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Sperm Morphological Characteristic and Mating Behaviour of Proviron® Treated West African Dwarf Bucks with Testicular Degeneration

Características Morfológicas de los Espermatozoides y Comportamiento en el Apareamiento de la Cabra Enana del Oeste Africano con Degeneración Testicular Tratadas con Proviron®

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SUMMARY: This study was conducted to determine the effect of scrotal insulation and post insulation Proviron® treatment on sperm morphological characteristics and mating behavior. Twelve healthy WAD bucks, free from any clinical or andrological disorder were used. Scrotal insulation was done using insulating bags for 30 days. After insulation, the bucks were divided into two groups. The treatment group received 100mg/head /week of Proviron® for 3 weeks. Semen collection was done using the electroejaculation method in all phases of the study. The proviron® treated bucks, when compared with insulation and post insulation untreated phases, showed significant reduction ($p<0.05$) in sperm cell abnormalities which reduced from 15.89±22.89 in post insulation untreated phase to 2.81±0.83 in post insulation treated phase. The Proviron® treated bucks also showed increased physical vigor by riding, mounting and fighting their untreated counterparts.

KEY WORDS: Morphology; Proviron®; Insulation; Testicular degeneration; Spermatozoa.

INTRODUCTION

The fully developed spermatozoon is an elongated cell consisting of a flattened head containing the nucleus, a tail containing the nucleus and a tail containing the apparatus necessary for cell motility. A neck connects the sperm head with the tail (flagellum), which is subdivided into the middle, principal and end pieces (Bardin, 1991).

About 25% or more of abnormal sperm leads to reduced fertility (Gyeongsang National University, 2005). Oyeyemi & Akusu, (1998) reported a significant difference ($p<0.05$) in the number of abnormal cells between control values and the first two weeks post vasectomy values in WAD bucks. Changes occurring in sperm morphology during migration have been correlated with the functional integrity of the testis and epididymis (Rao, 1971) and have led to the classification of sperm cell defects (Blom, 1983).

According to Blom (1983), first classification of sperm cell defects may be subdivided into three categories; Primary sperm abnormalities which are due to disturbance of spermatogenesis by congenital or hereditary factors, high ambient temperature or scrotal insulation and diseases. Examples are- twin, small and narrow heads, abaxially attached midpiece and rudimentary tail (Laing, 1979). Secondary sperm abnormalities which occur only after spermatogenesis and during epididymal journey of spermatozoa (Laing). Tertiary sperm abnormalities which arise from improper handling of semen sample.

Another classification is based on whether the abnormalities are major or minor. The third method classifies sperm abnormalities into head, midpiece or tail abnormalities.

Proviron® (Mesterolone, Schering AG, Germany) is a synthetic androgenic, anabolic steroid that constitutes a class of natural and synthetic hormones often referred to in medical texts as AAS (Anabolic/Androgenic Steroids). These steroids promote cell growth and division of several types of tissues including muscles, bones and germinal epithelium.

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The psychic effects of testosterone (main natural androgen) are difficult to define in man, but in experimental animals, androgens provoke boisterous and aggressive play (Ganong, 1997). Androgens cause a feeling of well being and an increased libido. They therefore may be responsible for sexual behavior since they contribute to aggressive behavior (Bhasin, 1993).

MATERIAL AND METHOD

Experimental animals. Twelve (12) healthy West African Dwarf (WAD) bucks were used for this study. All animals were clinically and andrologically examined for any probable congenital, acquired or anatomic defects such as cryptorchidism, testicular hypoplasia, testicular degeneration, sperm granulomas or inflammation. The age and body weight of the WAD bucks ranged from between 8 to 12 months and 6kg to 10kg. They were randomly assigned to 3 groups of 4 bucks per group.

Study Location and Management of Experimental Animals. The WAD bucks were housed in the Ward II of the Veterinary Teaching Hospital, University of Ibadan located between latitudes 07º and 20ºN and longitudes 030 and 500E with average humidity of 80%, average ambient temperature of 34ºC and total rainfall of 48''. The environment was devoid of radiation and chemical pollutants. They were kept on litters of wood shavings. They were fed on fresh succulent land cut grass and cassava peels supplemented with concentrate consisting of wheat offal, brewers grain and palm kernel cake at a rate of 0.5kh/head/day. Pens were swept clean everyday and fresh clean water served ad libitum. They were allowed to acclimate for 3 weeks during which they were dewormed with Albendazole® bolus (Phemix, Belgium) at a dosage of 2mg/10kg body weight, vaccinated against PPR and Tetanus using Peste de petite ruminantes vaccine (PPR) and Tetanus toxoid respectively two weeks after prophylactic antibiotic treatment with Long-acting Oxytet® (oxytetracycline L/A).

Scrotal Insulation. A double layered cellophane bag with 0.5mm thick cotton wool between the layers was used as the insulating material. It was wrapped around the scrotum, tied with thread and further sealed with adhesive for 30 days. Scrotal temperature was determined throughout the 30days of scrotal insulation.

Semen Collection. Semen samples were collected from all animals (12) as control in the experiment using the electro-ejaculation (EE) method on weekly basis for 3 weeks prior to insulation (pre-insulation phases). The normal baseline values of the semen characteristics of the 12 animals were obtained. The bucks served as self controls.

Semen collection resumed four days after the insulation and at weekly intervals during the 3 days of insulation phase.

After the removal of the insulation materials (1st day of post insulation phase), the remaining 8 animals were divided into two groups of 4 animals each. One group of 4 animals (experimental animals were treated with the test drug Proviron® (mesterolone) while the other group of 4 bucks served as control to the test group of 4 bucks.

Semen collection from each group resumed at weekly intervals after the test drug had been administered for 4 post-insulation. Semen characteristics were taken for each set of semen samples obtained.

The test drug was given at a dose rate of 4 tables (1 tablet = 25mg) per head per week in the test-drug group for 4 weeks post insulation.

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Cell Morphology. A drop of semen was placed on a clean, warm glass slide with two drops of Wells and Awa stain. These were gently mixed together and a smear was made on another clean warm slide and air dried. The slide was observed under light microscope at x40 objective. The number of abnormal sperm cells out of at least 600 sperm cells from several fields of the slide was taken. The number in percentages of abnormal sperm cells of total count was noted and recorded. Individual cells were examined and classified into three categories of abnormalities according to Blom (1973) – the primary, secondary and tertiary abnormalities.

RESULTS

Mating Behaviour. Normal libido was observed during the pre-insulation and insulation phases in all the WAD Bucks. The post insulation untreated bucks also exhibited similar normal degree of libido during post insulation untreated phase but Proviron® treated WAD buck showed increased physical vigor and aggressive behaviour, such as mounting, riding, butting and fighting their untreated counterparts of the post insulation untreated phase.

Morphological Changes. Sperm cell morphological characteristics were markedly affected by testicular heat during scrotal insulation but Proviron® treatment in post-insulation treated bucks greatly reversed the adverse effects of increased testicular temperature.

Sperm abnormalities included headless tails, tailless heads, rudimentary tails, looped tails, coiled tails, bent tails, curved midpiece and bent midpiece. However, prominent among the sperm abnormalities observed were bent tails (BT) curved mid-piece (CMP) and bent midpiece (BMP). The percentage of spermatozoa with BT pre insulation was 3.95 ± 2.30. This then increased significantly ($p<0.05$) to 15.89± 22.89 in post insulation untreated bucks and thereafter dropped significantly ($p<0.05$) to 2.82±0.83 in post insulation treated bucks.

The percentage of spermatozoa with Curved Midpiece (CMP) pre-insulation was 4.61±1.98. This significantly increased ($p<0.05$) to 17.39±24.91 in post insulation untreated bucks and thereafter dropped significantly to 3.2±0.87 in post insulation treated bucks. This value, although lower is however not significantly different ($p>0.05$) from the pre-insulation value.

The percentage value of Bent Midpiece (BMP) pre insulation was 3.94±1.69. This value increased significantly ($p<0.05$) to 19.75±22.63 at insulation and then decreased to 13.81±19.95 in post insulation untreated bucks although the difference is not significant ($p>0.05$). However, treatment with Proviron® post insulation significantly reduced ($p<0.05$) the percentage value of BMP to 2.41±1.00, a value lower than, but not significantly different ($p>0.05$) from the pre insulation value.

The percentage of total abnormal cells (TAC) pre insulation was 12.46±0.00. This increased significantly to 71.06±84.41 at insulation, reduced insignificantly ($p>0.05$) to 47.08±67.76 in post insulation untreated bucks and further reduced significantly ($p<0.05$) to 8.43±2.70 in post insulation treated bucks. This value is also similar to, but not significantly different ($p>0.05$) from the pre insulation values.

Percentage Total Normal Cells (TNC) reduced significantly ($p<0.05$) from 87.54±5.83 pre insulation to 28.94±84.41 at insulation. This value however increased insignificantly ($p<0.05$) to 52.92±67.76 in post insulation untreated bucks and further increased significantly ($p<0.05$) to 91.58±2.70 following Proviron® administration. This value is also similar to, but not significantly different ($p>0.05$) from the pre insulation TNC value.

DISCUSSION

All the WAD bucks displayed normal libido during scrotal insulation. This therefore implied that the heat so generated during scrotal insulation had little or no effect on the leydig cells which were the main producers of testosterone (Hafez, 1993; Ajala *et al.,* 2001) because the bucks were seen still courting females during the period. The Proviron treated bucks during post insulation showed increased physical vigor and aggressiveness in accordance with the psychic effects of androgens in experimental animals (Ganong).

The boisterous and aggressive play might increase libido and therefore be responsible for enhanced sexual behavior due to the androgenic effect of Proviron® (Bhasin). The manufacturer of Proviron®, Schering AG, claimed that some of the properties of Proviron® are: reducing or alleviating fatigue, lack of concentration, disturbances of libido and potency and overcoming depressive mood in human males. The claims by Schering AG might be true of having similar psychic effects in animals as Proviron® treated bucks displayed aggressiveness, mounting, riding, fighting and butting their control counterparts.

Morphological sperm cell defects that were prominent in this study at and post insulation were bent tails (BT) Curved midpiece (CMP) and Bent Midpiece (BMP). According to Blom (1983), these sperm cell defects could be classified as secondary sperm abnormalities which are defects which arise after spermatogenesis and during epididymal transit of spermatozoa (Laing). They could also be classified as minor sperm abnormalities which although may not be associated with infertility, may lead to subfertility. They are also comparable with secondary and tertiary abnormalities (Laing). This could be as a result of the increased epididymal temperature consequent upon scrotal insulation. At insulation, CMP had the highest value of 29.52±36.25% followed by BT (16.54±25.53%) and then BMP (19.75±22.63%). These values summed up to a total percentage of 71.06±84.41. These values exceed the percentage sperm cell defect allowed for fertility. Maximum allowed frequency of sperm head and midpiece abnormalities have also been put at 20% and 5% respectively (Laing). Figures exceeding these percentages result in reduced fertility. Maximum allowed tail abnormalities have also been put at 5% (Laing).

Following treatment, total abnormal cell values reduced to 8.43±2.70% which falls within the range of acceptable sperm cell abnormalities for optimum fertility to occur. Reduction of the percentage sperm abnormality post insulation could be due to return of testicular and epididymal temperature to normal after the removal of the insulation materials.

Consequently, scrotal thermoregulatory mechanism could function properly and the testicular temperature could be maintained below that of the body. Elevated body temperatures during periods of high ambient temperature or pyrexia from disease lead to testicular degeneration and reduce percentage of normal and fertile spermatozoa in the ejaculate (Hafez). Testicular regeneration following degeneration is a protracted process. In a study, Wildeus & Entwistle (1983), scrotal insulation for 48 hours raised subcutaneous scrotal temperature by 40C in hybrid Bos indicus x Bos taurus bulls, normal tail without head (headless) spermatozoa in the ejaculate increased significantly between 6 and 14 days, protoplasmic droplets and tail abnormalities between 20 and 23 days and spermatozoa with lost and damaged acrosomes increased significantly 12-23 days after insulation. At slaughter after 23 days, sperm production rates and gonadal reserves were markedly reduced particularly in the caudal epididymis.

In this study, treatment however with Proviron® post insulation cause a rapid regeneration of the testicles, return of spermatogenesis to almost normal, increased number of sperm cells and reduced number of sperm abnormalities. These positive changes could be due to the combined androgenic and anabolic properties of Proviron® by reversing testicular degeneration and also its androgenic activity without inhibiting gonadotropin secretion.

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RESUMEN: Este estudio se realizó para determinar el efecto de aislamiento escrotal y aislamiento posterior al tratamiento con Proviron® sobre las características morfológicas de los espermatozoides y el comportamiento del apareamiento. Fueron utilizadas 12 cabras enanas del Oeste Africano, sanas, libres de cualquier desorden clínico andrológico. El aislamiento escrotal se realizó utilizando bolsas de aislamiento durante 30 días. Después de aislamiento, los machos se dividieron en dos grupos. El grupo de tratamiento recibió 100mg/cada una por semana de Proviron® durante 3 semanas. La recolección de semen se realizó mediante el método de electroeyaculación en todas las fases del estudio. Las cabras tratadas con Proviron®, cuando se compararon con las fases post-aislamiento no tratadas, mostraron una reducción significativa ($p<0,05$) en las anormalidades de células espermáticas, las cuales se redujeron desde 15,89±22,89 en las fases post-aislamiento sin tratamiento a 2,81±0,83 en las fases post-aislamiento tratadas. Las cabras tratadas con Proviron® también mostraron un incremento en el vigor físico, la monta y la lucha contra sus homólogos no tratados.

PALABRAS CLAVE: Morfología; Proviron®; Aislamiento; Degeneración testicular; Espermatozoide.

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