Effects of dietary fumonisin B₁ on the onset of puberty, semen quality, fertility rates and testicular morphology in male rabbits

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Abstract

The influences of dietary fumonisin B₁ (FB₁), a metabolite of *Fusarium verticillioides*, on the onset of puberty, semen quality, fertility rates and testicular morphology in male rabbits (bucks) were studied. Forty male rabbits were randomly assigned and fed four diets containing 0.13, 5.0, 7.5 and 10.0 mg FB₁/kg, constituting diets 1 (control), 2, 3 and 4 respectively, for a period of 175 days in a completely randomized design. During the last week of the feeding trial, two untreated female rabbits were mated to each of the four treated bucks per treatment to assess the fertility rate of the treated bucks. Onset of puberty in animals fed diets 3 and 4 was significantly ($P < 0.05$) delayed by some 9–12 days. The weight at puberty, sperm concentration and total sperm/ejaculate were not significantly influenced by the dietary FB₁. Sperm mass activities, motility and live spermatozoa of the rabbits' semen significantly ($P < 0.05$) declined with an increase in the dietary FB₁. The highest sperm cell abnormalities were recorded in the animals fed 10.0 mg/kg FB₁, while the least was observed in the control animals. The conception rate, litter size and embryo survival rate were statistically the same among the dietary treatments. Embryo mortality was significantly ($P < 0.05$) higher in rabbits fed diets 3 and 4 than in others. Testicular elements were significantly ($P < 0.05$) impaired by the toxin in rabbits fed 7.5 and 10.0 mg FB₁/kg. This suggests that LOAEL of 7.50 mg/kg FB₁ delayed puberty, impaired semen quality and spermatogenesis and induced embryo mortality without a statistically adverse effect on the fertility rates of male rabbits.

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Introduction

Increased economic loss in livestock production due to the presence of mycotoxins in the feed given to animals is a major problem that is yet to be solved in Nigeria. The resultant effects of this are a short life span, retarded growth and poor reproductive performance on the side of male and female animals. One among such mycotoxins is fumonisin produced by *Fusarium verticillioides* as a secondary toxic metabolite. Fumonisin B₁ (FB₁) among other toxins produced by this fungus has been reported as the major fumonisin produced in culture (Bezuidenhout *et al.* 1988, Ross *et al.* 1992) as well as naturally occurring in maize and maize-based feeds and foods (Rheeder *et al.* 1992, Ross *et al.* 1992).

The carcinogenicity and hepatotoxicity as well as the effects on feed intake and body weight gain by dietary fumonisins in animals have been well documented (Marasas *et al.* 1988, Kellerman *et al.* 1990, Gelderblom *et al.* 1991, 1994, Ewuola *et al.* 2008a). Fumonisin-producing fungus grows readily in stored maize grains or *in situ*. The importance of this ingredient in the diets of man and his livestock cannot be underestimated. However, maize has been reported as the only

commodity that contains significant amounts of fumonisin (Shephard *et al.* 1996). Hence, the potential for fumonisin to be found in feeds and foodstuffs is high. Among other disturbances, fumonisin has been reported to have some adverse effects on reproduction in mice (Riley *et al.* 1996), rats (Voss *et al.* 1996), pigs (Harrison *et al.* 1990) and poultry (Bradlaw *et al.* 1994). Relatively little information is available on the effect of fumonisin on semen quality and fertility in farm animals, especially rabbits.

With the above in mind and the fact that the humid tropical conditions are very conducive to the growth of *F. verticillioides* in field or in stored maize grains, which form about 25–40% of livestock feeds, the present investigation was designed. The aims were to evaluate the effects of dietary FB₁ on the onset of puberty, semen quality, fertility rates and testicular morphology of male rabbits.

Results

Onset of puberty

Pubertal age and weight of rabbits fed fumonisin-contaminated diets are presented in Table 1. The result shows that the age at puberty of animals fed diets 1 and 2 was not significantly different in the animals.

Table 1 Pubertal weight and age and semen characteristics of male rabbits fed dietary fumonisin B₁ (mean ± S.E.M.; *n* = 10).

Parameters	Dietary fumonisin B ₁ levels (mg/kg)			
	0.13	5.0	7.5	10.0
Weight at puberty (g)	1725 ± 56.20	1703 ± 45.25	1690 ± 67.46	1716 ± 60.01
Age at puberty (days)	126.17 ± 3.61 [†]	127.00 ± 3.02 [†]	136.00 ± 4.01*	139.00 ± 3.98*
Semen volume (ml)	0.74 ± 0.05	0.73 ± 0.03	0.73 ± 0.03	0.74 ± 0.04
Semen colour	Creamy	Creamy	Creamy	Milky
Mass activities	+++	++	++	+
Sperm motility (%)	79.60 ± 3.26*	75.60 ± 3.22*	66.00 ± 2.92 [†]	65.20 ± 2.75 [†]
Sperm concentration (×10 ⁶ /ml)	5.38 ± 0.28	5.19 ± 0.19	5.04 ± 0.18	4.92 ± 0.28
Total sperm cells/ejaculate (×10 ⁶ /ml)	3.80 ± 0.25	3.79 ± 0.15	3.68 ± 0.16	3.62 ± 0.26
Live-dead sperm ratio	90:10 ± 1.83*	83:17 ± 2.55*	65:35 ± 3.87 [†]	63:37 ± 6.04 [†]
Sperm abnormality	5.00 ± 2.24 [†]	15.00 ± 3.26* [†]	23.00 ± 4.54*	28.00 ± 6.77*

*[†]Means in the same row with different superscripts are significantly (*P* < 0.05) different.

However, the pubertal age of animals fed diets 3 and 4 was significantly delayed by some 9–12 days. The weight at which the animals attained puberty was not significantly different among the treatments.

Semen characteristics

The data on semen characteristics of pubertal bucks fed fumonisin-contaminated diets such as volume, colour, sperm concentration, and live spermatozoa and morphological abnormalities are presented in Table 1. The results show that semen volume, sperm concentration and sperm number/ejaculate were not significantly different among the dietary treatments. The semen appearance in terms of colour was the same among the treatments 2, 3 and the control, while semen colour of the rabbits fed diet 4 containing 10.0 mg/kg FB₁ was milky. Mass activity of semen collected from the experimental animals declined from the control diet with turbulence motion/wave (+++) to animals on diet 4 with general oscillation (+). Percentage sperm motility was significantly (*P* < 0.05) influenced among the dietary treatments, with it being significantly (*P* < 0.05) lower in rabbits fed diets 3 and 4 than in those fed diet 2 and the control. The percentage live spermatozoa and morphological abnormalities were significantly (*P* < 0.05) influenced by the dietary treatments. The percentage live spermatozoa was inversely related to morphological abnormalities (Table 1), and this declined with an increase in the fumonisin concentration in the diets.

The least (63%) live sperm cells and the highest (28%) sperm cell abnormalities were recorded for the animals fed diet 4, while the highest (90%) and the least (5%) percentage live spermatozoa and spermatozoa abnormalities (Table 1) respectively were recorded for the animals fed the control diet.

Fertility assessment

The fertility of the male rabbits fed fumonisin-contaminated diets is presented in Table 2. The conception rate, litter size, embryo survival rate and embryo normalcy of does mated to treated bucks were not statistically different among the dietary treatments. Embryo mortality of does mated to bucks fed 7.5 and 10.0 mg/kg FB₁ was similar, but it was significantly higher (*P* < 0.05, *P* value 0.0396) than that of those fed 5.0 mg/kg FB₁ and the control diet.

Testicular morphology

The diameter of seminiferous tubules and volumetric proportions of spermatogenic elements of the experimental animals were significantly (*P* < 0.05) influenced among the dietary treatments (Table 3). Tubule diameter was highest (179.28 µm) in bucks fed control diet, and the least value (151.41 µm) was recorded for bucks fed diet 4 with 10.0 mg/kg FB₁. Type A spermatogonia and type B spermatogonia followed the same trend, and they significantly (*P* < 0.05) decreased with an increase in

Table 2 Fertility rate of pubertal rabbit bucks fed varied levels of dietary fumonisin B₁ (mean ± S.E.M.; *n* = 10).

Parameters	Dietary fumonisin B ₁ levels (mg/kg)			
	0.13	5.0	7.5	10.0
Ovulation rate	9.60 ± 0.15	9.00 ± 0.16	10.00 ± 0.14	9.20 ± 0.15
Conception rate (%)	100.00 ± 0.00	100.00 ± 0.00	87.50 ± 0.72	87.50 ± 0.72
Litter size	7.25 ± 0.19	7.00 ± 0.20	7.00 ± 0.35	6.50 ± 0.37
Embryo survival rate (%)	80.56 ± 2.91	77.78 ± 3.12	70.00 ± 2.98	70.65 ± 2.78
Foetal crown rump length (cm)	3.14 ± 0.23	3.01 ± 0.40	2.82 ± 0.31	2.98 ± 0.36
Embryo mortality (%)	24.48 ± 0.20 [†]	22.22 ± 0.25 [†]	30.00 ± 0.19*	29.35 ± 0.18*

*[†]Means in the same row with different superscripts are significantly (*P* < 0.05) different.

Table 3 Testicular weight, seminiferous tubule diameter and volumetric proportion of testicular elements of pubertal bucks fed dietary fumonisin B₁ (mean ± S.E.M.; *n* = 10).

Parameters	Dietary fumonisin B ₁ levels (mg/kg)			
	0.13	5.0	7.5	10.0
Paired testes weight (g)	3.50 ± 0.13 [†]	3.18 ± 0.14 [†]	4.07 ± 0.10*	3.40 ± 0.25 [†]
Seminiferous tubule diameter (µm)	179.28 ± 5.59*	164.19 ± 2.24* [†]	154.97 ± 5.37* [†]	151.41 ± 4.54 [†]
Volumetric proportion of testicular elements (%)				
Spermatogonia A	3.50 ± 0.41*	3.18 ± 0.26* [†]	2.92 ± 0.24 [†]	2.88 ± 0.39 [†]
Spermatogonia B	2.83 ± 0.36*	2.78 ± 0.09*	2.34 ± 0.13 [†]	2.30 ± 0.21 [†]
Primary spermatocytes	2.86 ± 0.42* [†]	3.00 ± 0.25*	2.84 ± 0.33* [†]	2.56 ± 0.24 [†]
Secondary spermatocytes	4.96 ± 0.27	4.86 ± 0.11	4.37 ± 0.15	4.18 ± 0.05
Round spermatids	5.40 ± 0.34*	5.09 ± 0.36*	4.15 ± 0.30 [†]	4.09 ± 0.24 [†]
Elongated spermatids	6.58 ± 0.42*	6.34 ± 0.43*	5.55 ± 0.77 [†]	4.91 ± 0.40 [†]
Spermatozoa	4.24 ± 0.38*	2.78 ± 0.32 [†]	2.62 ± 0.18 [†]	2.18 ± 0.23 [†]
Sertoli cells	3.67 ± 0.19*	3.07 ± 0.39 [†]	3.06 ± 0.27 [†]	2.50 ± 0.43 [†]
Leydig cells	4.28 ± 0.39	4.16 ± 0.33	4.04 ± 0.09	4.01 ± 0.29
Lumen	3.60 ± 0.09 [†]	3.98 ± 0.17 [†]	4.19 ± 0.21*	4.33 ± 0.08*

*[†],[‡] Means in the same row with different superscripts are significantly (*P* < 0.05) different.

the dietary FB₁ levels. The primary spermatocytes were significantly (*P* < 0.05) influenced among the dietary treatments, and the lowest values (2.84 and 2.56% respectively) were recorded for animals fed diets 3 and 4. Round and elongated spermatids and interstitial tissues followed the same trend, and they significantly (*P* < 0.05) decreased with an increase in the fumonisin levels in the diets. Highest spermatozoa (4.24%/rabbit) were obtained for control bucks, while the least value (2.18%/rabbit) was recorded for rabbits fed diet 4. Sertoli cells followed the same pattern and they decreased significantly (*P* < 0.05) from the control animals (3.67%/rabbit) to the animals placed on diet 4 (2.50%/rabbit). Lumen that was observed for rabbits fed diets 3 and 4 was significantly (*P* < 0.05) higher than that observed for those fed the control diet and diet 2 containing 5.0 mg FB₁/kg diet. The frequency of the stages of cycle of seminiferous epithelium of pubertal rabbits fed experimental diets (Table 4) was significantly (*P* < 0.05) affected by the treatments. Stages I, II, IV, VI and VII were significantly (*P* < 0.05) impaired in the rabbits exposed to 10 mg FB₁/kg diet as compared to those fed diet 2 and the control.

Discussion

Reproductive efficiency is recognized as the most costly and limiting constraint to efficient animal production as reproduction is the bedrock of animal production. Good semen quality with normal sperm concentration and high libido are the strength of rabbit production. In this study, the onset of puberty that was delayed by 9–12 days in animals fed 7.5 and 10.0 mg/kg FB₁ could be attributed to dietary FB₁ since all the animals were placed on the same plane of nutrition except for fumonisin levels that varied among the diets. This shows that the age at which animals attain puberty is directly related to their weight. These animals suffered a delay in the onset of puberty probably because of reduced growth rate, which was inhibited by the toxin (Bondy *et al.* 1998, Ewuola *et al.* 2007), and it may have also contributed to a delay in the maturation of the reproductive organs that determine puberty in the animals. Earlier studies have established that testicular weight and sperm production are highly related (Swierstra 1966, Egbunike *et al.* 1975). Besides, testicles of some of these animals suffered some degeneration especially at the Leydig cells, which is the site of

Table 4 Stages of seminiferous epithelial cycle (%) of pubertal bucks fed dietary fumonisin B₁ (mean ± S.E.M.; *n* = 10).

Parameters	Dietary fumonisin B ₁ levels (mg/kg)			
	0.13	5.0	7.5	10.0
Stage I	20.02 ± 0.13*	17.86 ± 0.14 [†]	16.71 ± 0.11 [‡]	16.12 ± 0.18 [§]
Stage II	18.30 ± 0.12*	18.01 ± 0.11*	17.86 ± 0.14*	15.38 ± 0.19 [†]
Stage III	16.24 ± 0.19 [§]	17.86 ± 0.14 [‡]	22.52 ± 0.21 [†]	25.00 ± 0.22*
Stage IV	16.57 ± 0.23*	14.29 ± 0.41 [†]	12.90 ± 0.35 [‡]	10.60 ± 0.38 [§]
Stage V	8.40 ± 0.18 [§]	10.71 ± 0.20 [‡]	14.29 ± 0.09 [†]	15.00 ± 0.12*
Stage VI	8.29 ± 0.31*	7.86 ± 0.28*	7.14 ± 0.29* [†]	6.07 ± 0.21 [§]
Stage VII	8.40 ± 0.23*	7.29 ± 0.25 [†]	5.57 ± 0.12 [‡]	4.79 ± 0.29 [†]
Stage VIII	6.79 ± 0.20 [†]	3.79 ± 0.19 [‡]	7.14 ± 0.21 [†]	9.02 ± 0.11*

*[†],[‡],[§] Means in the same row with different superscripts are significantly (*P* < 0.05) different.

testosterone production that is responsible for the onset of puberty and Sertoli cells – the nurse cells for testicular elements as reported by Ewuola (2009). The dietary toxin used in this study did not adversely affect the semen volume, sperm concentration and total sperm/ejaculate. This is probably due to the fact that quantitative semen characteristics are determined undermining whether the sperm cells are normal or abnormal, alive or dead (Kamar & Badreldin 1959).

The declined mass activity and sperm motility in animals fed 10 mg/kg FB₁ could be attributed to the toxin effect which may have probably inhibited cyclic 3'/5' AMP activity and calcium ion, which are believed, among other factors, to initiate motile ability of spermatozoa in the caput epididymis (Olson & Danzo 1981). On the other hand, the toxin had been reported to impede protein absorption and utilization (Ewuola *et al.* 2003, 2007), and this protein in the form of an acidic epididymal glycoprotein is said to be required for sperm maturation and to maintain motility of sperm cells from the caput epididymis (Brooks & Higgins 1980, Olson & Danzo 1981). The significantly influenced sperm motility agreed with the report of Egbunike & Oluyemi (1979) that sperm concentration was not related to both semen volume and progressive motility. Semen colour of bucks fed 10.0 mg FB₁/kg diet may be due to apparently low sperm concentration and increased secretion from the accessory glands. Sperm morphological abnormalities that were observed revealed that most sperm cells suffered secondary abnormalities such as the presence of cytoplasmic droplets on the tail or mid-piece, bent tails and detached head caps of the total abnormalities found in the semen of animals fed 7.5 and 10.0 mg/kg dietary FB₁. Spermatozoa with coiled tails were the most predominant. A similar observation was reported by Egbunike (1979) in rats given doses of aflatoxin B₁. In this study, it was also observed that the sperm concentration was negatively related to sperm cell abnormalities. Percentage live spermatozoa that declined with an increase in the FB₁ level in the diets may be attributed to the toxin being probably toxic to the sperm cells in the testicles since fumonisin has been reported to be widely distributed in the tissue of the animals following ingestion (Prelusky *et al.* 1996). In their studies, Goel *et al.* (1994), Gumprecht *et al.* (1995) and US NTP (1999) reported hepatotoxicity and nephrotoxicity of FB₁, and that relative to other tissues examined, the liver and kidney showed the greatest increase in the concentration of free sphinganine, a detectable biomarker for exposure to fumonisins (Riley *et al.* 1994). Rabbits have also been reported to be very sensitive to the toxic effects of FB₁ (LaBorde *et al.* 1997). This result corroborates the reports of Ogunlade *et al.* (2006), Gbore *et al.* (2007) and Gbore & Egbunike (2008) who reported that animals fed fumonisin-contaminated diets showed decreased testicular sperm reserves when compared to the controls. Conception rate, litter size, embryo survival rate and

embryo normalcy observed in this study were not adversely affected by the dietary treatments. In total, 73.49% of the embryos survived the 10-day gestation period resulting in a litter size of 6.94. A result similar to this observation was obtained by Gbore *et al.* (2007) when microdoses of dietary FB₁ were fed to adult rabbits. This result also lends support to the findings of Panda *et al.* (1970) and Egbunike (1979) on a mycotoxin, who reported that litter size, conception rates and embryo survival rate were unaffected in rats after both sexes had been treated with aflatoxin B₁. The significant difference that was observed in embryo mortality among the treatments could probably be attributed to the toxin effect (LaBorde *et al.* 1997).

The significantly depressed volumetric proportion of spermatogenic elements in animals fed diets containing high FB₁ could be attributed to the toxin effect. Fumonisin has been reported to inhibit biosynthesis of sphingolipid, which controls body cholesterol level (Fincham *et al.* 1992, Merrill *et al.* 1993), which probably results in the increased cholesterol-rich lipid in the body including testes (Ewuola *et al.* 2008b). A decrease in cell size and number of spermatogenic elements and continuous accumulation of lipid-cholesterol suggests that the synthetic activity of Leydig cells was affected in fumonisin-treated animals. The overall effect of the toxin on the reproductive parameters examined in this study may be attributed to both direct and indirect effects of FB₁ on the animals. Apart from testicular degeneration, hepatic and renal lesions reported in the same animals (Ewuola 2009) significantly elevated aspartate amino transferase and alanine amino transferase, and alkaline phosphatase observed in the treated animals (Ewuola & Egbunike 2008) ascertained disruption done to their livers and kidneys. It is well established that fumonisin toxins disrupt glycosphingolipid metabolism (Yoo *et al.* 1992, WHO 2000), and that the lipid signalling molecules serve as important contributors to the regulation of gametogenesis. The sphingolipid molecules involve in the mediation of stress- or damage-induced apoptosis in the germ line, which is an event that is most likely associated with impaired gonadal function and infertility in animals.

Conclusion

Based on the results obtained from this study, the LOAEL of 7.5 mg FB₁/kg in the diet of rabbits delayed puberty, impaired semen quality and spermatogenesis and induced embryo mortality with potential to depress reproduction in animals that will be used for breeding purposes. Therefore, such an ingredient contaminated with *F. verticillioides* that would liberate up to 7.50 mg/kg FB₁ should be avoided in ration formulation for rabbits.

Materials and Methods

Experimental materials

This study was approved by our institutional committee on the care and use of laboratory animals. Forty-eight crossbred (New Zealand White × Chinchilla) male rabbits that were 49 days old with an average weight of 757.50 ± 0.50 g were sourced from Rabbitry Unit, Olabisi Onabanjo University, Ayetoro, Ogun State, Nigeria, out of which 40 were used for this investigation. The animals were housed individually in metal cages, each $75 \times 36 \times 30$ cm in dimension, and were fed *ad libitum* on fumonisin – contaminated diets containing corn (as the contaminated grains), 30%; rice husk, 23%; wheat offal, 27%; soybean meal, 15%; fish meal, 2%; calcium diphosphate, 2%; salt, 0.5%; vitamin-premix, 0.45%; methionine, 0.03%; and lysine, 0.02%, for a period of 175 days.

Four dietary treatments 1 (control) 2, 3 and 4 were formulated to contain 0.13, 5.0, 7.5 and 10.0 mg FB₁/kg of diet respectively by substituting ground maize cultured with *F. verticillioides* (MRC 826, Marasas 2001) for ground, non-cultured maize in various proportions. The fungus isolates were characterized and grown on acidified potato dextrose agar under a control environment in an incubator for a period of 7–14 days. After the incubation period, the microconidia were washed into a suspension with distilled water and were used to inoculate the autoclaved maize grains used for this experiment at the Plant Pathology Unit, International Institute for Tropical Agriculture (IITA), Ibadan, according to the method of Nelson *et al.* (1991). Samples of homogenously mixed experimental diets were quantified in replicates for FB₁ using fumonisin quantitative Veratox test kit (Neogen Corps., Lansing, MI, USA), a competitive direct ELISA (CD-ELISA) method, and were also screened for other toxins which were found to be negligible. The diets contained ~16% crude protein, 11% crude fibre and 2.55 kcal DE/g. The animals were randomly allotted to the diets in a completely randomized design (CRD), with each treatment having ten rabbits.

Onset of puberty and semen evaluation

Animals were assessed for the attainment of puberty as from the age of 12–15 weeks on a weekly basis and at 72 h interval thereafter by examining their preputial fluid smeared on a glass slide under a microscope for sperm cells. Pubertal age was established when at least 50% of them showed the presence of sperm cells. At puberty, they were subjected to a training period of 4 weeks with ready does (mature female rabbits) using artificial vagina for semen collection. After the animals became used to the system, they were allowed a resting period of 1 week, following which they were ejaculated at 72 h interval and five ejaculates were collected from each animal mostly in the morning for about 2 weeks. The data for each of the semen quality parameters were averaged for each animal and were then averaged for the group.

Semen evaluation of the ejaculate was done immediately after collection for physical characteristics such as colour, volume, mass activity, sperm progressive motility, sperm concentration, percentage live–dead sperm cells and percentage sperm abnormalities. Semen volume was read in

a calibrated microsyringe to the nearest 0.01 ml. Semen colour was visually appraised directly under natural light, and the rating was from watery to creamy colour. A drop of fresh semen was placed on a clean glass slide and was examined with a microscope under $\times 10$ objective lens to determine mass activity. The mass activity was scored subjectively according to the intensity of the wave motion from the absence of wave motion (+) to very turbulent motions (+++). To assess sperm motility, a drop of the diluent (sodium citrate) was added to a drop of freshly collected semen on a glass slide, covered with a coverslip and examined with a microscope under $\times 40$ objective lens. The percentage progressively motile spermatozoa were estimated, and the motility score was subjectively rated between 0 and 100. Sperm concentration was determined using a haemocytometer by taking 0.02 ml of the undiluted semen and by mixing it with 4.0 ml of formal saline (90% physiological saline + 10% formalin). This mixture was thoroughly shaken and was allowed to run by capillary action under the coverslip on the haemocytometer. The sperm cells were allowed 3 min to settle before counting. Sperm cells in the five squares of the ruled area of the haemocytometer were counted diagonally. The total sperm cell counts were multiplied by the dilution factor, area of the haemocytometer, and the number of the square that was counted, and then divided by the depth of the haemocytometer.

The proportion of live–dead spermatozoa was determined by adding a drop of undiluted semen to a drop of the staining solution (Nigrosin–Eosin) on a clean slide, mixing gently and by preparing a smear. The slide was air-dried and examined with a microscope under oil immersion. The proportion of abnormal sperm cells was evaluated under the microscope by random evaluation of at least 100 spermatozoa on the slide prepared for the live–dead sperm cells estimation. Sperm output was estimated as a product of semen volume and sperm concentration of the same animal.

Fertility assessment

Eight untreated pubertal does were assigned to four treated bucks for each dietary treatment for the mating trial in the ratio of 2:1 (female:male). Each female was mated twice daily in the morning and the evening for 2 days consecutively. The does were killed on the 11th day into gestation, and their uteri were cut opened longitudinally to check for conception and to count the embryos therein. Corpora lutea counts were taken to represent the ovulation rates, while the number of surviving embryos, expressed as the percentage of the ovulation rate, was considered as the embryo survival rate. Embryo mortality rate is estimated to be the algebraic differences between ovulation rate and number of embryos expressed as the percentage of the ovulation rate. At the end of the mating trial, treated bucks were anaesthetized and killed, and testes were carefully dissected, removed, weighed and processed for testicular histometry.

Testicular histometry

The tissues were weighed and fixed in Bouin's fixative for 72 h before dehydration in ten changes in ethanol of different concentration ranges from 70 to 100% at 1-h interval.

After dehydration, the tissues were cleared in two changes of chloroform before infiltration/embedding in molten wax at 58–60 °C for 12 h (overnight). Thereafter, the tissues were removed and blocked using paraffin wax and were later sectioned using a microtome. Permanent slide of each section was made for microscopic analysis to determine the volumetric proportion of testicular elements using 25-point ocular graticules (Egbunike *et al.* 1975). The histological aspect of this study has been published already (Ewuola 2009).

Statistical analysis

The design used for this experiment is a CRD. Data collected on sperm concentration, volumetric proportion of testicular elements, seminiferous tubule diameter and stages of cellular association were subjected to statistical analysis using ANOVA procedure of SAS (1999). The significant means were compared using the Duncan multiple range test of the same software. Other parameters in both semen characteristics and fertility were subjected to a descriptive analysis.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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