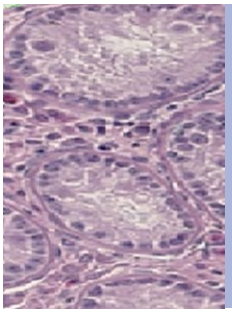

Comparison of Fine-needle Aspiration and Tissue Sections to Determine Sertoli and Germ Cell Counts in Prepubertal Beef Bulls

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At present, no methodology can predict potential daily sperm production and fertility in the bull. The size of the Sertoli cell (SC) population established in the testis before puberty is one of the most important determinants of daily sperm production in the bull. Fine-needle aspiration (FNA) has been used for many decades as a diagnostic tool, but its value to determine Sertoli and germ cell counts in bulls remains unexplored.

The objective of this study was to compare three sampling techniques (22G needle FNA vs. tissue samples collected using a 14G needle with vacuum pressure [14G ASP] and tissue cuts) and two stains (immunohistochemistry with GATA4 [IHC] vs. HE) to determine SC density in prepubertal bulls.

Fourteen age-matched prepubertal Angus bulls from the Central Grasslands Research Extension Center near Streeter, N.D., were castrated and testicular parenchyma samples were obtained for cytology. At castration, body weight, scrotal circumference, and testicular and dissected testicular weights were determined. From the same testis side, parenchyma samples for histology were collected using tissue sections and 14G ASP, and smears were produced using 22G FNA.

Two smears and tissue sections of each sampling method were stained using immunohistochemistry with anti-GATA4 as a specific SC marker (IHC-GATA4) and two with conventional HE. Complementary mirrored tissue sections were produced and stained with IHC-GATA4 and HE for individualized ST cross section cell count comparison.

Scrotal circumference was highly correlated with intact (P = 0.010) and dissected testicular weights (P = 0.018). Furthermore, intact and dissected testicular weights were correlated with IHC-GATA4 SC density determined in mirrored tissue sections (P = 0.018 and P = 0.016, respectively).

A high correlation was observed between stains for SC (P < 0.0001) and GC (P < 0.0001) counts performed on individualized ST cross sections. A significant correlation existed between GC counts and SC counts (P = 0.027) and density (P = 0.032) in IHC -GATA4 mirror tissue sections.

Nevertheless, no significant correlation existed between techniques for SC density in IHC-GATA4 sections (P =0.587). The high correlation observed between scrotal circumference and testis weight underlines the importance that scrotal circumference has as a routine measurement in breeding bulls.

The use of mirrored tissue sections allowed us to specify individual ST and assess the value of GATA4 as a SC marker. No significant correlation existed for SC density performed in IHC-GATA4 22GFNA, 14G ASP and tissue sections; nevertheless, a high correlation was observed between GC and SC cross section counts and density, highlighting the role SC play in determining the number of GC in the testis of prepubertal bulls.

Introduction

Potential daily sperm production of the bull is determined by the size of the Sertoli cell (SC) population in the testicle (Berndston, 1987). Sertoli cells replicate at specific windows of time during the life of the individual (Moura and Erickson, 1997).

Once puberty is reached, SC stop multiplying, fixing the ceiling of daily sperm production of the bull (O'Shaughnessy and Fowler, 2011). The incorporation of an accurate assessment method of SC population size could identify those subfertile individuals that escape current detection in routine breeding soundness exams (BSE) (Rajak et al., 2013).

Fine-needle aspiration (FNA) has been used for many decades as a diagnostic technique for neoplasms (Martin, 1930), reproductive pathologies (Aridogan et al., 2003), and infertility in humans (Craft et al., 1977) and animals (Leme and Papa, 2010). When compared with open biopsy, FNA presents several advantages, such as low invasiveness and no complications (Heath et al., 2001).

We hypothesized that Sertoli cell density determined using 22G needle FNA vs. 14 G with vacuum pressure vs. tissue sections are highly correlated. The objectives were to compare three sampling techniques (22G FNA vs. 14G needle with vacuum pressure and tissue sections) and two stains (HE vs. IHC-GATA4) to determine SC density in prepubertal bulls.

Materials and Methods

Fourteen aged-matched crossbred Angus bulls of 90 to 97 days of age and a bodyweight (±SEM) of 159.82 ± 3.67 kilograms (kg) were castrated surgically using an open-knife surgical procedure (IACUC #A18077). Fine-needle aspiration samples and tissue sections were collected from the same testis side.

The following stains were performed:

2 slides x 22G smears with HE

2 x 22G smears with IHC-GATA4

2 x 14G tissue sections with HE

2 x 14G tissue sections with IHC-GATA4

1 x conventional tissue section with HE

1 x conventional tissue section with IHC-GATA4

For stain comparison, mirrored tissue cuts were produced, allowing cell counts in complementary seminiferous tubule cross sections (Figure 1).

Immunohistochemistry-GATA4 and HE images from tissue sections and smears were captured using a Zeiss Imager M2 epifluorescence microscope equipped with a Zeiss piezo automated stage and AxioCam HRm camera (Carl Zeiss International, Jena, Germany). From each IHC and HE smear and tissue section, five different images from randomly chosen fields were captured at 20-x magnification. Cell counts were performed using the Image Pro Premier 3-D software (Media cybernetics, Rockville, Md.).

Statistical Analysis

Data for birthweight, testicular parameters and cytology were analyzed using the PROC CORR and PROC REG procedure in SAS (SAS version 9.4; SAS Inst. Inc., Cary, N.C.).

Significance levels for all data comparison were determined and considered significant when $P<0.05$.

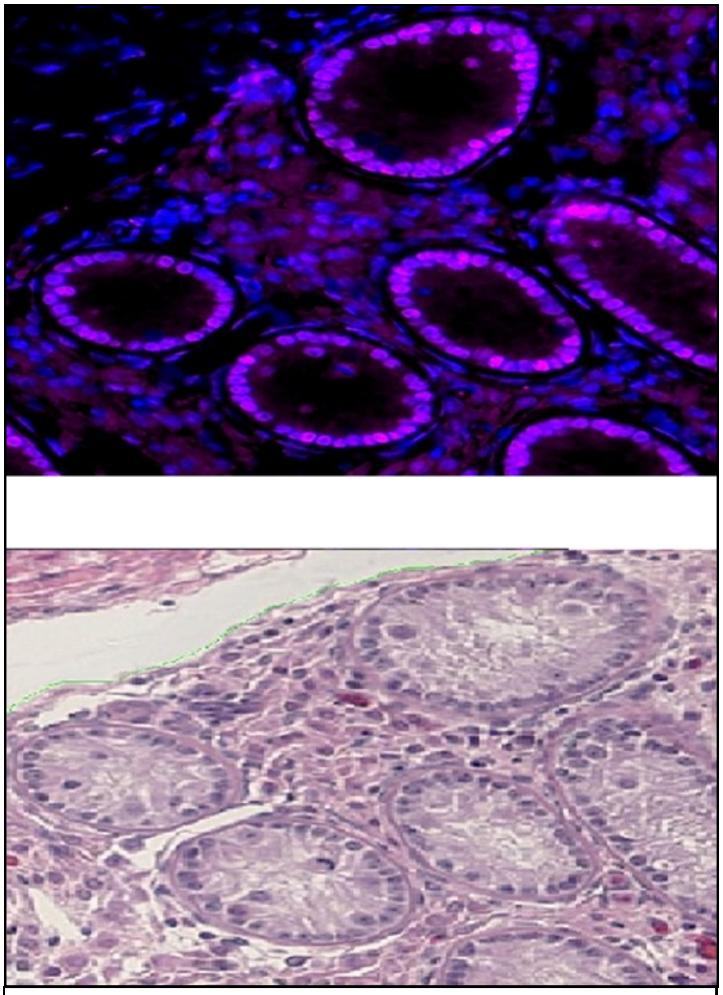


Figure 1. Images correspond to complementary mirror tissue sections stained with IHC-GATA4 (top) and HE (bottom). Stars of the same color correspond to the same ST cross section.

Table 1. Pearson's correlation coefficient and significance levels between Sertoli and germ cell counts per seminiferous tubule cross section on IHC-GATA4 or HE in prepubertal beef bulls (90 to 97 days of age).

	Pearson's correlation coefficient			
Parameter	SC IHC auto	SC HE count	GC IHC count	GC HE count
SC IHC auto[a]		0.945***	0.587*	0.550*
SC HE count[b]			0.526*	0.447
GC IHC count[c]				0.948***
GC HE count[d]				

[a]SC IHC auto = SC automatic count per ST cross section on IHC-GATA4 mirror section.

[b]SC HE count = SC manual count per ST cross section on HE mirror section.

[c]GC IHC count = GC count per ST cross section on IHC-GATA4 mirror section.

[d]GC HE count = GC count per ST cross section on HE mirror section.

*P<0.05; **P<0.01; ***P<0.0001.

Results and Discussion

A high significant correlation was observed between GC HE counts per ST cross sections and intact (r = 0.607; *P* = 0.021) and dissected testicular weight (r = 0.594; *P* = 0.024). Furthermore, intact and dissected testicular weights were correlated with IHC-GATA4 SC density determined in mirrored tissue sections (r = 0.616; *P* = 0.018) and (r = 0.628; *P* = 0.016, respectively).

Sertoli cell counts conducted in the same individual ST cross section using IHC-GATA4 and HE were highly correlated for automatic (*P* < 0.0001) and manual counts (*P* < 0.0001) (Table 1), showing the usefulness and value of GATA4 as a specific SC marker (Figure 2). A similar scenario was found for GC, with highly correlated counts per ST cross sections for IHC and HE (*P* < 0.0001).

Sertoli cells are the only somatic cells within the ST that provide the hormonal, nutritional and physical support to germ cells (Moura and Erickson, 1997). In agreement with this, significant correlations existed between automatic and manual SC and GC counts done per ST cross sections on IHC-GATA4 (*P* = 0.027) and HE sections (*P* = 0.041) (Table 1).

When GATA4+ cell density determined in smears obtained by 22G FNA was compared with GATA4+ cell density done on tissue sections or in 14G ASP sections, no significant correlation was found (*P* = 0.587). The absence of ST micro-anatomical references hindering the cytological reconnaissance of immature SC combined with the heterogeneous distribution and mixture of different types of loose cells observed in 22G FNA smears might contribute to the absence of significant correlations for SC density between smears and tissue sections.

Furthermore, intrinsic aspects of technique, such as uneven application of vacuum, syringe and needle control, and hand force might be causing the lack of consistency observed between FNA samples for SC density (Haseler et al., 2011).

Conclusions

The high correlation observed between SC and HE counts done in the same ST cross sections using IHC-GATA4 and HE confirms the value of GATA4 as a specific SC marker in prepubertal bulls.

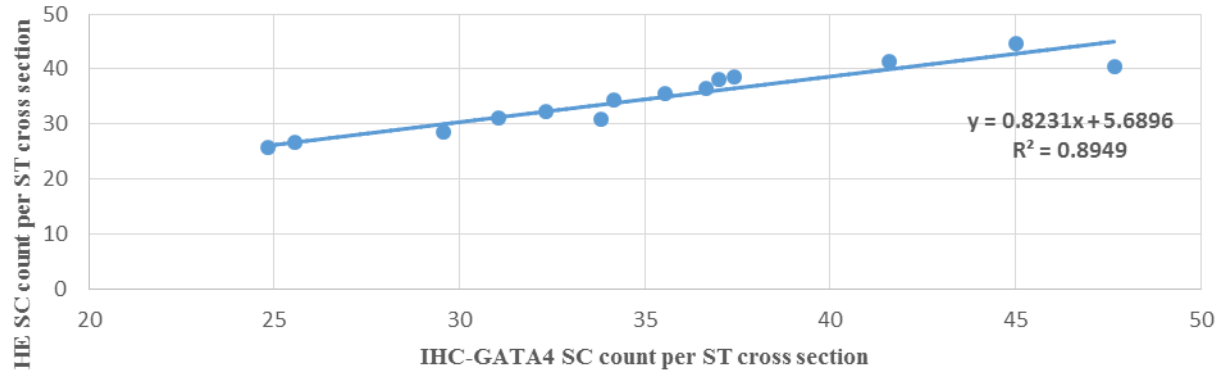
The high correlations seen between manual and automatic SC and GC counts performed on the same individual ST reflects the high accuracy of the automatic cell count using an image processing software tool.

The significant correlation detected between SC counts done in individual ST cross sections or as SC density with GC counts highlights the important role SC play in determining GC counts in prepubertal bulls.

The role of SC population size as a determinant of potential daily sperm production in the bull was confirmed by the relationship observed between SC densities and testis weights.

The lack of significant correlations for SC density among IHC-GATA4 22G FNA, 14G ASP and tissue sections observed in the present study reflects differences in cell counts among the sampling methods.

Figure 2. The relationship between Sertoli cell (SC) counts done on seminiferous tubule (ST) cross sections stained with immunohystochemistry with GATA4 (IHC-GATA4) and hematoxylin and eosin (HE) in prepubertal beef bulls (90 to 97 days of age).



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