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Effect of Clomiphene Citrate (Clomid®) Fertility Drug on Sperm Production Rate Gonadal and Extragonadal Sperm Reserves of Nigerian Yankasa Rams

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Abstract: Three groups of 6 healthy mature Yankasa rams, aged 1.5-2.5 years weighing between 30 and 50 kg were assigned to either 12.25 mg (CDI) or 24.5 mg (CD2) clomiphene citrate as Clomid® (Bruno Pharmaceutici S p A, Rome) daily for 5 days. Another group of 6 rams was given normal saline during the same period to serve as control (CON). All treatments were given to study the effect of the drug on daily sperm production, gonadal and extragonadal sperm reserves. All the solutions were given as oral douches. The results indicate daily sperm production (10^9) for the control (CON) 2.15±0.64 differed significantly ($p<0.05$) from (CDI) 5.16±0.12 and (CD 2) 4.85±0.16. Daily sperm production/gram/testis (10^9) were similar between treatments. Gonadal sperm reserves (10^9) for (CD1) 17.45±1.64 differed significantly ($p<0.05$) from (CD2) 14.25±1.45 and (CON) 12.15±1.15. Mean caput sperm reserves (10^8) for (CD1) 4.10±0.06 differed significantly ($p<0.05$) from (CD2) 3.64±0.04 and (CON) 2.25±0.03. Mean corpus sperm reserve (10^8) for (CD1) 5.10±0.08 differed significantly ($p<0.05$) from (CD2) 4.65±0.05 and (CON) 3.25±0.06. Mean caudal sperm reserve (10^8) for (CD1) 6.25±0.54 was similar ($p>0.05$) to (CD2) 5.48±0.62 but they differed significantly ($p<0.05$) from (CON) 4.15±0.21. Ductus deferens sperm reserve was similar ($p>0.05$) between treatment groups. High correlations were found between testis weight and daily sperm production and testicular sperm reserves. The gonadal and extragonadal reserves and daily sperm production values obtained in this study are within the range reported for exotic breeds.

Key words: Nigerian yankasa ram, sperm reserve, sperm production, clomiphene citrate (clomid®)

INTRODUCTION

Yankasa is the predominant breed of sheep indigenous to the Guinea and Sudan Savannah belt of West Africa (Iheukwumere and Okere, 1990). According to Mgbeoji and Orji (1984), the use of Yankasa rams to upgrade the smaller village sheep in the habitat has extended this breed to southeastern Nigeria. The Nigerian Yankasa rams are typically tall, exceeding a height of 50-70 cm at withers and weighs 30-50 kg with an outstanding sexual agility, hence they have been widely used for artificial insemination programmes (Osinowo *et al*., 1992). Several aspects of the physiology of reproduction of rams have been documented (Iheukwumere *et al*., 2001; Osinowo, 1990; Ahemen and Bitto, 2007). Measurable criteria such as Scrotal dimensions, Sperm production rate, gonadal and extragonadal sperm reserve have been extensively studied in some Nigerian breeds (Osinowo *et al*., 1992; Kwari and Waziri, 2001; Ahemen and Bitto, 2007). Few of such reports are however, available in the Yankasa rams, the breed that is abundant in Nigeria and resistant to some local diseases (Iheukwumere and Okere, 1990). It has been observed that the reproductive capacity of Yankasa rams is low (Osinowo, 2006) when compared with the exotic breed of rams.

There is cause to stimulate sperm production using inexpensive preparations with an aim to ensuring high conception rates in both naturally mated and artificially inseminated ewes.

Clomiphene citrate is a mixture of the Cis and trans isomers of 1-Chloro-2[4-(2-diethylamino ethoxy) phenyl] 1-1, 2-diphenyl ethylene citrate. The drug is a human fertility drug marketed under the trade name Clomid® (Bruno Farmaceutici SpA, Rome) or the generic name Clomiphene citrate (Medical and Chemical Agency Srl, Milan). The drug is soluble in water at 20°C. The drug has been mentioned for its possibility in the stimulation of spermatogenesis (British Pharmaceutical Society, 1973).

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Herbert *et al.* (2002) investigated the use of this drug in induction of spermatogenesis in male rabbit bucks. Clomiphene citrate acts in males and females by stimulating the production of pituitary gonadotrophins (British Pharmaceutical Society, 1973), possibly through the stimulation of the secretion of gonadotrophin releasing hormone (GnRH) by the hypothalamus. Herbert *et al.* (2000) observed that the 2 variants of the drug induced superovulation in West African dwarf ewes.

There is paucity of information on the use of clomiphene citrate in the induction of spermatogenesis in the Nigerian Yankasa rams: This study was therefore designed to investigate the effect of this fertility drug on sperm production rate, gonadal and extragonadal sperm reserves of Nigerian Yankasa rams. The information is essential in the determination of male/female ratio during natural mating and artificial insemination programmes (Ahemen and Bitto, 2007) and also in evaluating male reproductive efficiency of a breed.

MATERIALS AND METHODS

Management of animals: Fifteen healthy sexually mature Nigerian Yankasa rams weighing between 30 and 50 kg and aged 1.5-2.5 years were used for this study. The animals were purchased from the local markets and housed individually in pens used for this study. The animals were dewormed and fed *ad libitum* with a mixture of hay and silage. A concentrate ration of maize and palm kernel cake was fed to the rams. The rams were daily allowed to browse and exercise in fenced paddocks planted with pasture consisting of giant star grass (*Cynadom nlemfluensis*) and elephant grass (*Penniset um purpereum*), Fresh drinking water and salt lick as mineral supplement were provided.

Experimental design and drug administration: The fifteen rams were divided into 3 treatment groups consisting of 5 rams per each group. These groups were assigned to 3 experimental treatments as follows: Control (CON), Single dose of clomid® (CD1) containing 12.25 mg of active Clomiphene citrate, double dose of Clomid® (CD2) containing 24.5 mg of active clomiphene citrate. One half of a 50 mg tablet of the drug was dissolved in 3 mL of pre-warmed physiological solution at 20°C. Each ram in the CD1 group received 2 mL of reconstituted drug by oral douching every day for 5 days, while those in the CD2 group received 4 mL of the drug for the same period, by the same route.

Semen collection and evaluation: Gonadal and extragonadal sperm reserves were estimated following the homogenized count using a haemocytometer (Bitto and Egbunike, 2006). Before the weighing of the testes and the 3 parts of the epididymis (caput, corpus and candal) the connective tissue that adhered to each part was separated. Parenchyma (1 g) of each testis was sectioned and homogenized in 10 mL of normal saline. One gram of caput, corpus, caudal epididymis and ductus deferens were also minced separately in 10 mL of normal saline with a pair of scissors for 5 min. The testicular and epididymal homogenates were then filtered through 2 layers of loosely netted bandage.

The spermatozoa and clongated spermatide numbers in the testicular and epididymal samples were determined using an improved Neubauer Chamber; 2 counts per each sample was performed and the mean used in the analysis to obtain the sperm reserves.

Daily Sperm Production (DSP) was estimated for testicular homogenates by dividing the gonadal sperm reserves by a time divisor of 3.66 corresponding to the time in days of the duration of the seminiferous epithelium cycle (Bitto and Egbunike, 2006). Daily sperm production per gram testis (DSPG) was determined by dividing the DSP by the weight of testicular parenchyma (Bitto and Egbunike, 2006).

Data analysis: All the data collected from this study were analyzed using the analysis of variance (Steel and Torrie, 1980). Means where significant were separated by the Duncan's New Multiple Range Test as described by Obi (1990).

RESULTS AND DISCUSSION

The results of scrotal circumference, weight of the testis, epididymis, epididymal segments and ductus deferens are shown in Table 1. Mean values of scrotal circumference weights of the testes and epididymis obtained in this study are higher than values reported for other Nigerian breeds of similar ages by Ahemen and Bitto (2007) in West African dwarf rams, Kwari and Waziuri (2001) in Balami rams. This could be attributed to the size of the Yankasa rams. The Yankasa ram had been

Table 1: Scrotal circumference, testicular, epididymal and ductus deferens weight in mature Yankasa rams

Parameters	Mean±SEM
Scrotal circumference (cm)	22.40±0.54
Paired testes weight (g)	154.25±2.48
Paired epididymal weight (g)	20.14±0.34
Weight of epididymal segments	
Caput (g)	8.54±0.21
Corpus (g)	4.21±0.32
Caudal (g)	8.00±0.07
Ductus deferens weight (g)	2.35±0.16

Table 2: Sperm production rate, gonadal and extra gonadal sperm reserves in Yankasa rams treated with Clomiphene citrate (Clomid®)

	Treatments		
Variables	CON	CD1	CD2
Daily sperm production ($\times 10^9$)	2.15±0.04^a	5.16±0.12^a	4.85±0.16^a
Daily sperm production/gram/testis (10^9)	0.64±0.01	0.98±0.05	0.86±0.04
Gonadal sperm reserve ($\times 10^9$)	12.15±1.5^c	17.45±1.64^a	14.25±1.45^b
Caput sperm reserves ($\times 10^8$)	2.25±0.03^c	4.10±0.06^a	3.64±0.04^b
Corpus sperm reserves ($\times 10^8$)	3.25±0.06^c	5.10±0.08^a	4.65±0.05^b
Caudal sperm reserves ($\times 10^8$)	4.15±0.21^b	6.25±0.54^a	5.48±0.63^a
Ductus deferense sperm reserves ($\times 10^8$)	0.45±0.02	0.65±0.04	0.62±0.01
Relative epididymal sperm distribution (%)			
Caput 27.85			
Corpus 13.80			
Caudal 58.35			

abc: Means within row with different superscripts are significantly different ($p<0.05$); CON = Control; CD1 = Single dose of clomiphene citrate (Clomid®); CD2 = Double dose of clomiphene citrate (Clomid®)

Table 3: Correlations between testicular morphometry, sperm production rate, gonadal and extragonadal sperm reserves in Yankasa rams

	Daily sperm prod.Per gram testis	Daily sperm production	Cauda sperm reserve	Corpus sperm reserve	Caput sperm reserve	Testis sperm reserve	Cauda weight	Corpus weight	Caput weight	Testicular weight
Testis wt	-0.02	0.83^{xx}	0.11	0.65^x	0.83^{xx}	0.82^{xx}	0.74^{xx}	0.91^{xx}	0.83^x	
Caput weight	-0.28	0.56^x	-0.32	0.31	0.67^x	0.55^x	0.25	0.88	-	
Corpus weight	-0.17	-0.64^{xx}	-0.23	-0.55^x	0.56^x	-0.65^x	-0.56	-		
Cauda weight	0.06	0.56^x	0.57^x	0.87^{xx}	0.46^x	0.54^x				
Testicular sperm reserves	0.57	0.11	0.05	0.21	0.85^{xx}	-				
Caput sperm reserve	0.21	0.86^{xx}	0.22	0.31	-					
Corpus sperm reserve	0.45	0.22	0.65^x	-						
Cauda sperm reserve	-0.22	0.05								
Daily sperm production	0.58^x									
Daily sperm prod. Per gram testis										

x Significant ($p<0.05$); xx Significant ($p<0.01$)

described as the largest in size of all indigenous breeds of sheep in Nigeria (Iheukwumere *et al*., 2001; Osinowo *et al*., 1992).

The results of Yankasa rams treated with varying doses of Clomiphene Citrate (Clomid®) on sperm production rate, gonadal and extragonadal sperm reserves are shown in Table 2. The daily sperm production of rams treated with 12.25 mg single dose of Clomid® CD1, 5.16±0.12 ($\times 10^9$) and double dose 24.5 mg Clomid® CD2, 4.85±0.126 ($\times 10^9$) were similar ($p>0.05$), however, they differed significantly ($p<0.05$) different from the control regroup CON 2.15±0.04 ($\times 10^9$) in daily sperm production. The higher numerical value obtained in the CD1 treatment group falls within the range 5.29×10^9 reported by Martinez *et al.* (1994) in the temperate breeds.

The values in the gonadal and extragonadal sperm reserves were also higher than 2.15±0.05 for gonadal and 1.79±0.05 for extragonadal sperm reserves reported by Ahemen and Bitto (2007) in West African dwarf rams and also higher in extragonadal sperm reserves obtained by Kwari and Waziri (2001) in Balami rams. The observed differences in this study could be attributed to genotype, testicular size, technique of estimation (Ahemen and Bitto, 2007) and drug administration (Herbert *et al*., 2002).

The sperm reserves of the caput in rams treated with 12.25 mg single dose Clomid® 4.10±0.06 ($\times 10^8$) differed significantly ($p<0.05$) from rams treated with 24.5 mg double dose of Clomid® 3.64±0.04± ($\times 10^8$) and the control group 2.25±0.03 ($\times 10^8$). However, the double dose of 24.5 mg clomid® differed significantly ($p<0.05$) from the control group. The corpus sperm reserves followed the same pattern as the caput sperm reserves. The caudal sperm reserves in rams treated with 12.25 mg single dose Clomid® 6.25±0.54 ($\times 10^8$) and rams treated with 24.5 mg Clomid® 5.48±0.63 ($\times 10^8$) double dose of Clomid® were similar (_ 0.05); but they differed significantly ($p<0.05$) from the control group 4.15±0.21 ($\times 10^8$).

The sperm reserves of the ductus deferens were similar ($p>0.05$) between the treatment groups. The sperm reserves of the caput epididymis represented 27.85% of the total reserve of the organ, while the corpus and caudal accounted for 13.80 and 58.35%, respectively. The distribution of epididymal sperm reserves observed in this study is similar to what has been reported for other breeds (Kwari and Waziri, 2001; Osinowo, 2006, Ahemen and Bitto, 2007). It is generally agreed that the caudal epididymis contain most of the epididymal sperm reserves and hence, it is the major site of sperm storage (Kwari and Waziri, 2001).

In this study, it was observed that Clomiphene citrate induced spermatogenesis in the treated groups. The mechanism for this action is partly described. It is

common knowledge that LH as intersticial cell stimulating hormone (ICSH) stimulates the intersticial cell of leydig to produce testosterone which facilitates the process of spermatogenesis (Herbert *et al*., 2002). However, in a similar study, Herbert *et al*. (2002) had indicated differences in the serum testosterone levels showed slightly higher values for the Clomid® treated group (CD) than the CON (control) group but were not significant-ly different ($p>0.05$). This implies that it may not be through increased production of testosterone under the influence of ICSH alone that may be responsible for improved sperm production rates in treated animal (Herbert *et al*., 2002). Herbert *et al*. (2002) also reported that FSH mediates in the maturation of sperm cells prior to ejaculation. It has also been reported that exogenous administration of testosterone itself leads to a negative feedback on the hypothalamus thus reducing the sperm production process (Adamopoulous *et al*., 1990; Jeyakumar *et al*., 1997). The observation in this study, that the gonadal and extragonadal sperm reserves in the CD1 group was higher ($p<0.05$) than in the CD2 group which received higher dose of the drug suggests that a high dose rate of the drug such as 24.5 mg/ram/day for 5 days given in this study could excite suppressive effect on the hypo-thalamus. A negative feedback may have been established that worked against further increases in testosterone levels and in turn slowed down the process of spermatogenesis. Similar results have been given by Li-Jian *et al*. (1997) and Mungail *et al*. (1997). Table 3 shows correlation betweens testicular morphometry, sperm production rate, gonadal and extragonadal sperm reserves. High correlations were observed between testicular weight and testicular sperm reserves ($r = 82$, $p<0.05$), Corpus weight and corpus sperm reserves ($r = 67$, $p<0.05$), Cauda weight and cauda sperm reserves ($r = 57$, $p<0.05$). These high and relative correlations observed are suggestive of high spermatozoa reserves per unit mass of the testis and epididymal segments.

CONCLUSION

The values obtained for gonadal and extragonadal sperm reserves fall within the range reported in the literature (Osinowo, 2006; Kwari and Waziri, 2001). Hence the administration of 12.25 mg of clomid would improve sperm production in the rams without any deleterious effects on the gonadal and extragonadal sperm reserves in Nigerian Yankasa rams.

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