Sperm abnormality traits can contribute to the genetic evaluation for male and female reproduction in tropical beef genotypes

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Summary

Genetic parameters for traits describing the incidence of sperm cell abnormalities (proximal droplets (PD), abnormalities of the sperm cell mid-piece and tails and heads) were estimated for 1,234 Brahman (BRAH) and 1,914 Tropical Composite (TCOMP) bulls at 12, 18 and 24 months of age. At 12 months, only 8.3% of BRAH bulls were sufficiently mature to produce an analysable sample, compared to 50.4% for TCOMP. PD, associated with samples from peripubertal bulls, were the most common abnormality, with highest incidence for BRAH at 18 months (24.6%) and TCOMP at 12 months (22.6%) of age. Heritabilities for PD tended to be higher than for other abnormalities, at 0.39 for BRAH at 24 months, and 0.45 for TCOMP bulls at 12 months. Genetic correlations of PD with female age at puberty (AP) and lactation anoestrous interval (LAI) were moderate for BRAH bulls at 18 (r_g = 0.51 and 0.35 respectively) and 24 months (r_g = 0.22 and 0.49 respectively). These relationships were generally weaker for TCOMP, though PD at 12 months showed a comparable genetic correlation with LAI of 0.51. AP and LAI are difficult and expensive to measure for genetic evaluation. The potential for PD to be exploited as a direct and early descriptor of male puberty, and an indicator trait for female reproductive performance, presents new opportunities to apply selection to improve reproductive performance in tropical beef breeds.

Keywords: sperm abnormalities, bull fertility, female reproduction, genetic correlation.

Introduction

Corbet *et al*. (2013) presented genetic parameters for traits evaluated as part of the Bull Breeding Soundness Evaluation (BBSE), described by Chenoweth (1980) in tropically adapted Brahman (BRAH) and Tropical Composite (TCOMP) bulls, which included laboratory analyses to determine percent normal sperm (PNS). The study found that while PNS could be heritable for both BRAH and TCOMP (h^2 = 0.00 to 0.41), age at measurement (12, 18 and 24 months) significantly impacted the amount of additive variance for the trait and the resultant heritability. Johnston *et al*. (2014*b*) reported moderate genetic correlations of higher PNS with lower female age at puberty (AP) and lactation anoestrous interval (LAI) (r_g = -0.05 to -0.48). The study concluding that PNS could be measured relatively early (compared to female LAI and lifetime reproductive performance), had the advantage of being expressed in males, and presented opportunities as a genetic indicator for female reproductive performance.

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In evaluating PNS, specific abnormalities were attributed to each abnormal cell (Burns *et al*., 2013). This study aimed to estimate heritabilities for sperm abnormality traits, and to establish whether these could provide useful information to the genetic evaluation of reproduction traits in young, tropically adapted beef bulls and females.

Material and methods

Bull management and sperm morphology traits

Management and data collection protocols for the 1,234 BRAH and 1,914 TCOMP bulls which generated the data for this study were described by Burns *et al*. (2013). These were the progeny of cows bred for the Beef CRC northern breeding project described by Johnston *et al*. (2009, 2014*a*). The TCOMP genotype comprised ~50% tropically adapted Bos indicus or African Sanga, and 50% non-adapted Bos taurus genetics (Barwick *et al*., 2009). Bulls were bred on five properties across central, northern and western Queensland over 7 years using sires selected to ensure representation of industry populations and allocated to achieve genetic linkage across years and properties of origin. At weaning, bull calves were relocated from breeding locations to one of two research station (see Burns *et al*., 2013).

Bull breeding soundness examinations (BBSE) were conducted on young bulls when contemporary groups averaged 12, 18 and 24 months of age. Mean age in days ± s.d. were 374 ± 28.2, 526 ± 27.7 and 704 ± 25.5 for BRAH and 398 ± 28.7, 551 ± 29.5 and 728 ± 24.4 for TCOMP, As part of this procedure, semen samples were collected using an Electrojector® (CGS Products Pty. Ltd., Trafalgar, Vic. Australia) from any bull with a scrotal circumference of greater than 20cm. A sample from the resulting ejaculate was fixed using phosphate-buffered saline (PBS), and transported to a laboratory for sperm morphology analysis (Table 1). Table 1 presents a brief description of the sperm abnormality traits analysed for this study.

Table 1. Description of sperm abnormality traits measured at 12 , 18 and 24 month (all traits are percentages of sperm cells evaluated which display the specified abnormality).

Trait	Code[1]	Description[2]
Knobbed acrosomes	KA	Abnormal development of the acrosome which produces a flattened or indented apex to the sperm cell.
Pyriform heads	PH	Sperm cells which develop with an atypical (pear shaped) head which affects the cell nucleus and fertilising capacity.
Abnormal mid pieces	MP	Includes several abnormalities of the sperm cell mid-piece.
Proximal and distal droplets	PD	Spherical adhesions to the proximal or distal segment of the sperm cell tail, most commonly observed in peripubertal bulls (Hopper 2015).
Swollen acrosomes	SA	Atypical enlargement of the acrosome.
Abnormal tails and heads	TH	Includes several abnormalities of the sperm cell tails and heads, including lose or detached heads and bent tails.
Vacuoles and teratoids	VT	Includes voids and irregular surfaces of the sperm cell indicative of severe disturbance in spermatogenesis.

[1] Descriptors of sperm abnormalities were produced for bulls at 12, 18 and 24 months of age. Age specific acronyms describe the class of abnormality and the age at which samples were collected.

[2] Adapted from Burns *et al*. (2013).

Female management and reproduction traits

The females evaluated for this study were part of the Beef CRC's Northern Breeding Project, and comprised 1,030 BRAH and 1,130 TCOMP heifers and the dams of the bulls evaluated for sperm abnormality traits. Breeding and management of the tropically adapted females evaluated for this study up to their first annual mating was described by Barwick *et al*. (2009). Johnston *et al*. (2009) described ultrasound scanning of females to identify age at first corpus luteum (CL), which was interpreted as identifying age at puberty (AP). Females were first mated, to calve as 3 year olds, at an average age of 25 months (Johnston *et al*., 2009). At the start of the second annual mating period, ultrasound scanning to identify the presence of a CL re-commenced for lactating cows (N = 629 BRAH and 872 TCOMP) to identify the re-commencement of oestrous in lactating females, and allow the calculation of lactation anoestrous interval, as the days from the beginning of the second annual mating period (bull in) to the identification of a CL by ultrasound scanning.

Statistical analyses.

For each of the sperm abnormality traits analysed, models were developed separately for BRAH and TCOMP, with the significance of fixed effects tested using linear mixed modelling procedures (proc mixed) in SAS (SAS Institute, Cary, NC, USA). Initial models included the fixed effects of weaning year (2004–10), birth location (five properties), birth month (Sept. to Jan.), post-weaning location, the management group of the dam, dam age (3–9 years) and her previous lactation status (wet or dry) where appropriate. Terms for sire group and dam group were included to account for additive and possible non-additive breed effects for TCOMP. All first order interactions were initially tested in models with sire fitted as random. Terms were sequentially removed, in order of non-significance ($P > 0.05$), to yield the final model for each trait. Consistent with the methods described by Corbet *et al*. (2013), variance components for sperm abnormality traits were estimated using ASReml (Gilmour *et al.,* 2009) in models which contained significant fixed effects, with animal fitted as random and relationships between animals described by a three generation pedigree. Genetic correlations of sperm abnormality traits (with heritability > 0.10) with female AP and LAI were estimated from bivariate analyses separately for each genotype.

Results and Discussion

Viable sperm samples were only obtained from 103 BRAH bulls at 12 months of age (Corbet *et al*., 2013), and sperm abnormality traits for animals of this class were removed from subsequent analyses. KA, PH, SA and VT at all ages displayed very low proportions of non-zero records for both BRAH and TCOMP (< 5%), and were also removed from the current study. Summary statistics, additive variances and heritabilities for sperm cell abnormality traits are presented in Table 2. Means show that for both genotypes, PD was the most common abnormality observed in bulls from their first viable sample (18 months for BRAH and 12 months of age for TCOMP). Amann *et al*. (2000) and Hopper (2010), reported that PD was common in peripubertal bulls, which was supported by the decrease in the incidence of the abnormality at subsequent measurement times for both genotypes. Means for MP abnormalities were reasonably consistent across measurement ages, (15.0 and 11.3% in BRAH at 18 and 24 months, and 12.7, 11.6 and 10.0% in TCOMP at 12, 18 and 24 months), with TH abnormalities present at levels below 10% for bulls of both genotypes, at each age

evaluated.

The heritability for sperm abnormality traits can be compared to those reported by Corbet *et al*. (2013) for percent normal sperm (PNS), which pooled results for all of the specific abnormalities analysed for this study. For BRAH, PNS had a heritability of 0.00, 0.25 and 0.15 at 12, 18 and 24 months respectively, while for TCOMP bulls, these were slightly higher (0.41, 0.20 and 0.27). It is expected that heritabilities of the individual sperm abnormalities analysed in the current study would be distributed around that for PNS at each measurement time. This was consistently the case, with heritabilities for MP18, TH18 and PD18 in TCOMP (0.14, 0.28 and 0.09), for example, reflecting that reported for PNS in 18 month old TCOMP bulls of 0.20 (Corbet *et al*., 2013). Estimates of variance components for sperm abnormality traits in the literature are scarce, and this is particularly so for tropical beef genotypes. Kealey *et al*. (2006) and Roberts *et al*. (2010) reported heritabilities for Hereford and composite (Red angus X Charolais X Tarentaise) bulls respectively, which ranged from 0.08 to 0.37 for proximal droplets and 0.16 to 0.37 for tail abnormalities. Kealey *et al*. (2006) reporting a heritability of 0.02 for KA and Roberts *et al*. (2010) a heritability of 0.16 for MP, all of which were consistent with the results for tropical genotypes, from the current study.

Table 2. Number of records analysed (N), mean and standard deviation (s.d.), with additive variance (σ_a^2) and resultant heritability (and associated standard error (s.e.)) for sperm abnormality traits in young tropically adapted bulls.

Trait[1]	Brahman						Tropical Composite					
(%)	N	Mean	s.d.	σ_a^2	h^2	s.e.	N	Mean	s.d.	σ_a^2	h^2	s.e.
MP12							967	14.4	12.7	38.9	0.27	0.10
MP18	826	15.0	12.6	21.3	0.12	0.08	1793	12.6	11.6	18.0	0.14	0.05
MP24	1234	11.3	12.3	6.2	0.04	0.04	1914	10.0	10.4	18.9	0.17	0.05
PD12							967	19.1	22.6	219.6	0.45	0.11
PD18	826	24.6	26.7	177.7	0.30	0.10	1793	6.8	11.5	10.7	0.09	0.04
PD24	1234	7.7	15.6	92.3	0.39	0.09	1914	4.2	7.5	3.5	0.06	0.04
TH12							967	6.0	8.6	17.0	0.24	0.09
TH18	826	6.1	9.2	22.5	0.28	0.10	1793	8.0	11.4	37.2	0.28	0.07
TH24	1234	5.6	9.0	13.0	0.16	0.07	1914	6.2	10.2	38.9	0.38	0.07

[1] See Table 1 and Burns *et al*. (2013) for a description of sperm abnormality traits.

Research by Johnston *et al*. (2009 and 2014*a*) showed that an accurate measure of AP in the tropically adapted heifers was moderately heritable (h^2 = 0.57 and 0.52 for BRAH and TCOMP respectively), and that selection to improve the trait would have positive consequences for lifetime reproductive performance. Results of the current study show that a correctly timed analysis of sperm morphology (12 months for TCOMP and 18 – 24 months for BRAH) can be exploited to improve the trait by selection, and conclusions in the literature suggest this may be descriptive of variation in male puberty (Amann *et al*., 2000 and Hopper 2010). The heritabilities for MP and TH abnormalities (h^2 = 0.04 to 0.38) also suggest that opportunities exist to reduce the incidence of these by selection in tropical beef genotypes.

Table 3 presents genetic correlations of sperm abnormality traits with AP and LAI; with the genetic relationships of PNS with AP and LAI (reported by Johnston *et al*., 2014*b*) presented for reference. As PNS increases with the proportion of normal (rather than abnormal) cells, it is expected that signs for relationships of that trait with AP and LAI will be reversed when compared to the traits evaluated for the current study.

Table 3. Genetic correlations of sperm abnormality traits with heifer age at puberty (AP) and lactation anoestrous interval (LAI).

Sperm Abnormality Trait (%)[1]	BRAH				TCOMP			
	AP		LAI		AP		LAI	
	r_g	s.e.	r_g	s.e.	r_g	s.e.	r_g	s.e.
MP12					-0.17	0.20	-0.04	0.28
MP18	-0.13	0.25	0.20	0.30	0.22	0.21	0.39	0.29
MP24					0.07	0.18	0.16	0.25
PD12					0.13	0.16	0.51	0.22
PD18	0.51	0.20	0.35	0.25				
PD24	0.22	0.14	0.49	0.18				
TH12					-0.05	0.20	0.10	0.27
TH18	0.05	0.20	0.12	0.25	0.16	0.15	-0.17	0.20
TH24	0.25	0.19	0.04	0.25	0.09	0.13	0.02	0.18
PNS12[2]					-0.05	0.16	-0.34	0.25
PNS18[2]	-0.48	0.21	-0.52	0.31	-0.24	0.17	-0.30	0.25
PNS24[2]	-0.27	0.20	-0.65	0.24	-0.11	0.14	0.05	0.20

[1] See Table 1 and Burns *et al*. (2013) for a description of sperm abnormality traits.
[2] Results previously reported by Johnston *et al*. (2014*b*).

Johnston *et al*. (2014*b*) concluded that the strength of the relationship of PNS18 with AP (r_g = -0.48) in BRAH was sufficient to exploit the trait as a genetic indicator for female age at puberty. Genetic relationships of the component abnormalities with AP in BRAH suggest that this relationship is likely to be driven by associations of PD18 with AP (r_g = 0.51). PD18 and PD24 both showed moderate genetic relationships with LAI, suggesting that selection to reduce the incidence of the abnormality would have favourable consequences for LAI (r_g = 0.35 and 0.49 respectively). These were not quite as strong as the relationships observed by Johnston *et al*. (2014*b*) for PNS, which may indicate that, the pooled trait, describing the incidence of all sperm abnormalities, will provide more information about genetic LAI than PD alone in the Brahman genetic evaluation.

For TCOMP, the genetic relationships of sperm abnormality traits with AP were similar to those observed for BRAH though the age at which relationships were strongest tended to be lower, consistent with results reported by Johnston *et al*. (2014*b*) for PNS. Lower PD12 was genetically associated with shorter (more favourable) LAI (r_g = 0.51), with MP18 showing similar associations (r_g = 0.39). These correlations were both higher than those reported for PNS, (r_g = 0.05 to -0.34), suggesting that including individual sperm abnormalities in the genetic evaluation for TCOMP breeds may provide additional information about female reproduction compared to PNS alone.

Conclusions

The results of this study showed that the proportion of sperm cells displaying proximal droplets, mid-piece, tail or head abnormalities could be improved in tropical adapted beef

bulls by selection.
Lower incidence of proximal droplets displayed moderate and favourable genetic correlations with female age at puberty and lactation anoestrous interval, though the age at which these relationships were strongest was younger for Tropical Composites than Brahmans. Direct measurement of female AP and LAI by serial ultrasound scanning are labour intensive and expensive undertakings, and require a progeny test to generate results for genetic evaluation. Exploiting sperm morphology traits as genetic indicators for female reproduction presents opportunities to reduce the lag associated with progeny testing, and to improve rates of gentic gain for reproductive performance in tropical beef breeds.

List of references

Amann, R.P., G.E. Seidel & R.G. Mortimer, 2000. Fertilizing potential of semen from young beef bulls containing a higher or lower percentage of sperm with proximal droplet. Theriogenology. 54: 1499 – 1515.

Barwick, S.A., D.J. Johnston, H.M. Burrow, R.G. Holroyd, M.L. Wolcott, & M.T. Sullivan, 2009. Genetics of heifer performance in 'wet' and 'dry' seasons and their relationships with steer performance in two tropical beef genotypes. *Anim. Prod. Sci*. 49: 367 - 382.

Burns, B.M., N.J. Corbet, D.H. Corbet, Y. Li, J.M. Crisp, B.K. Venus, D.J. Johnston, M.R. McGowan & R.G. Holroyd, 2013. Male traits and herd reproductive capability in tropical beef cattle. 1. Experimental design and animal measures. *Anim. Prod. Sci*. 53: 87–100.

Chenoweth, P.J. (1980). Bull fertility. *Modern Veterinary Practice* 61: 987–991.

Corbet, N.J., B.M. Burns, D.J. Johnston, M.L. Wolcott, D.H. Corbet, B.K. Venus, Y. Li, M.R. McGowan & R.G. Holroyd, 2013. Male traits and herd reproductive capability in tropical beef cattle. 2. Genetic parameters of bull traits. *Anim. Prod. Sci*. 53: 101 - 113.

Gilmour, A.R., B.J. Gogel, B.R. Cullis & R. Thompson, 2009. 'ASReml user guide. Release 3.0.' VSN International: Hemel Hempstead, UK.

Hopper, R.M. (editor), 2015. Bovine Reproduction. John Wiley & Sons. Ames, Iowa, USA.

Johnston, D.J., S.A. Barwick, R.G. Holroyd, G. Fordyce, M.L. Wolcott, & H.M. Burrow, 2009. Genetics of heifer puberty in two tropical beef genotypes in northern Australia and associations with heifer- and steer-production traits.. *Anim. Prod. Sci*. 49: 399 – 412.

Johnston, D.J., S.A. Barwick, G. Fordyce, R.G. Holroyd, P.J. Williams, N.J. Corbet, 2014*a*. Genetics of early and lifetime annual reproductive performance in cows of two tropical genotypes in northern Australia. *Anim. Prod. Sci*. 54: 1 - 15.

Johnston, D.J., N.J. Corbet, S.A. Barwick, M.L. Wolcott & R.G. Holroyd, 2014*b*. Genetic correlations of young bull reproductive traits and heifer puberty traits with female reproductive performance in two tropical beef genotypes in northern Australia. *Anim. Prod. Sci*. 54: 16 - 27.

Kealey, C.G., M.D. MacNeil, M.W. Tess, T.W. Geary, & R.A. Bellows, 2006. Genetic parameter estimates for scrotal circumference and semen characteristics of line 1 Hereford bulls. *J. Anim. Sci*. 84: 283 – 290.

Roberts, C.A., T.W. Geary, M.D. MacNeil, R.C. Waterman, A.J. Roberts & L.J. Alexander. 2010. Factors affecting spermatozoa morphology in beef bulls. *Proc. West. Sect. Am. Soc. Anim. Sci.* 61: 122-125.