

Sperm Morphological Studies of the West African Dwarf Buck Treated with Pumpkin Plant (*Cucurbita pepo*)

Estudio Morfológico del Esperma de la Cabra Enana del Oeste Africano Tratado con Planta de Calabazas (*Cucurbita pepo*)

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SUMMARY: Four healthy bucks of the West African Dwarf breed aged between 24 and 30 months were used for this study. The bucks were first used as control and later as experimental animals upon being fed daily with oiled pumpkin plant for the period of six months. The objective of the study was to investigate the effect of the pumpkin plant on the morphology of the spermatozoa of the bucks. There were significant differences ($p < 0.05$) between the control and experimental values for both primary and secondary morphological spermatozoa abnormalities: the pyriform head has a control value of 6 (0.42%) and post-feeding value of 0 (0%), the beat tail; 14(0.97%) and 2 (0.16%) for the control and post feeding values respectively. The curved mid piece: 17 (1.18%) and 1 (0.08%) for the control and post-feeding values respectively. The bent mid piece also differed significantly ($p < 0.05$) between control value of 16 (1.11%) and post feeding value of 3 (0.23%). All through the stages of the study, there were significant reductions in the number of sperm cells with abnormalities consequent upon daily feeding of the animals with pumpkin plant. The plant is therefore recommended for both prevention and treatment of male infertility in man and animals.

KEY WORDS: Pumpkin plant; Morphology; Male infertility; Spermatozoa.

INTRODUCTION

Reproductive ability in the male comprises the production of semen containing normal spermatozoa (quality) in the adequate number (quantity), together with the desire and ability to mate (Oyeyemi & Ubiogoro, 2005). The study of reproductive organs has been always reported by Massanyi *et al.* (1997; 2003); Oke *et al.* (1988, 1989, 1995); Oke & Aire (1996); Cigankova *et al.* (1993; 1996), Oyeyemi & Ubiogoro and Oyeyemi & Babalola (2006).

The pumpkin plant (*Cucurbita pepo*), believed to have originated in the North America, had been found by different cultures to be of great value in the prevention and treatment of prostrate ailments and impotence in man (Bodytalkhealth, 2005). The West African Dwarf (WD) goats occurring in the tropical forest belt of West Africa are small sized breeds ranging from between 20-30kg weight (Epstein & Herz, 1964; Chang & Landauer, 1950; Otesile, 1993). The WAD goat is very important in developing countries (Awotwi & Fynn, 1992), being able to thrive in adverse conditions (Devendra, 1971;

Oyeyemi 1997) and has a high fertility rate with a short generative interval allowing for a possible increase in population than cattle in West Africa (Williamson & Payne, 1965; Mackenzie, 1970).

The sperm cell can be broadly divided into the head, and tail with the tail further divisible into 4 regions: the neck, the middle piece, the principal piece and end piece (Hammer, 1970). The head is covered by a protoplasmic cap known as the *Galea capitis* having shapes varying according to species. It is avoid in the bull, ram, rabbit while in man it is round. The middle piece of the spermatozoa provides energy for motility (Setchell, 1977), while its distal end piece consists of inner axial filaments resembling the flagellum of a ciliated cell.

Low plane of nutrition has been identified to delay slightly, the onset of puberty and increase sperm abnormality (Oyeyemi & Akusu, 1998). High quality semen should not have more than 10% of abnormal sperm cells. This study was

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aimed at investigating the effect of the pumpkin plant (*Cucurbita pepo*) on the morphology of the spermatozoa of the WAD buck thus contributing to information on the effect of herbs in male reproductive performance.

MATERIAL AND METHOD

Four WAD bucks with age ranging between 24 and 30 months, were used for the study. They were kept at the Large Animals Ward II of the Veterinary Teaching Hospital (VTH), University of Ibadan, located between latitude 150N and 300S with relative humidity ranging from 50 – 80%, rainfall is about 70 inches *per annum* and temperature between 28°C and 34°C. The bucks were first used as control (Group A) and later as experimental (Group B) upon being fed with boiled pumpkin plant. Both groups were maintained on a daily ration of Guinea corn offal of about 1 kg *per animal* supplemented with dried cassava peels (*Manihot* species). Water was provided *ad libitum*. The animals were dewormed using both Albendazole and Ivermectin while Levamisole was used to control endo and ectoparasites. Vaccination against pests de petit ruminants (PPR) disease was carried out using the PPR vaccine (NVRI, VOM). Other necessary Veterinary attentions were given throughout the whole period of the experiment.

Semen Collection. Semen was collected from the goats using the electrojaculation method as described by Zemjanis (1977), on a weekly basis before and after the onset of feeding with boiled pumpkin fruits and was sustained for five weeks.

Morphological Studies. On a clean, warm glass slide, a drop of semen was placed as well as two drops of Wells and Awa stain. The semen and stain were thoroughly mixed together with a smear made on another clean and warm slide. The smear was air-dried and observed using the light microscope starting with low power to high magnification. The presence of abnormal cells out of at least 400 sperm cells from several fields on the slide was counted and their total percentage estimated.

Statistical Analysis. Paired comparisons were done using the “t” test where applicable. Analysis of variance was used where means was significantly differed; separation of means was also done using Duncan’s multiple range test.

RESULTS

The results of the spermatozoa morphology obtained are presented in Tables I -V. The morphological abnormalities

of sperm cells observed in the course of the study were pyriform head, small head, narrow head, rudimentary tail, coiled tail, bent tail, twin head, curved mid-piece, tailless head, headless tail, bent mid piece and looped tail. There were considerable reduction in the number of sperm abnormalities (Tables I-V).

There was a significant difference ($p < 0.05$) between the control value, 6 (0.42%) of spermatozoa with pyriform head and the post experiment the 0 (0%) values obtained for weeks 2, 3 and 4 (Tables I-V). There was a significance difference ($p < 0.05$) between the control value 14 (0.97%) of spermatozoa with bent tail and the post experiment value 2 (0.16%) as shown in Tables I and V.

Also, a significant difference ($p < 0.05$) was observed between the control value 17 (1.18%) of spermatozoa with curved mid piece and those weeks 2, 3 and the post experiment being 13 (0.82%), 7 (0.41%) and 1(0.08%) respectively (Tables I, III, IV and V). For the spermatozoa with bent mid piece, there was a significant difference ($p < 0.05$) between the control value 16 (11%) and the post experiment value of 3 (0.23%) as shown in Tables I and V. There was asinificant difference ($p < 0.05$) between the control value for total normal spermatozoa, 1333 (92.51%) and the post experiment value of 1240 (96.8%) as shown in tables I and V. However, there were no significant differences between the values obtained for the control experiment for the small head, narrow head, rudimentary tail coiled tail, twin head tailless head and the post experiment headless tail, and looped tail.

DISCUSSION

The ejaculate obtained in the study using the electroejaculation method agrees with the findings of Bearden & Fuquay (1997) in terms of its colour being creamy/ milky. The volume of sperm obtained fell within the range of 0.31 and 0.38ml as obtained by Oyeyemi *et al.* (1996) showing that the electroejaculation method does not have a negative influenced on the volume of the ejaculate.

The spermatozoa morphology of the bucks used for this study was similar to earlier reports relating to the WAD bucks by Akusu (1982); Oyeyemi *et al.* (2000). It also corresponds to the earlier report of Oyeyemi & Akusu (1998) on the characteristics of the semen of the WAD bucks. The presence of abnormal forms of spermatozoa in this study is consistent with the report of Moss *et al.* (1979) that a number of abnormal forms of spermatozoa are normally encountered in all ejaculates. It is only when they are present is large

Table I. Morphological examination, control. PH: Pyriform; SH: Small head; NH: Narrow head; RT: Rudimentary tail; CT: Coiled tail; BT: Bent tail; TT: Twain tail; CM: Curved midpiece; TH: Tailless head; HT: Headless tail; BM: Bent midpiece; LT: Looped tail; TNC: Total normal cells; TAC: Total abnormal cells; TCC: Total cells counted.

S/N	PH	SH	NH	RT	CT	BT	TT	CM	TH	HT	BM	LT	TNC	TAC
K1	1(0.24)	2(0.48)	1(0.24)	1(0.24)	3(0.72)	4(0.72)	0(0)	5(1.20)	8(1.92)	6(1.44)	5(1.20)	1(0.24)	386(92.34)	33(7.89)
K2	1(0.33)	0(0)	0(0)	1(0.33)	0(0)	3(0.99)	0(0)	3(0.99)	4(1.32)	4(1.32)	2(0.66)	1(0.33)	285(94.37)	17(5.63)
K3	4(0.88)	0(0)	0(0)	2(0.44)	0(0)	6(1.33)	0(0)	6(1.33)	7(1.56)	6(1.44)	6(1.33)	7(1.55)	408(90.67)	42(9.33)
K4	0(0)	0(0)	0(0)	1(0.36)	2(0.74)	2(0.74)	0(0)	3(1.11)	5(1.85)	3(1.11)	3(1.11)	0(0)	254(93.73)	17(6.27)
Total	6(0.42)	2(0.14)	1(0.07)	5(0.35)	5(0.35)	14(0.97)	0(0)	17(1.18)	24(1.67)	19(1.32)	16(1.11)	9(0.62)	1333(92.51)	109(7.56)

Table II. Experiment (Week 1). Feeding of pumpkin fruit.

S/N	PH	SH	NH	RT	CT	BT	TT	CM	TH	HT	BM	LT	TNC	TAC
K1	0(0)	0(0)	0(0)	0(0)	1(0.22)	5(1.08)	0(0)	1(0.22)	8(1.72)	1(0.22)	8(1.72)	3(0.65)	438(94.19)	27(5.81)
K2	0(0)	1(0.24)	0(0)	1(0.24)	1(0.24)	6(1.44)	0(0)	3(0.72)	9(2.15)	6(1.44)	5(1.20)	2(0.48)	384(91.86)	34(8.13)
K3	0(0)	0(0)	0(0)	0(0)	3(0.75)	4(1.00)	0(0)	4(1.00)	5(1.24)	12(2.99)	4(1.00)	0(0)	372(92.54)	32(7.96)
Total	0(0)	1(0.08)	0(0)	1(0.08)	5(0.39)	15(1.17)	0(0)	8(0.62)	22(1.71)	19(1.48)	17(1.32)	5(0.39)	1194(92.92)	93(7.24)

Table III. Experiment 2 (Week 2). Feeding of pumpkin fruit.

S/N	PH	SH	NH	RT	CT	BT	TT	CM	TH	HT	BM	LT	TNC	TAC
K1	0(0)	0(0)	0(0)	0(0)	0(0)	3(0.81)	0	1(0.27)	3(0.81)	3(0.81)	1(0.27)	1(0.27)	359(96.77)	12(3.23)
K2	0(0)	0(0)	0(0)	0(0)	0(0)	2(0.51)	0	4(1.01)	8(2.02)	10(2.53)	4(1.01)	1(0.25)	367(92.28)	29(7.32)
K3	0(0)	0(0)	0(0)	0(0)	3(0.72)	4(0.97)	0	4(0.72)	2(0.48)	2(0.48)	3(0.72)	0(0)	397(95.89)	17(4.11)
K4	0(0)	0(0)	0(0)	2(0.49)	1(0.24)	3(0.73)	0	5(1.22)	4(0.98)	2(0.49)	6(1.46)	2(0.49)	385(93.90)	25(6.10)
Total	0(0)	0(0)	0(0)	2(0.13)	4(0.25)	12(0.75)	0	13(0.82)	17(1.07)	17(1.07)	14(0.88)	4(0.25)	1508(94.78)	83(5.22)

Table IV. Experiment 3 (Week 3). Feeding of pumpkin fruit.

S/N	PH	SH	NH	RT	CT	BT	TT	CM	TH	HT	BM	LT	TNC	TAC
K1	0(0)	0(0)	0(0)	0(0)	2(0.50)	2(0.50)	0(0)	1(0.25)	4(0.99)	3(0.74)	1(0.25)	1(0.25)	389(96.53)	14(3.47)
K2	0(0)	1(0.23)	0(0)	1(0.23)	1(0.23)	0(0)	0(0)	3(0.68)	28(6.36)	16(3.60)	3(0.68)	0(0)	387(87.95)	53(12.05)
K3	0(0)	0(0)	1(0.23)	1(0.23)	2(0.46)	4(0.92)	0(0)	2(0.46)	3(0.69)	3(0.69)	4(0.92)	0(0)	416(95.41)	20(4.59)
K4	0(0)	0(0)	0(0)	3(0.69)	1(0.23)	1(0.23)	0(0)	1(0.23)	2(0.46)	2(0.46)	1(0.23)	0(0)	425(97.48)	11(2.25)
Total	0(0)	1(0.06)	1(0.06)	5(0.29)	6(0.35)	7(0.41)	0(0)	7(0.41)	37(2.16)	24(1.40)	9(0.52)	1(0.06)	1617(94.29)	98(5.71)

Table V. Post Experiment (Week 4).

S/N	PH	SH	NH	RT	CT	BT	TT	CM	TH	HT	BM	LT	TNC	TAC
K1	0(0)	0(0)	0(0)	1(0.24)	1(0.24)	1(0.24)	0(0)	1(0.24)	1(0.24)	3(0.71)	0(0)	0(0)	414(98.10)	8(1.90)
K2	0(0)	0(0)	0(0)	2(0.47)	2(0.47)	0(0)	0(0)	0(0)	9(2.13)	6(1.42)	1(0.24)	0(0)	403(95.27)	20(4.72)
K3	0(0)	0(0)	0(0)	2(0.46)	3(0.69)	1(0.23)	0(0)	0(0)	3(0.89)	2(0.46)	2(0.46)	0(0)	423(97.02)	13(2.98)
K4														
Total	0(0)	0(0)	0(0)	5(0.39)	6(0.47)	2(0.16)	0(0)	1(0.08)	37(2.26)	11(0.86)	3(0.23)	0(0)	1240(96.80)	41(3.20)

numbers that they are associated with impaired fertility. The morphological abnormalities observed in the study were similar to those described by Zemjanis (1970), where the value of spermatozoa with pyriform head was found to reduce significantly ($p < 0.05$) from 6 (0.42%) to 0 (0%) after 4 weeks of supplementary feeding with boiled pumpkin.

The pyriform head abnormality of spermatozoa has been described as a primary abnormality and a range above 15% of such abnormality poses a threat to fertility. Mid piece abnormalities observed in the study reduced significantly, this could be attributed to the fact that the pumpkin plant is rich in protein and zinc, which are essential elements in the formation of the mid piece during spermatogenesis. Occurrence of the mid piece spermatozoa abnormality has been traced to the period of storage in the epididymis (Oyeyemi & Babalola, 2006). Mid piece abnormalities are secondary and had been traced to the deficiency of zinc. Zinc and folate are involved in the synthesis of DNA and RNA although the extract pathophysiology of zinc deficiency leading to clinical symptoms of decreased spermatogenesis and impaired male fertility has not been known but it has been shown to cause impaired male fertility in the form of reduced sperm motility, reduced percentage motility of sperm, morphological abnormalities and reduced spermatogenesis (Wong *et al.*, 2000). The tail abnormalities observed in the study showed no significant difference ($p < 0.05$) with the

feeding of pumpkin fruit. The increase in the values for the tail and mid piece abnormalities observed in the control experiment may not be unconnected with high ambient and scrotal temperatures. This agrees with the reports of Oyeyemi *et al.* (2000) and Larsen (1986), that spermatogenesis is impaired in species of animals with non-pendulous animal. However, there was a progressive reduction of sperm abnormalities consequent on the continued feeding of the pumpkin fruit. It was also demonstrated that such changes were sustainable since the values in the post experimental stage of the study also showed a significant difference ($p < 0.05$) with either the control or the previous experimental stage. This corresponds to the report of Bodytalkhealth that the pumpkin plant is not only rich in zinc but also contains minerals and vitamins like magnesium, iron, phosphorus, calcium, vitamin A, vitamin B complex and folic acid, all required for normal spermatogenesis. It can be concluded that the pumpkin plant (*Cucurbita pepo*) causes a significant reduction in the amount of sperm cells with primary and secondary abnormalities thereby establishing further its wealth of zinc and protein. Therefore the pumpkin plant is recommended for both the treatment and prevention of infertility in male animals. Also further work on the plant could result in the extraction its essential elements that could be of great resource in the pharmaceutical industry.

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RESUMEN: Cuatro cabras de la raza enana del oeste de África, con edades comprendidas entre los 24 y 30 meses, fueron utilizadas para este estudio. Las cabras sirvieron primero como control y, posteriormente, como animales de experimentación los que se alimentaron diariamente con aceite de planta de calabaza por un período de seis meses. El objetivo del estudio fue investigar el efecto de la planta de calabaza sobre la morfología de los espermatozoides de las cabras. Hubo diferencias significativas ($p < 0,05$) entre los valores control y experimental en las anomalías morfológicas primarias y secundarias de los espermatozoides: la cabeza tuvo un valor de control de 6 (0,42%) y post alimentación valor 0 (0%), el movimiento de la cola un valor de 14 (0,97%) y 2 (0,16%) para el control y post alimentación, respectivamente. La curvatura media: valores de 17 (1,18 %) y 1 (0,08 %) para el control y post-alimentación, respectivamente. La inclinación media también difería significativamente ($p < 0,05$), con un valor entre 16 (1,11%) el control y 3 (0,23%) post-alimentación. Hasta el final de las etapas del estudio, hubo una reducción significativa en el número de células de espermatozoa con anomalías como consecuencia de la alimentación diaria de los animales con la planta de calabaza. Por lo tanto, la planta se recomienda para la prevención y el tratamiento de la infertilidad en hombres y otros animales.

PALABRAS CLAVE: Planta de calabaza; Morfología; Infertilidad masculina; Espermatozoides.

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