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SEASONAL DIFFERENCES IN REPRODUCTIVE CHARACTERISTICS OF *Bos indicus* AND *Bos taurus* BULLS IN TROPICAL NORTHERN AUSTRALIA

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Seasonal differences in sperm morphology and composition of seminal vesicle secretions between wet and dry seasons were studied in material collected from 300 bulls at slaughter. Blood plasma concentrations of glucose, blood urea nitrogen, protein and albumin were significantly lower in dry season samples. Spermatozoal and acrosomal morphology was significangly affected by season and the percentages of spermatozoa with abnormal tails, protoplasmic droplets and detached acrosomes were 12.8 versus 16.8, 10.8 versus 18.0 and 13.9 versus 23.0, respectively, in wet versus dry season. Fructose and sodium concentrations in seminal vesicular fluid were significantly higher in wet versus dry season, being 683 versus 527 mg/100 ml and 178 versus 154 mequiv/l, respectively. There was a significant genotype by season interaction with markedly increased citric acid concentrations in *Bos indicus* cross bulls during the dry season. Differences between the two seasons imply that nutrition is the major factor influencing seasonal variation in reproductive function of bulls in the tropics. Genetic factors may influence the expression of these differences.

Key words: Bulls, seasonal differences, sperm morphology, seminal vesicles, blood metabolites.

Environmental conditions in northern Australia are characterised by two distinct seasons. The summer months, from October to March, are typified by high rainfall (range 400 to 1250 mm) and mean maximum temperatures ranging from 30 to 39 °C. In the winter months, May to September, temperatures vary from 15 to 25 °C, with virtual absence of rainfall, and as a result there is a decline in pasture quality as the year progresses. In this environment there are no marked differences in minimum and maximum day length as occurs in lower latitudes.

Donaldson (1963) suggested that these environmental conditions may affect reproductive performance of bulls in this region, but little information is available on the relative contribution of high ambient temperatures and poor nutrition to this apparent seasonal variation in reproductive function. The study reported here, using material obtained at slaughter from bulls originating in this region, was designed to examine possible changes in sperm morphology and sex gland secretions due to wet season (high temperatures, good nutrition) and dry season (lower temperatures, poor nutrition) environmental conditions in northern Australia.

Materials and Methods

Origin of animals and collection procedures: The bulls sampled in this study were derived from a number of coastal, subcoastal and inland

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herds in the tropical regions of northern Australia. Material was collected from a random sample of 300 bulls, slaughtered at a Townsville abattoir, either during the period March to April (wet season group, n = 148) or September to October (dry season group, n = 152). Collection procedures at slaughter for ampullae, seminal vesicles and blood samples were similar to those described by Ladds et al (1973). At slaughter animals were classified as either young (<3 years) or mature (>3 years) by dentition (Bellenger, 1971). Breed type was recorded from phenotypic appearance of the animal as *Bos taurus*, *Bos indicus* cross or high grade *Bos indicus*. Due to the small number of high grade *Bos indicus* bulls (n = 24) they were excluded from subsequent breed type comparisons and only *Bos taurus* and *Bos indicus* cross bulls were compared.

Blood samples were collected at slaughter into lithium-heparinised tubes, centrifuged (2000 G/20 min) and the plasma stored at -15°C for subsequent analysis. Seminal vesicles and ampullae were dissected free of connective tissue, examined on gross morphological appearance and all abnormal glands discarded. Vesicular fluid was recovered fron normal seminal vesicles by incision and aspiration of the exudate, after fluids on the surface had been carefully removed to avoid contamination. Care was taken to avoid damage to blood vessels and all samples were centrifuged (2000 G/20 min) to remove any cellular debris prior to storage at -15°C. Spermatozoa were removed in a similar fashion from the ampullae and aliquots fixed in 0.2% PBS-glutaraldehyde (Johnson et al 1976 for examination of sperm morphology under phase-contrast microscopy. Semen smears were also Giemsa stained (Watson, 1975) for acrosome evaluation under oil-immersion microscopy (x1000).

Analytical procedures: The concentrations of blood plasma glucose, urea nitrogen (BUN), total protein and albumin were determined with an Autoanalyser II (Technicon Instruments, Tarrytown, New York) using a standard technique for simulataneous analysis of concentrations in relation to a reference plasma containing known amounts of glucose, BUN, total protein and albumin (Skeggs and Hockstrasser, 1964). Sodium (Na) and potassium (K) concentrations in seminal vesicular fluid were determined by atomic absorption spectrophotometry (AA-275, Varian Techtron, Springvale, Australia) as described by Quinn et al (1965). Fructose concentrations in seminal vesicular fluid were determined using a colorimetric technique (Mann 1946), adapted to a microlitre scale, using 50 µl aliquots of sample. Citric acid in seminal vesicular fluid was also determined by a colorimetric technique, as a modification of the technique described by Lindner and Mann (1960) for seminal vesicular tissue.

Statistical analysis: Data were analysed by least-squares analysis of variance procedures. The initial mathematical model included season, day of collection within season, genotype, age and a season by genotype interaction term. Age was found not to significantly affect any of the reproductive characteristics examined and was excluded from the final model. Simple correlation coefficients were calculated between variables within seasons.

Results

Blood plasma metabolites were determined to provide an indication of possible between season differences in nutritional status of the bulls. Wet season means are however based on only 15 observations, due to technical problems. During the dry season, concentrations of glucose, total protein and albumin were lower ($P < .01$) and BUN declined markedly ($P < .001$) between wet and dry season samples (Table 1). Only BUN concentrations varied significantly between genotypes, with higher ($P < .001$) concentrations in *Bos taurus* cross bulls during the dry season

Table 1:

Mean (± SEM) levels of blood metabolites, spermatozoal morphology and seminal vesicular fluid composition during wet and dry season.

Characteristics	Wet season	Dry season
n	148	152
Blood plasma metabolites[1]		
Glucose (mg/100 ml)	175 ± 10	148 ± 3**
Blood urea nitrogen (mg/100 ml)	25.3 ± 1.7	18.6 ± 0.4***
Total protein (mg/ml)	127 ± 3	119 ± 1**
Albumin (mg/ml)	44 ± 1	40 ± 1**
Sperm morphology (%)		
Abnormal heads	10.8 ± 1.0	14.0 ± 1.3
Abnormal tails	12.8 ± 1.1	16.3 ± 1.2*
Protoplasmic droplets	10.8 ± 1.0	18.0 ± 1.4***
Acrosome morphology (%)		
Ruffled	21.0 ± 1.2	18.0 ± 1.3
Broken	4.5 ± 1.0	1.0 ± 1.3***
Detached	13.9 ± 1.2	23.0 ± 1.9***
Seminal vesicular fluid		
Fructose (mg/100 ml)	683 ± 18	527 ± 24***
Citric acid (mg/100 ml)	942 ± 28	1063 ± 33*
Sodium (mequiv/l)	178 ± 5	154 ± 5**
Potassium (mequiv/l)	44 ± 3	43 ± 3

* ($P < .05$); ** ($P < .01$); *** ($P < .001$) difference between season.
[1] Mean blood plasma metabolite concentrations for wet season are based on 15 observations only.

(Table 2). In general, age had no effect on the characteristics examined, with the exception of glucose which was higher ($P < .05$) in younger than in older bulls, 147 and 101 mg/100 ml, respectively.

The percentage of morphologically abnormal spermatozoa was lower in samples collected during the wet season compared to the dry season (Table 1), primarily as the result of a high incidence during the dry season ($P < .001$) of spermatozoa with protoplasmic droplets. The percentage of sperm tail abnormalities (bent and coiled) was also higher in the dry compared to the wet season ($P < .05$), as were the percentages of deformed

Table 2[1]

Mean (± SEM) levels of blood plasma metabolites, spermatozoal morphology and seminal vesicular fluid between Bos indicus cross and Bos taurus genotypes within season.

Characteristics	Wet season		Dry season	
	Bos indicus	*Bos taurus*	*Bos indicus*	*Bos taurus*
n	89	46	69	72
Blood plasma metabolites[2]				
Glucose (mg/100 ml)	174 ±20	176 ±13	149 ±4	145 ±4
BUN (mg/100 ml)	28.4 ±2.8	23.8 ±2.0	16.9 ±0.5	19.8 ±0.5***
Total protein (mg/ml)	132 ±3	124 ±4	117 ±2	120 ±1
Albumin (mg/ml)	41 ±2	45 ±2	39 ±1	41 ±1
Sperm morphology (%)				
Abnormal heads	9.5 ±1.1	11.8 ±2.1	12.4 ±1.9	15.8 ±1.8
Abnormal tails	13.0 ±1.8	13.9 ±1.0	14.7 ±1.6	16.9 ±1.8
Protoplasmic droplets	10.8 ±1.4	11.3 ±1.6	20.9 ±2.2	15.2 ±1.9
Acrosome morphology (%)				
Ruffled	20.5 ±1.6	21.6 ±2.3	21.5 ±2.1	15.2 ±1.5*
Broken	4.8 ±0.6	4.3 ±0.7	1.3 ±0.2	0.6 ±0.1
Detached	7.4 ±1.0	16.2 ±3.0*	23.1 ±2.6	28.8 ±3.0
Seminal vesicular fluid				
Fructose (mg/100 ml)	699 ±23	646 ±31	550 ±34	494 ±35
Citric acid (mg/100 ml)	908 ±34	986 ±52	1155 ±50	978 ±45
Sodium (mequiv/l)	174 ±6	180 ±8	147 ±8	162 ±8
Potassium (mequiv/l)	39 ±3	56 ±6**	42 ±5	48 ±4

* ($P < .05$); ** ($P < .01$); *** ($P < .001$) difference between genotypes within seasons.
1 Designates *Bos indicus* cross bulls, excluding 24 high grade *Bos indicus*.
2 Blood plasma metabolite concentrations are base on only 9 and 6 animals in *Bos indicus* cross and *Bos taurus* groups, respectively.

and detached heads. The latter difference was not statistically significant.

There were no significant differences in morphologically abnormal spermatozoa between *Bos indicus* and *Bos taurus* genotypes, either during the wet or the dry season, although the percentage of abnormal heads and tails was higher in samples from *Bos taurus* than from *Bos indicus* cross bulls during the dry season (Table 2). There was a pronounced increase from 10.8 to 20.9 % in the incidence of spermatozoa with protoplasmic droplets between wet and dry season in *Bos indicus* cross bulls. The data on acrosome morphology (Table 1) were somewhat conflicting and whereas the percentage of detached acrosomes was higher during the dry season ($P < .001$), spermatozoa with broken acrosomes were found more frequently during the wet season ($P < .001$). There were indications of a breed by season interaction in the percentage of abnormal acrosomes, resulting from the increase in detached acrosomes in *Bos indicus* cross bulls during the dry season, which was significantly lower ($P < .05$) in these genotypes during the wet season (Table 2). The percentage of ruffled

acrosomes was lower ($P < .05$) in *Bos taurus* than in *Bos indicus* cross bulls in the dry season.

Seasonal differences in seminal vesicular fluid composition were found in fructose, citric acid and Na, but not in K concentrations, with fructose ($P < .001$) and Na concentrations ($P < .01$) being higher during the wet season (Table 1). The differences in citric acid concentrations ($P < .01$) were observed primarily in *Bos indicus* cross bulls with higher concentrations during the dry season and a significant ($P < .01$) genotype by season interaction was observed (Table 2). K concentrations were higher in *Bos taurus* bulls during the wet season ($P < .01$), but this was not reflected in a genotype by season interaction.

During the dry season, blood glucose was positively correlated ($P < .05$) with seminal vesicular fructose and Na concentrations, but negatively correlated with K concentrations. In contrast, BUN was negatively correlated ($P < .05$) with seminal vesicular fructose, citric acid and Na. Correlations between sperm morphology and seminal vesicular fluid composition were also primarily evident during the dry season, with fructose and citric acid concentrations being negatively ($P < .01$) and K concentrations positively ($P < .01$) correlated with sperm head abnormalities.

Discussion

The use of slaughter bulls for this survey enabled sampling of material from a large number of animals drawn from a number of regions. The procedure also had the advantage of uniform, standardised collection procedures, thereby eliminating differences between bulls which can occur with semen collection procedures under field conditions (Chenoweth and Osborne, 1978). The approach, however, was limited to the comparison of two different bull populations in each season and by the absence of any pre-slaughter reproductive history of the animals. The latter limitation meant that some bias in the data may have occurred, as some bulls could have been sent for slaughter due to poor reproductive performance.

As the bull population in this study was heterogenous with respect to age, weight and size, estimates of the importance of seasonal nutritional differences were restricted to monitoring blood metabolites. Metabolic parameters, such as blood glucose, BUN and albumin have been found to be sensitive indicators of changes in energy and protein intake in cattle (Blowey et al 1973). Levels of both energy (glucose) and protein metabolites (BUN, total protein and albumin) were depressed during the dry season, suggesting that bulls experienced deficiencies in both energy and protein intake during this period. Similar differences have been observed in bulls on different planes of nutrition under both temperate (Kitchenham et al 1977; Manston et al 1977) and tropical (Ndama et al 1953) climatic conditions. Genotype differences in response to nutritional stress during the dry season were apparent in BUN levels, which were markedly lower in *Bos indicus* cross bulls, but not *Bos taurus* genotypes. These differences possibly reflect a more efficient

utilisation of BUN for protein synthesis in *Bos indicus* cattle under nutritional stress (Vercoe 1967). The lower glucose levels in older bulls found here also confirm published results of decreasing blood glucose concentrations with increasing age (Manston et al 1977).

Sperm morphology was clearly affected by season, with a decline in the percentage of normal spermatozoa in bulls sampled during a period of poor nutrition in the dry season. The effect on testicular function *per se*, as indicated by the small increase in sperm head abnormalities, was less evident than would be expected from published data (Kumi-Diaka and Zemjanis 1978), which suggested an impaired spermatogenic function in bulls experiencing seasonal undernutrition. More pronounced dry season effects, however, were found in secondary sperm abnormalities, predominantly spermatozoa with protoplasmic droplets and bent tails, which would suggest that nutritional stress may mediate its effect primarily through changes in the fluid environment of the excurrent ducts. Similar seasonal variations of increased percentages of secondary sperm abnormalities were reported by Igboeli and Rakha (1971) but as the dry season in that study coincided with periods of elevated ambient temperatures, these authors concluded that heat stress was the major cause of secondary abnormalities. The present results, obtained in material from bulls not subjected to temperature stress, suggest that seasonal undernutrition may be a significant factor in the expression of secondary sperm abnormalities, in addition to its effect on overall testis sperm production (Ndama et al 1983).

Overall differences in sperm morphology between genotypes did not reach statistical significance and thus confirm trends reported earlier (Chenoweth and Osborne 1978). Genotypes varied however in specific types of abnormalities, i.e.the higher percentage of abnormal sperm heads, but lower percentage of spermatozoa with protoplasmic droplets in *Bos taurus* compared to *Bos indicus* cross bulls. The nature of these sperm abnormalities would suggest that they result from damage at the testicular level (sperm head abnormalities) in *Bos taurus*, while those of *Bos indicus* bulls originate in the epididymis (retained protoplasmic droplets). A hypothesis may be advanced that testicular, but not epididymal function is more susceptible to temperature and nutritional stress in *Bos taurus* than in *Bos indicus* bulls. This theory is supported by genotypic differences in the proportion of detached acrosomes, but requires further experimental verification.

The interpretation of data on changes in acrosome morphology is dependent on the definition of these abnormalities as either stationary, independent forms, or as intermediate stages of continuous acrosomal disintegration, resulting in complete loss of the acrosome (Saake and Marshall, 1968). When adopting the latter definition, changes in acrosome structure would be the result of varying degrees of ageing, due to changes in the fluid environment of the excurrent ducts. From the data of this study, in which a high percentage of spermatozoa with detached acrosomes were found during the dry season, one could infer that nutritional stress initiated a faster rate of acrosomal ageing, resulting

in a decreased frequency of intermediate stages of acrosome disintegration. This hypothesis could also explain the apparent discrepancies in the higher percentage of intermediate stages of acrosome deterioration in *Bos indicus* bulls during the dry season, while lower percentages of detached acrosomes were noted than in *Bos taurus* genotypes. Hence nutritional stress during the dry season may affect the rate of acrosomal disintegration to a lesser degree in *Bos indicus* genotypes, possibly because of their greater environmental adaptation.

The influence of nutritional stress on seminal vesicle secretions is well documented (Mann 1974) and the observed decline in fructose concentrations during the dry season is in agreement with findings of Igboeli and Rakha (1971) under similar conditions. An unexpected result was the increase in citric acid concentrations, since previous reports have indicated a combined reduction of fructose and citric acid under nutritional stress (Mann and Walton, 1953). The high concentrations of citric acid were exclusively associated with material from *Bos indicus* cross bulls, suggesting a possible genotypic basis for this difference. Na in seminal plasma has been reported to decline under conditions of nutritional stress (Hiroe et al 1965) and these findings were confirmed in the present study, in which decreasing Na and stable K concentrations were noted. The results are in contrast however to previous observations of seasonal variations in *Bos indicus* bulls (Singh 1969; Igboeli and Rakha 1971), in which lower Na concentrations were found during periods of high ambient temperatures. Two explanations may be offered for this discrepancy. First, under conditions of high ambient temperatures and nutritional stress, present in the latter studies, specific effects of androgen control on seminal vesicular function may mask the individual effects of either one of these stresses, and such an explanation has been suggested by Igoboeli and Rakha (1971). Secondly, a significant effect of genotype on K concentrations was noticed here, which resulted in lower dry season K levels in *Bos taurus*, but not *Bos indicus* cross bulls. This factor may have masked general seasonal trends in cation concentration, as Na and K concentrations are inversely related (Cragle et al 1958).

Possible nutritional effects on seminal vesicle function are only partially explained by correlations between blood plasma metabolities and seminal vesicular fluid composition. The correlation of fructose and Na with plasma glucose during the dry season suggests an interaction of plane of nutrition and accessory sex gland function, in agreement with observations by Mann (1974). However, BUN concentrations were negatively correlated with fructose, citric acid and Na, and this inconsistency is likely to be of genotypic origin, since BUN concentrations differed markedly between genotypes during the dry season.

Conclusions

The results of this study representing data from one year for samples obtained during both wet and dry seasons indicate that poor nutrition, rather than elevated ambient temperatures, has the greater influence on a number of aspects of reproductive function in bulls in tropical northern

Australia. Both spermatogenic and accessory sex gland function were affected by poor nutrition to a similar degree. Furthermore, the results indicate that there are differences in reproductive function between *Bos indicus* and *Bos taurus* genotypes in response to seasonal variation. These differences have been suggested to be associated with the superior thermoregulatory capacity of *Bos indicus* genotypes (Johnston et al 1963; Kumi-Diaka et al 1981), but the present results suggest that the response may also involve increased adaptability to nutritional stress.

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