculated by dividing the total number of germ cells by the total number of Sertoli cells within each tubule. Total means were obtained for each testicle and analyzed using the same statistical procedure. Significant differences were considered when $P < 0.05$.

Results and Discussion

No differences were observed between treatments (Table 1) for measures of scrotal circumference ($P = 0.13$), and weights of the testicles ($P = 0.83$), parenchyma ($P = 0.61$) or epididymis ($P = 0.11$; Table 1). In addition, no differences were observed between treatments in the number of Sertoli cells per ST ($P = 0.44$), number of germ cells per ST ($P = 0.20$) or the ratio of germ cells/Sertoli cell ($P = 0.16$).

This preliminary experiment indicated that the administration of vitamin A in young peri-pubertal bulls did not have a beneficial effect in testicular development. The lack of effects of vitamin A on the different testicular cell types could be the

Table 1. Effect of vitamin A on testicular development in young beef bulls.

	Vitamin A		Control		
Item	Mean	SEM	Mean	SEM	*P* Value
No. bulls	8		6		
Difference in SC (cm)	1.63	0.26	2.17	0.17	0.13
Testicular weight (g)	228.17	17.00	221.79	24.98	0.83
Testicular parenchyma weight (g)	141.02	11.82	131.46	14.49	0.61
Epididymis weight (g)	12.01	1.10	9.43	1.01	0.11
Sertoli cells/ST	45.56	3.14	48.04	2.18	0.56
Germ cells/ST	217.29	26.83	156.04	16.75	0.10
Ratio (germ cells/Sertoli cell)	5.03	0.93	3.30	0.43	0.1

SEM = standard error of the mean; SC = scrotal circumference; ST = seminiferous tubules.

reflection of an already differentiated and stable Sertoli cell population because previous studies indicate that the end of Sertoli cell division occurs before 30 to 40 weeks of age in the bull (Rawlings et al., 2008).

The lack of information about precise time frames for obtaining beneficial effects on testicular development in the bull by the application of different treatment and management strategies highlights the need for further research.

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