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A Preliminary Investigation on the Semen Characteristics of the Split Ejaculates of Unilateral Cryptorchid Boars

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Abstract: The semen profile in two cryptorchid boars and two age-matched (aged 10 months and two years old) boars were evaluated using the split ejaculate method. Sperm concentrations, sperm morphology, seminal alpha-glucosidase and seminal fructose were analysed. A disparity in sperm concentrations and percentage normal sperms was observed in split ejaculate fractions of healthy boars compared to cryptorchid boars ($p < 0.001$) in both age groups. This study documents for the first time in literature, some data on seminal alpha-glucosidase activity (range 11.4 to 12.5 mU mL⁻¹) and seminal fructose (range 7.2-10.8 mg 100 mL⁻¹) concentration in the split ejaculates of boars. The testicular ectopia accompanied by epididymal ectopia of unilateral cryptorchidism causes sperm morpho-abnormalities. Cryptorchidism also causes impairment of seminal vesicular functions but not epididymal secretory functions.

Key words: Pig, cryptorchid, semen, split ejaculate

INTRODUCTION

Cryptorchidism is a sex-linked autosomal gene abnormality leading to a developmental defect whereby one or both testes fail to descend into the scrotum. The condition occurs most frequently in pigs, horses and humans but is relatively uncommon in sheep and cattle (McEntee, 1990). The structure of sperm (Guraya, 1987) and the time of testicular descent into the testes (Mc Mahon *et al.*, 1995) in humans and boars are similar. It has also been proposed that it is more appropriate to use the boar as an animal model to the study of cryptorchidism than the use of rodents (Pinart *et al.*, 1998). In humans, cryptorchidism results in alterations in sperm morphology (Mieusset *et al.*, 1995) and the occurrence of different types of sperm malformations (Pinart *et al.*, 1998). However, reports on the effects of cryptorchidism on sperm parameters and biochemical constituents in various fractions of the split ejaculate are scarce. The split ejaculate technique has proved most valuable in tracing the origin of various compounds in humans (Ndovi *et al.*, 2007) and Boars (Pena *et al.*, 2006). The present study was designed to provide a preliminary investigation of the alterations in sperm concentration, sperm morphology, seminal fructose and seminal alpha-glucosidase in various fractions of the ejaculate of the cryptorchid boars by employing split ejaculate technique and comparing them with healthy boars.

MATERIALS AND METHODS

This study was performed using two unilateral cryptorchid boars with the right testis in an abdominal position aged 10 months and two years. Two age matched controls were randomly selected from the stock at the Pig Industry Board, Arcturus, Zimbabwe beginning in 2001. The boars were subjected to a semen collection rhythm of once per week for three weeks to allow for sexual rest as suggested by earlier workers (Pinart *et al.*, 1996). Semen was collected by using the gloved-hand technique (Thiengtham, 1994).

Split-ejaculate technique in boars: Pre-trials were carried out on the gloved-hand technique and the same collector carried out the procedure to minimize variations between collections. Collections were done during the same season between 09:00 and 10:00 h each time to minimize environmental effects on the boar and semen between collections. Before collection of semen, the boars were teased and aroused by exposure for 30 min to sows exhibiting standing heat (oestrous). Using a gloved-hand and lubrication, the boars penis was manipulated until the pig ejaculated. The split ejaculate technique involved filtering the semen through a gauze (to remove the gel fraction) and collecting the semen fractions in four separate containers at 30 sec intervals as suggested in previous reports (Hafez, 1993).

Processing of samples for sperm concentration and sperm morphology: Each of the four fractions from each collection was analysed for its sperm concentration. Semen was diluted 1 in 20 in a test tube using semen diluting fluid (5 g sodium bicarbonate in 1 mL formaldehyde and made up to 100 mL using distilled water). The two chambers on the Improved Neubauer counter were filled with the diluted semen and both chambers were counted and the mean values obtained.

Sperm morphology was evaluated by using the negrosin-eosin stain. The stained smears were then viewed under the microscope at 1000X magnification under oil immersion. At least 200 spermatozoa were observed and assessed for abnormalities of the sperm head, neck, mid-piece and end-piece. Using a counting machine, the normal and abnormal spermatozoa were grouped.

Processing of samples for accessory sex gland function: Seminal fructose was analysed by the modified Roe's method (Foreman and Gaylor, 1973). The Episcreen kit (Ferti-Pro, N.V. Belgium) was used for the determination of seminal alpha-glucosidase. Each test was replicated and the results were expressed as mg 100 mL⁻¹ and mU mL⁻¹ respectively.

Data analysis: The data was analysed using Minitab data analysis, software 7.2 (1989) non-parametric statistics. Mean values were calculated using data from three collections from the two healthy boars and the two cryptorchid boars. Differences between mean values were assessed using the Students' two sample unpaired t-test. The data on sperm concentration, sperm morphology, fructose concentration and alpha-glucosidase activity were also subjected to Tukey Kramer multiple analysis and comparisons were made between fractions 1, 2, 3 and 4 of healthy and cryptorchid boars.

RESULTS

Table 1 compares the sperm parameters and accessory sex gland functions in various fractions of the split ejaculate of the 10 month old healthy and cryptorchid boars. The sperm concentration in fraction 2 was lower in cryptorchid (p<0.001), and higher in fraction 3 than in the healthy boar (p<0.001). The percentage of normal sperms were lower in fractions 3 and 4 of the cryptorchid as compared to the healthy boar (p<0.01). The seminal fructose was lower in fraction 3 and 4 of the cryptorchid.

For the 2 year old healthy and cryptorchid boars (table 2) the sperm concentration in fraction 2 (p<0.01) and 3 (p<0.001) was higher compared to the healthy boars, while in fraction 4, it was lower compared to the healthy

Table 1: Sperm and semen characteristics in the split ejaculates of a 10 month old boar compared to an age matched normal boar (Values are mean±SD, n = 3 collections for each)

Parameters	Healthy boar	Cryptorchid boar
Sperm concentration (10⁶ mL⁻¹)		
Fraction 1	0.0	0.0
Fraction 2	108±7	10±4**
Fraction 3	23±7	81±13**
Fraction 4	126±23	121±25
Sperm morphology (% normal)		
Fraction 1	84± 1	77±5
Fraction 2	85±1	76±6
Fraction 3	83±2	70±1*
Fraction 4	86±1	71±2*
Seminal fructose (mg 100 mL⁻¹)		
Fraction 1	8±0.2	8±0.4
Fraction 2	9±0.2	8±0.5
Fraction 3	10±0.4	8±0.1*
Fraction 4	10±1	8±0.4*
Seminal alpha-glucosidase (mU mL⁻¹)		
Fraction 1	11.7±0.4	11.9±0.4
Fraction 2	11.7±0.3	12.0±0.3
Fraction 3	12.5±1.3	11.7±0.3
Fraction 4	11.8±0.5	12.5±0.0

* p < 0.05 ** p < 0.001

Table 2: Sperm and semen characteristics in split ejaculates of a 2 year old cryptorchid boar compared to an age matched healthy boar (Values are mean±SD, n = 3 collections per boar)

Parameters	Healthy boar	Cryptorchid boar
Sperm concentration (10⁶ mL⁻¹)		
Fraction 1	2±1.0	2±0.4
Fraction 2	77±36	100±10*
Fraction 3	35±15	90±8**
Fraction 4	165±32	62±8**
Sperm morphology (% normal)		
Fraction 1	83±3	72±0.4**
Fraction 2	81±4	78±6
Fraction 3	83±3	77±4
Fraction 4	81±4	74±1*
Seminal fructose (mg 100 mL⁻¹)		
Fraction 1	10±0.5	10±0.9
Fraction 2	9±0.3	8±0.5
Fraction 3	10±1	8±0.5
Fraction 4	9±1	8±0.1
Seminal alpha-glucosidase (mU mL⁻¹)		
Fraction 1	11.5±0.4	12.0±0.4
Fraction 2	11.7±0.3	11.6±0.9
Fraction 3	11.9±0.8	12.2±0.1
Fraction 4	11.5±0.6	12.2±1.0

*p < 0.01 ** p < 0.001

boars (p<0.001). The percentage of normal sperms were significantly lower in the cryptorchid in fraction 1 (p<0.001) and fraction 4 (p<0.01) compared to the healthy boars. The seminal fructose was consistently lower in the cryptorchid compared to the healthy boars in fractions 2, 3 and 4. No differences were observed in sperm parameters and in the accessory sex gland functions between the different collection days of 10 month old and 2 year old healthy and cryptorchid boars.

DISCUSSION

The present study documents novel data on the effects of unilateral cryptorchidism on the sperm

parameters in various fractions of the split ejaculate in boars. While fraction 1 contained few sperms, fractions 2, 3 and 4 showed generally increasing sperm-rich portions though there were fluctuations. These fluctuations could be attributed to a number of factors, like the adaptation of the boar to gloved-hand technique, experience of the collector, the status of the estrous sow (whether it was on standing heat or not) and the environment (Hafez, 1993). Furthermore previous studies in normal boars have shown that the sperm quality post-cryopreservation differs depending on the fraction of seminal fluid the spermatozoa are contained in and there is variation between boars so they have to be assessed individually to optimise which fraction of the ejaculate is suitable for best results with cryopreservation (Pena *et al.*, 2006). The authors acknowledge that a major limitation of the present study is the small sample size, although the number of collections from each boar has proved to be quite adequate to delineate the changes attributed to cryptorchidism.

Generally the boar requires about 90 sec to ejaculate 80% of the total spermatozoa and needs 40 sec to ejaculate 3×10^9 spermatozoa, a number that is frequently quoted as being sufficient for satisfactory fertility (Thiengtham, 1994). Each collection in this study lasted approximately 120 sec. Therefore the time requirement was sufficient to obtain the majority of spermatozoa in the ejaculate and also almost all seminal constituents. Since boars expel large volumes of sperm in each ejaculate and deplete their epididymal reserves quickly (Hafez, 1993), a short sexual rest of at least 3 days is recommended to allow for testicular sperm production (Mieusset *et al.*, 1995). No differences were observed in sperm concentrations between collections due to the mandatory one week sexual rest and possibly due to the pre-trials conducted before the actual study.

In a previous study, a decrease in the testicular sperm production was reported in unilateral abdominal cryptorchidism (Pinart *et al.*, 1999) which is confirmed in the present study. This could be attributed to the lower total surface area of the seminiferous epithelium of the cryptorchid boars compared to the healthy boars. The sperm concentration of both 10 month old boars were nearer the lower limit of the normal range, while in 2 year old they were nearer the upper limit of the range, which is in line with the observation that sexual maturation in boars occur around 18 months of age (Pinart *et al.*, 1999). The number of abnormal sperms was greater in the cryptorchids possibly due to some degree of impairment in spermatogenesis and spermiogenesis (Pinart *et al.*, 1998). Primary malformations occur in the testis during

spermatogenesis and secondary malformations occur in the epididymis along the sperm maturation process (Briz *et al.*, 1996).

In the present study both primary and secondary abnormalities were observed in the cryptorchid boars. Microcephalic head is an example of a primary abnormality, while reflex tails and cytoplasmic droplets are secondary abnormalities. Cytoplasmic droplets were mostly present in the cryptorchid boars and more so the 10 month old healthy boar. It is known that shedding of the cytoplasmic droplets from the boar spermatozoa is induced by fructose originating from the seminal vesicular fluid (Harayama *et al.*, 1996), while bending of the sperm tail in the live sperm could be due to environmental changes, especially temperature, as it is known that heat stress leads to increased frequency of abnormal sperms in boars (Spinaci *et al.*, 2006). Pre-heated containers were used in the present study and the slides for morphology studies were prepared within 30 minutes after collection to minimize these tertiary abnormalities. These changes however occur almost within a few minutes of spermatozoal exposure to adverse conditions and are irreversible. The presence of immature spermatozoa with proximal or distal droplets is due to abnormalities in the epididymis and develops in the epididymal duct (Briz *et al.*, 1996). Experiments with cryopreservation of spermatozoa have shown that there is a need to optimize the cryopreservation conditions, cooling rates and warming rates of spermatozoa from ejaculates with poor freeing ability (Hernandez *et al.*, 2007).

Seminal fructose (an indicator of seminal vesicular function), in the split ejaculate of boars varied in the different fractions and in 10 month old boars, differences between the healthy one and cryptorchid were observed. This may be attributed to seminal vesicle obstruction as a result of either vesiculitis or hypoplasia of the seminal vesicles or Leydig cell deficit (Hafez, 1993). The seminal alpha-glucosidase activity, an indicator of epididymal function, ranged from 11.4-12.5 mU mL⁻¹. No differences were observed between healthy and cryptorchid boars, suggesting that the epididymal functional impairment may be minimal in unilateral cryptorchidism. This is in contrast to reports where testicular ectopia associated with structural changes in the epididymal ectopia has been shown (Pinart *et al.*, 1998).

Although cryptorchid epididymides keep a temperature similar to the body temperature, a reduction of diameter and length of cauda and the transit time in the cauda epididymis has been reported (Foldesy and Bedford, 1982). However, the present study suggests that epididymal secretory activities were unaffected in cryptorchidism.

In conclusion, in this study fraction 1 of the ejaculate contained almost no spermatozoa and sperm-rich fractions follow thereafter and the percentage of normal sperms in cryptorchid boars is lower compared to healthy boars. Testicular ectopia and the epididymal ectopia induced by the cryptorchidism also caused significant morphoabnormalities in the cryptorchid boars. The seminal fructose concentrations suggest that cryptorchidism appears to impair seminal vesicular function however the seminal alpha-glucosidase activity suggests that monorchidism did not affect epididymal secretory function.

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