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Effect of breed, age, season and region on sperm morphology in 11,387 bulls submitted to breeding soundness evaluation in Australia

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ABSTRACT

This study reports the distribution of sperm morphology defects by breed, age, season and region of 11,387 bulls in 500 herds in Australia and near Pacific Islands during annual BBSE. Bull location was divided into 4 broad climatic regions based upon temperature, vegetation and climatic risk. Taking into account the impact of age, season, region, and breed there were differences between breeds in both percent morphologically normal sperm and in some individual categories of sperm abnormality ($P < 0.001$). Independent of breed, season and region, proximal droplets were significantly increased in bulls less than 20 months of age. This is the first study to comprehensively collect data from this wide geographical area and compare sperm morphology profiles among the *Bos indicus* and *Bos taurus* breeds. The findings of this study will act as a guide for veterinary practitioners and cattle breeders in the proportion of bulls that can be expected to pass the PNS test, by breed, age and region, based on a robust data set.

1. Introduction

The current Australian Cattle Veterinarians (ACV) Bull breeding soundness examination (BBSE) guidelines support the laboratory assessment of sperm morphology by accredited appropriately trained technicians as part of five key components used to identify risk factors in bull fertility [1]. This assessment system [2], determines the threshold levels of sperm abnormalities based upon published research into the effects of each abnormality upon bull fertility. These thresholds consider whether the abnormality precludes affected spermatozoa from fertilizing the ovum, i.e. the abnormality does not allow transport to the ova or attachment to the ova [3]. In this case increasing the number of spermatozoa in the ejaculate may therefore, compensate for such abnormalities, and the fertility of the bull will increase with these increased numbers of spermatozoa. Such abnormalities include those with abnormal or nil motility, which are filtered out in the female tract [4], and abnormal head shape, which reduces the ability to penetrate the zona pellucida [5]. The threshold for such abnormalities is set at 30% [2,6,7]. Increasing the number of spermatozoa in the ejaculate cannot, however, compensate for abnormalities which, initiate fertilization and/or embryo development, but this development is not sustained. The threshold for these abnormalities is, therefore lower at 20% [2,6]. This method of analysis of morphology differentials, based on the current understanding of the severity and consequence of the various spermatozoon abnormalities [8], was used in this study as it yields a more accurate breeding soundness classification.

The determination of the percentage morphologically normal sperm is highly repeatable and is strongly correlated with days to conception and calf output in both dairy herds [9–11] and beef herds [12]. A major hurdle in the uptake of morphology testing by the cattle industry, however, is the observed variation in results due to environmental effects. It is established that any environmental stress sufficient to cause elevation in circulating cortisol is sufficient

Abbreviations: QSML, Queensland sperm morphology laboratory; ACV, Australian cattle veterinarians; PNS, percent normal sperm; SEM, standard error of the mean; CI, confidence interval.

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to affect sperm morphology [12,13]. High environmental temperatures [14,15] and often associated high humidity which occurs during the late spring-summer-early autumn period in Northern Australia, may transitorily affect morphology as may nutrition [16], with both of these possible stressors being influenced by season. The age of the bull, particularly around puberty, also affects sperm production and morphology whereas the reported effect of breed upon morphology appears inconsistent [17–19].

The objective of this epidemiological study is to establish robust data sets that will aid prognosis, benchmarking and herd management by investigating the incidence of sperm defects associated with the risk factors of breed, age, season and region of collection. These observations will allow practitioners in the field to relate this information to their final BBSE classification.

2. Methods

2.1. Semen collection and analysis

The 11,387 semen samples evaluated in this study were sourced from samples across 500 herds submitted by 95 clinicians between 2012 and 2016 to the Queensland Sperm Morphology Laboratory (QSML). Semen samples were collected using the standard electro-ejaculation technique recommended by the ACV [6,20]. The bulls consisted of 21 breeds (n = 1599 Angus; 1006 Brahman; 26 Braford; 216 Brangus; 156 Belmont Red; 253 Charbray; 345 Charolais; 1799 Droughtmaster; 104 Holstein-Friesian; 375 Hereford; 115 Jersey; 115 Limousin; 83 Murray Grey; 55 Red Angus; 148 Romagnola; 110 Senepol; 4477 Santa Gertrudis; 39 Simmental; 232 Simbrah; 84 Shorthorn; 50 Wagyu) and were sourced from the 4 regions detailed below (Region 1: n = 678; Region 2: n = 6580; Region 3: n = 2773; Region 4: n = 1396 cattle, respectively).

2.2. Morphology

Assessments of sperm abnormalities were made using methodology and standards as described by Fordyce, Entwistle [1]. Briefly, one to two drops of semen were placed into a vial containing 1 mL vial of phosphate-buffered formal saline. These vials were sent to a single laboratory and assessed by one of three trained morphologists, who were periodically reviewed under the same accreditation system to reduce individual bias.

The morphology of 100 individual spermatozoa in each sample, considered to contain sufficient sperm for examination, was assessed using 1000× magnification under differential interference contrast microscopy (Nikon 80i, Nikon Instruments Tokyo, Japan). The sperm morphology traits were individually classified into eight categories [1] detailed below, with total remaining normal sperm noted as percent morphologically normal sperm (PNS) per ejaculate.

This study categorized sperm into the eight categories in the ACV BBSE scheme. Bulls who achieve, less than 70% normal sperm, or more than the respective tolerance levels in each category, fail the morphology test. These categories and tolerance levels are; normal sperm- (which includes abnormalities not reported to affect conception rates), proximal droplets (20%), midpiece abnormalities (30%), loose heads and principal piece (tail) abnormalities (30%), pyriform heads (20%), knobbed acrosomes (30%), vacuoles and teratoids (including abnormalities of DNA condensation) (30%), and swollen acrosomes (including those sperm with lost acrosomes) (30%) [1].

2.3. Breed, age, region and season; coding and analysis

2.3.1. Breed

Bulls of known breed were filtered for those tested more than once, with only the highest PNS count for each bull included in the analysis and repeat analyses at a different age excluded. As both age and climate have been reported to alter PNS, all bulls where region, season or age, were unknown, were subsequently removed from the analysis.

Less than 30 Braford and Simmental remained when age, season, region were taken into account. These breeds were therefore omitted in the post-hoc analysis.

The small number of bulls that did not pass the sperm morphology test on PY (Pyriform head) test alone (five animals), meant that no inferences could be drawn from the data.

2.3.2. Season

Season of sample collection was divided into three categories; summer, winter and spring/autumn (2), in accord with the known effects of temperature and rainfall pattern upon both the parasite burden of the bull, and the direct effect of temperature stress.

2.3.3. Region

Similarly geographical area was divided into four broad regions based upon temperature, vegetation and parasite exposure (illustrated in Fig. 1). North (region 1) equates to a location north of the northern tick line, North East (region 3) east of the Australian tick line [21] (including the near Pacific Islands). The junction of these two areas was determined by the 30 °C average daily maximum temperature isotherm. South (region 4) was determined as below the 24 °C average daily maximum isotherm, while Interior (region 2) equates to that area of mainland Australia not included in localities 1, 3 and 4.

2.3.4. Age

Age was divided into three brackets <20 mths, 21–30 mths and >31 mths, respectively. These divisions were used based on the known effects of puberty upon sperm morphology in bulls <20

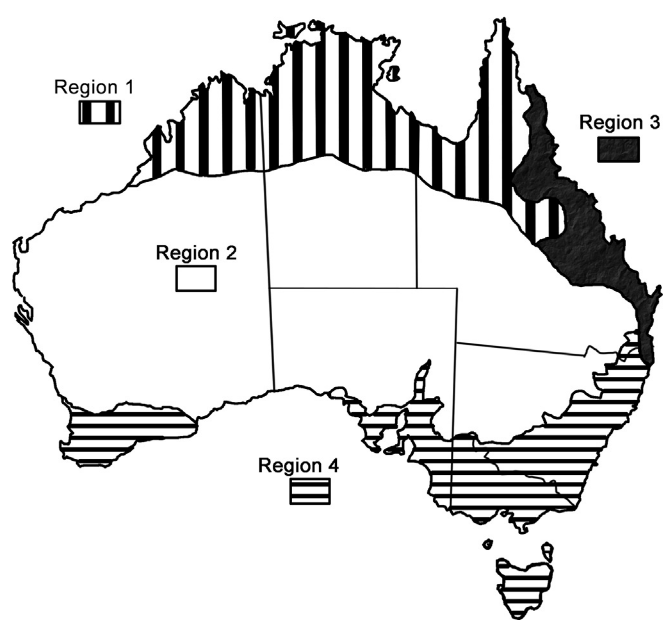

Fig. 1. Australian climatic regions used in the study based upon temperature, vegetation and parasite exposure.
▮▮ = Region 1 (North) equates to a region north of the northern tick line, ■ = Region 3 (North East) east of the Australian tick line [21] (including the near Pacific Islands). The junction of these two areas was determined by the 30 °C average daily maximum temperature isotherm. ☰ = Region 4 (South) was determined as below the 24 °C average daily maximum isotherm. □ = Region 2 (Interior) equates to that area of mainland Australia not included in localities 1, 3 and 4.

mths and reflect previous studies in the area [17].

2.4. Statistical analysis

Binary logistic regression (LR) was used to model the relationships between bull fertility [as measured by pass/fail cut-off of percent normal sperm (PNS)] with bull breed, region, age and season of testing, based on 7284 observations. The binary response is the outcome of the spermiogram analysis (PNS test), being either pass or fail. This was based on the established threshold cut-off point of at least 70% morphologically normal sperm being a pass, and otherwise a fail [1].

Similarly, binary logistic regression was used to model the relationship between bull fertility (as defined as the binary response outcome either pass/fail) with the same four risk factors (breed, region, age, season) for each of the individual abnormalities using the published thresholds: proximal droplet (<20%), midpiece (<30%), head and tail (<30%), pyriform (<20%), knobbed acrosome (<30%), vacuole and teratoid (<20%), and swollen acrosomes (<30%) [1]. For example; a bull sample containing less than 20% sperm with a proximal droplet was considered a pass, and otherwise a fail.

A multivariable binary logistic regression was used for each bull fertility outcome, in which we have assumed the logit transformation of the binary response outcome. Using this model, all four risk factors are taken into account to determine whether there is a statistically significant difference in sperm morphology between breeds overall (using a global P value) and between each breed compared with Angus (reference breed) (post-hoc comparison P values).

Goodness of fit for each multivariable binary logistic model was confirmed using a Hosmer_Lemeshow goodness-of-fit test. All analyses were completed using Stata v14.0 (StatCorp, Texas USA). A probability of $P < 0.05$ was taken as the level of significance in all analyses.

3. Results

The effect of; breed, region, season and age upon the number of bulls passing the sperm morphology test, are presented in Table 1.

3.1. Breed

Differences existed among breeds in the percentage of morphologically normal sperm (PNS) (global P value < 0.0001; Table 1): Of the breeds that differed, (Brahman, Brangus, Belmont Red, Charbray, Droughtmaster, Friesian, Romagnola and Santa Gertrudis) all except Friesians had lower odds of passing the sperm morphology test at the 70% PNS threshold compared to the reference breed; Angus.

3.2. Region

Table 2 illustrates that the North region has the least number of

Table 1
The effect of; breed, region, season and age upon the number of bulls passing the sperm morphology test.

Model Term		Odds Ratio	P > z	[95% CI]		Total	% Fail	Global P value
Breed								<0.0001
1	Angus(reference)					1599	20.1	
2	Brahman	0.32	<0.001	0.23	0.43	1006	35.8	
3	Braford**	0.15	<0.001	0.05	0.45	26	38.5	
4	Brangus	0.57	0.030	0.34	0.96	216	25.9	
5	Belmont Red	0.35	<0.001	0.22	0.54	156	28.2	
6	Charbray	0.22	<0.001	0.15	0.32	253	50.2	
7	Charolais	0.62	0.010	0.43	0.89	345	22.3	
8	Droughtmaster	0.26	<0.001	0.20	0.33	1799	39.4	
9	Friesian	3.45	0.010	1.36	8.72	104	4.8	
10	Hereford	0.96	0.860	0.63	1.46	375	20.8	
11	Jersey	1.04	0.910	0.54	2.01	115	17.4	
12	Limousin	1.30	0.440	0.67	2.54	115	11.3	
13	Murray Grey	1.05	0.900	0.47	2.34	83	21.7	
14	Red Angus	0.76	0.490	0.35	1.66	55	18.2	
15	Romagnola	0.62	0.030	0.40	0.96	148	29.1	
16	Senepol	0.64	0.100	0.37	1.10	110	23.6	
17	Santa Gertrudis	0.36	<0.001	0.29	0.46	4477	35.7	
18	Simmental**	1.51	0.460	0.51	4.43	39	12.8	
19	Simbrah	0.79	0.250	0.53	1.18	232	21.6	
20	Shorthorn	1.33	0.600	0.46	3.89	84	23.8	
21	Wagyu	2.36	0.250	0.55	10.16	50	6.0	
Region								<0.0001
1	North(reference)					678	38.6	
2	Interior	0.96	0.740	0.74	1.24	6580	33.8	
3	North East	1.53	<0.001	1.17	1.99	2733	29.5	
4	South	0.9	0.550	0.64	1.27	1396	21.7	
Age								<0.0001
1	<20mths(reference)					2577	33.6	
2	21-30mths	0.86	0.020	0.76	0.98	3237	32.9	
3	>31mths	1.24	0.010	1.06	1.46	1470	26.2	
Season								0.5002
01	Summer (reference)					2512	35.4	
2	Spring & autumn	0.97	0.740	0.84	1.14	4310	31.7	
3	Winter	1.05	0.500	0.91	1.21	4565	29.4	

Legend Results from a multivariable binary logistic regression model with outcome PNS coded as either pass or fail, versus four predictor (independent) categorical variables: breed, region (4 categories; see Fig. 1), age group and season of collection. Results presented include odds ratios, 95% confidence intervals, global P values and post-hoc comparison P values. ** Indicates breeds dropped from the regression model due to insufficient numbers. Bulls without age data were omitted from the logistic regression leaving 7284 bulls. The columns of total numbers and % fail include all bulls (n = 11,387).

Table 2
The distribution of bulls across each region.

Age Category	Age	Region				Total
		1	2	3	4	
1	<20mths	269(10%)	1549(60%)	598(23%)	161(6%)	2577(100%)
2	21-30mths	185 (6%)	1308(40%)	1242(38%)	502(15%)	3237(100%)
3	>31mths	163 (11%)	710(48%)	347(24%)	250(17%)	1470(100%)
Total number of bulls		617	3567	2187	913	7284

Legend Numbers of bulls in each region (4 categories; Region 1 (North) equates to a region north of the northern tick line, Region 3 (North East) east of the Australian tick line (including the near Pacific Islands). The junction of these two areas was determined by the 30 °C average daily maximum temperature isotherm. Region 4 (South) was determined as below the 24 °C average daily maximum isotherm. Region 2 (Interior) equates to that area of mainland Australia not included in localities 1, 3 and 4. Age group (3 categories; <20 months, 21–30 months and >31 months) with frequency distribution by age.

bulls likely to pass the PNS test. The North East region is significantly different to the reference region North for all bulls (comparison P value < 0.001). Bulls in the North East have 53% higher odds of passing the sperm morphology test than a bull from the North (OR = 1.53, 95% CI: 2.73, 29.5). The actual percentage of bulls failing the morphology test in each region are: 38, 34, 29 and 22 in regions 1 to 4 respectively. The overall effect of region on PNS is statistically significant (chi2 (3df) = 50.79, $P < 0.0001$). Table 3 illustrates the distribution of breed type (*Bos taurus* or *Bos indicus* infused) across the 4 regions. The North and North East regions had a similar percentage of *Bos indicus* infused bulls at 97 and 95% respectively.

3.3. Age

Older bulls (21–30 mths and >31 mths) are significantly different ($P < 0.05$) from younger bulls (<20 mths, reference group): A bull of age 21–30 mths is 14% less likely to pass the sperm morphology test than a bull of <20mths (OR = 0.86, 95% CI: 0.76, 0.98), whereas a bull >31mths is 24% more likely to pass the sperm test than a young bull < 20 mth old (OR = 1.24, 95%CI: 1.06,1.46).

3.4. Season

There was no overall effect of season on the PNS test ($P = 0.5002$).

3.5. Individual sperm abnormalities

Risk factors and the number of bulls passing the threshold, for each individual sperm abnormality.

3.5.1. Proximal droplets (PD)

Overall the semen samples of 8.7% of bulls had greater than the PD defect threshold. There was a overall effect of breed on the PD morphology test, when adjusting for locality, age and season (global P value < 0.0001). Brahman, Belmont Red, Charbray, Droughtmaster, Senepol and Santa Gertrudis bulls were less likely than Angus to pass the sperm morphology test based on PD threshold alone. Bulls tested in the North East region were more likely to pass the PD test (58%) than bulls tested in the North ($P = 0.022$). Older bulls (21–30 mths and >31 mths) were more likely to pass the PD test (34% and 92% respectively) than bulls <20 mths. Fig. 2 illustrates the relationship between percentage PD and increasing age. There was no effect of season on the levels of PD defects.

3.5.2. Midpiece abnormalities (MP)

Overall the semen samples of 5.2% of bulls had greater levels of MP defects than the threshold. There was a difference between breeds in the odds of failing versus passing the sperm morphology test based on MP threshold alone (global P value < 0.0001). Brahman and Droughtmaster bulls were 50% less likely to pass the MP test than Angus. There was also an effect of season of testing, with lowered odds of passing the MP test in winter or spring/autumn compared with summer (32% and 40% less likely, respectively).

3.5.3. Vacuoles and teratoids (VT)

Adjusting for breed, locality, age and season, overall the semen samples of 11.9% of bulls had greater levels of VT defects than the threshold: Brahman, Brangus, Belmont Red, Charbray, Charolais, Droughtmaster, Limousin, and Santa Gertrudis were significantly

Table 3
The distribution of breed type across each region.

Region	Breed type				Total by Region
	Btaurus		Bindicus (x)		
	% by Breed	% by Region	% by Breed	% by Region	
1 (North)	19		659		678
	3%	0.05%	97%	8%	
2 (Interior)	1909		4671		6580
	29%	58%	71%	58%	
3 (North East)	135		2598		2733
	4%	4%	95%	32%	
4 (South)	1236		160		1396
	88%	38%	12%	2%	
Total by breed type	3299		8088		11387

Legend Numbers of bulls of each breed type in each region (The 4 categories are; Region 1 (North) equates to a region north of the northern tick line, Region 3 (North East) east of the Australian tick line (including the near Pacific Islands). The junction of these two areas was determined by the 30 °C average daily maximum temperature isotherm. Region 4 (South) was determined as below the 24 °C average daily maximum isotherm. Region 2 (Interior) equates to that area of mainland Australia not included in localities 1, 3 and 4. Breed type is divided into *Bos taurus* (Btaurus) or *Bos indicus* including *Bos indicus* infused breeds (Bindicus x) with frequency distribution by region. (NB We observe Bindicus x bulls at 97% in region1, 95% in region 3, 71% in region 2 and 12% in region 4.)

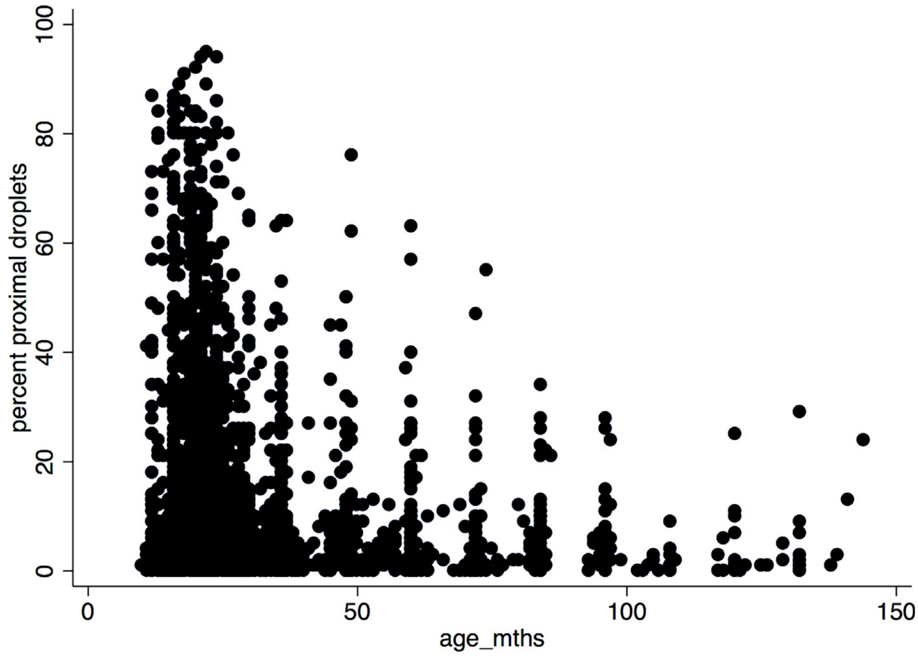

Fig. 2. The distribution of percent of proximal droplets as bulls aged (n = 7284).

less likely to pass the sperm morphology test based on VT threshold alone than Angus bulls.

Bulls older than 20 months were less likely (41% and 36% respectively) to pass the VT test than younger bulls (<20 mths). Bulls tested in spring/autumn or winter, were more likely to pass the VT test (54% and 104%, respectively) than bulls tested in summer.

3.5.4. Heads and tails (HT)

Overall the semen samples of only 2% of bulls had greater than the HT threshold. There was a difference between breeds in the odds of passing the sperm morphology test based on HT ($P < 0.05$) with Brahman, Charbray, Droughtmaster, and Shorthorn having lower odds of passing the HT threshold test than Angus bulls.

3.5.5. Knobbed acrosome (KA)

Overall less than 1% of bulls had greater than the KA threshold. Older bulls, (21–30 mths and >31 mths) had significantly reduced odds of passing the sperm test based on KA compared to younger bulls (OR = 0.025 (95%CI: 0.002,0.260) and OR = 0.023 (95%CI: 0.002,0.274) respectively]. The odds of passing the KA threshold test were significantly increased when bulls were tested in Spring/Autumn or Winter than in Summer (OR = 6.35 (95%CI: 1.46,27.7) and OR = 11.72 (95% CI: 2.49,55.02), respectively.

3.5.6. Swollen acrosomes (SA)

Adjusting for breed, locality, age and season, overall less than 1% of bulls overall failed on the SA threshold alone. Only Wagyu bulls had reduced odds of failing the SA test compared to Angus r (OR = 0.02, 95% CI: 0.001,0.43).

4. Discussion

This is the first study to comprehensively collect data from a wide geographical area and compare sperm morphology profiles among the *Bos indicus* and *Bos taurus* breeds, assessed following routine BBSE, as part of routine management programs. Differences were observed between breeds, ages, region and season of collection. The findings of this study will act as a guide for veterinary practitioners and cattle breeders in the proportion of bulls that can be expected to pass the BBSE PNS test, by breed, age and region, based on a robust data set.

The observed significant negative relationship between age and the occurrence of proximal droplets agrees with previous reports associating this abnormality with pubertal bulls and decreasing with increasing age [22,23]. Conversely, there was no association between age and other sperm traits such as midpiece abnormalities across breeds whilst there was a small increase in the numbers of bulls of <20 mths of age passing on vacuolation and knobbed acrosomes thresholds compared to older age groups. This may reflect the recommended practice of culling of individual bulls when they consistently display these abnormalities at an early age [20].

Previous reports suggest that the incidence of knobbed acrosome [9], midpiece [18], and proximal droplet [24] abnormalities vary between breeds. Our study supports these findings as we found differences among breeds in the proportion of these abnormalities. Furthermore, the observed increased incidence of vacuolation defects in bulls in this datset with *Bos indicus* genetics is in agreement with previous reports suggesting that this genotype is more susceptible to this abnormality. This has previously been suggested by other authors but not confirmed [25]. This susceptibility may also be induced in these bulls in situations of environmental stress as we have recently reported [16]. This is in accord with reports that vacuolation may be associated with decreased production of testosterone from the Leydig cells after a stress induced cortisol rise reducing circulating LH [2,15]. This abnormality may therefore be transient [26] and associated with nutritional and seasonal stresses [19]. We note that bulls were observed to fail on the vacuole threshold more frequently in summer, which suggests excessive heat may be a causative stress factor as previously reported [27].

Brahman, Brangus, Belmont Red, Charbray, Droughtmaster, Romagnola and Santa Gertrudis breeds had lower odds of passing the sperm morphology test at the 70% PNS threshold compared to the reference breed, Angus. All of these bulls are from breeds with *Bos indicus* content (with the exception of Romagnola).

Sperm abnormalities were increased in those bulls collected between 21 and 30 mths of age compared with the reference age (<20 mths). This may seem counterintuitive as peri pubertal elevation in sperm abnormalities in bulls <20 mths would be expected to increase abnormalities [13,19]. Many young bulls, however, are grain fed prior to sale which may lead to subacute ruminal acidosis previously reported to may have a deleterious affect upon sperm morphology [16] in this age group. High energy feeding to bulls prior to sale is commonly observed by the veterinary practitioner and is a major cause failure in the BBSE through its negative effect upon morphology [16]. This effect may also reflect increased abnormalities in later maturing *Bos indicus* bulls tested at this age. Mature bulls were significantly more likely to pass the sperm morphology test than bulls <31mths as previously reported in other studies in *Bos taurus* bulls [17].

Environmental conditions such as those present in Northern Australia have been reported to alter profiles of FSH and testosterone [13,16] sufficient to affect spermatogenesis [28]. The percentage of bulls that failed the morphology test in each region were: 38 (North), 34 (Interior), 29 (North East) and 22 (South) (Table 2), the greater number passing in the South and the least in the North. The numbers of bulls tested in the North East are 50% higher than in the South and this North East region has significantly lower 95% confidence interval than the Inland or South region. It is unclear why the North East (coastal with higher temperatures than the south) is the only region significantly different to the North in the number of bulls passing the sperm morphology test. Additionally, Table 3 shows that a higher percentage of *Bos indicus* bulls occurred in these tropical and humid regions (North and North East). *Bos indicus* bulls have been reported to show lower PNS than *Bos taurus* bulls [29,30] and it has previously been suggested that these less favourable (high temperatures and humidity with higher tick burden) locations and *Bos indicus* genotype may interact to lower PNS. However, the North East Coastal region, for example, had as large a proportion of *Bos indicus* bulls (95%) as the Interior (71%), yet the number of bulls passing the morphology test was significantly higher in the former. The role of less favoured, harsher environmental region, in lowering PNS of *Bos indicus* bulls is, therefore, arguable. Furthermore, in the statistical model employed, all independent variables (including region, age and season) are taken into account when the statistical difference between breeds in sperm morphology was examined. We can therefore state the observed difference, for example between Droughtmaster and Angus, is significant as the other independent variables including region, have been accounted for in the model.

Despite the lack of significant differences between seasons in the proportion of bulls passing the PNS test there was a difference observed in some individual abnormalities: The odds of passing both vacuole and knobbed acrosome thresholds decreased when bulls were tested in summer. This may reflect a deleterious effect of available feed in winter and autumn particularly in the tropical and subtropical regions upon the number of these abnormalities observed. The opposite effect was observed with midpiece abnormalities where the odds of passing the threshold were increased if bulls were tested in summer.

Sperm morphology remains a critical test of bull fertility [9,11], however semen is often tested only for total sperm numbers and sperm motility. Even in large collection centres microscopy is often replaced by flow cytometry and assessment of proportions of membrane-intact spermatozoa due to the enhanced speed and automation [3]. The relevance of the sperm morphology evaluation relates to its indicative role for the presence of deviations in the processes of spermatogenesis and sperm maturation. Correctly performed and interpreted morphology remains a vital tool in the assessment of bull fertility. The Australian guidelines in bull sperm morphology examination and reporting [1] have become widely accepted among practitioners and cattle producers because this standardized approach offers uniformity to clinicians and producers. This study builds upon this framework of clinical practice by providing contextual information to practitioners on the distribution of sperm defects between breeds whilst taking into consideration the risk factors of age, season and region in Australia. This data will, enable benchmarking, aid prognosis and contribute to herd management.

5. Conclusion

This study compares sperm morphology profiles among the *Bos indicus* and *Bos taurus* breeds assessed following routine BBSE. The differences observed between breeds, ages, region and season of collection will act as a guide for veterinary practitioners and cattle breeders, in the proportion of bulls that can be expected to pass the BBSE PNS test, by breed, age and region, based on a robust data set. These observations will afford practitioners a more accurate assessment of a bull's performance in the final breeding soundness classification.

Conflicts of interest

The authors declare that they have no competing interests.

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