AENSI Journals

Advances in Environmental Biology

ISSN-1995-0756 EISSN-1998-1066

Journal home page: http://www.aensiweb.com/AEB/

Sperm Morphological Characteristics of Clomiphene Citrate Treated West African Dwarf Bucks with Testicular Degeneration

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ARTICLE INFO

Article history:
Received 15 June 2014
Received in revised form
8 July 2014
Accepted 4 September 2014
Available online 20 September 2014

Keywords:
Spermiogram, Testicular degeneration, Clomiphene citrate, Bucks.

ABSTRACT

Twelve (12) sexually matured bucks were used for this study weighing between 9 to 12 kg 2and fed compounded feed supplemented with dried cassava peels and freshly cut grass daily. 3Water was given ad libitum. They were stabilized and scrotal insulation was done as recommended. The insulating 5materials were subsequently removed and the animals were randomly divided into 3 groups 6of four (4) each. Group 1 served as the control and did not receive any treatment. Group 2 7and 3 were treated orally with Clomiphene citrate 50mg daily for 5 days and 10 days 8respectively. Semen samples were collected via electroejaculation method. The results showed that scrotal insulation results in testicular degeneration and there was a 10significant decrease ($P \leq 0.05$) in the spermiogram parameters at the pre insulation phase and 11day 1-post insulation. However, a progressive increase in all the parameters (except the 12volume) was observed from day 5 after the beginning of treatment to day 23. There was a 13significant difference ($P \leq 0.05$) in the percentage sperm abnormalities. From the study above, Clomiphene citrate, a drug indicated for follicle stimulation in 15females has shown significant morphological regeneration in sperm cells thus can be 16encouraged for use in male animals.

To Cite This Article: Oluwaseun Olanrewaju ESAN., Olasumbo Oluwayemisi ESAN., Matthew Olugbenga OYEYEMI., Sperm Morphological Characteristics of Clomiphene Citrate Treated West African Dwarf Bucks with Testicular Degeneration. ***Adv. Environ. Biol., 8(12),*** *594-598, 2014*

INTRODUCTION

The West African Dwarf (WAD) goat is widely distributed across the rainforest belt of southern Nigeria where it makes significant contributions to the livelihoods of impoverished families. The potentials of WAD goats in poverty alleviation programme is well-recognized, but is still largely untapped. This breed is known to display a wide range of qualitative variations in coat colour (black, brown, white, pied, mottled, mixed, etc; [1, 2],

There are still a lot of challenges impeding maximum output by the breed. Such challenges include various diseases and infertility. Infertility is responsible for a large percentage of farm animals culled annually; including goats [3]. The causes of infertility include anatomical and genetic defects, physiological, pathological, and management factors. Infectious causes of buck infertility are rare, but significant problems do result from genetically determined intersexuality, testicular hypoplasia, cryptorchidism, sperm granulomas and testicular degeneration [4].

Testicular degeneration involves progressively retrogressive changes in the germinal epithelium of the seminiferous tubules. Great variations in severity are seen from cases showing mild defects of spermatogenesis to those showing gross testicular atrophy in which only the spermatogonia and Sertoli cells remain [5]. The condition can be unilateral or bilateral depending on whether the cause is local or systemic [6].

Causes of testicular degeneration include thermoregulatory dysfunction of the scrotum. Other causes include: local infections, pyrexia and inflammatory disease of the either or both testicles or scrotum. Systemic infections involving bacteria and blood protozoan parasites that may cause pyrexia also result in testicular degeneration [5].

Presence of 25% or more of abnormal sperm leads to reduced fertility [7]. Bishop *et al*., [8] observed that any semen sample with effects exceeding 20% will result in reduced fertility. Changes occurring in sperm

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morphology during migration have been correlated with the functional integrity of the testis and epididymis [9] and has led to the classification of sperm cell defects [10].
Sperm abnormalities can be classified into three categories;

Primary abnormalities which are due to disturbance of spermatogenesis by congenital or hereditary factors, high ambient temperature or scrotal insulation and diseases [11, 12].

Secondary abnormalities includes abnormalities which only occur after spermatogenesis and during epididymal journey of spermatozoa [11, 12]. Tertiary abnormalities arise from improper handling semen sample involving water toxicity of stain, cold/heat shock, unfavorable PH [12]. Other classifications according to Blom [13] are major or minor sperm abnormalities.

The use of anabolic steroid Proviron® has been found to reverse the effect of testicular degeneration in WAD bucks [14], however, this study is aimed at discovering other non-steroidal means of reversing testicular generation in WAD bucks.

CLOMID (clomiphene citrate tablets USP) is an orally administered, nonsteroidal, ovulatory stimulant designated chemically as 2-[p-(2-chloro-1,2-diphenylvinyl) phenoxy] triethylamine citrate (1:1). It has the molecular formula of $C_{26}H_{28}C1NO \bullet C_6H_8O_7$ and a molecular weight of 598.09.

It is capable of interacting with estrogen-receptor-containing tissues, including the hypothalamus, pituitary, ovary, endometrium, vagina, and cervix. It may compete with estrogen for estrogen-receptor-binding sites and may delay replenishment of intracellular estrogen receptors. Clomiphene citrate initiates a series of endocrine events culminating in a preovulatory gonadotropin surge and subsequent follicular rupture. The first endocrine event in response to a course of clomiphene therapy is an increase in the release of pituitary gonadotropins. This initiates steroidogenesis and folliculogenesis, resulting in growth of the ovarian follicle and an increase in the circulating level of estradiol. It is used mainly in the female but an extra-label use has been described in the male due to its ability to induce steroidogenesis.

MATERIALS AND METHOD

2.1 Experimental animals:

Twelve healthy West African Dwarf (WAD) bucks were purchased in Ibadan. All the animals were clinically examined to be sure they are free from obvious reproductive problems. Breeding soundness examination including libido test was conducted for any probable congenital or acquired anatomical defects such as cryptochidism, testicular hypoplasia, adhesions or degeneration. Age and weight of the WAD bucks varied between 12 to 18 months and 9 to 12kg respectively.

The animals were housed in experimental unit of the Veterinary Teaching Hospital, University of Ibadan, Relative humidity and average temperatures were 80% and 34°C respectively. The bedding was made from wood shavings and they were fed on feed concentrate supplemented with freshly cut elephant grass and dried cassava peels daily. Water was also served *ad-libitum*. The animals were allowed to acclimatise for two weeks during which they were dewormed with ivermectin, at a dosage of 0.02mg per kg body weight and treated prophylactically with oxtetracycline (20%) and vaccinated with pestes de petit ruminantae (PPR).

2.2 Scrotal insulation:

A double layered black cellophane bag with 0.5mm thick cotton wool between the layers was used as the insulating material. It was wrapped around the testes and further sealed with adhesive for three weeks.

2.3 Post insulation and Treatment phase:

Insulation material was removed. Animals were randomly divided into 3 groups of 4 animals each.

Group A did not receive any treatment; group B were treated orally with Clomiphene citrate 50mg daily for 5 days and group C were treated with the same dose for 10days. Treatment commence immediately after removal of insulating material. Semen sample was collected and analysed from the 3 groups at day 1, 5, 19, 23 and 65 after the beginning of treatment. Blood sample was also collected for haemogram at these periods.

2.4 Semen collection:

Ejaculate was collected via electro-ejaculation method and was kept warm at temperatures between 35ºC and 38ºC. Evaluations of the semen sample were done by the method described by [15, 16]. Data were subjected to general linear models procedure, Duncan's multiple test and the student t-test were used to analysis the work using a statistical tool [17]. The levels of significant differences were taken to be at the 95% confidence interval ($P \leq 0.05$).

3.0 Results:

The libido of all the animals was normal at every stage of the experiment. It was unchanged during the scrotal insulation phase, insulation phase and even the post insulation phase. Following removal of the

insulating material, the testes were swollen, softer and flabby in consistency. There was extensive alopecia of the scrotum and also marked increase in the penduloucity of the scrotum.

The highest scrotal temperature recorded for the insulating period was 40ºC while the highest rectal temperature recorded was 38.7ºC. The colour of the semen changed from creamy/milky to milky/opalescent immediately after removal of insulation material and then changed back to milky/creamy as the days progressed but more quickly in the group treated for 5 day.

3.1 Spermiogram results:

Table 1: Comparison between pre insulation and Day 1 after removal of insulation.

Treatment	Mass Activity	Motility %	Live/Dead ratio %	Volume (ml)	Count ($x10^6$sperm/ml)
Pre insulation	1.95±0.27	85.45±2.38	96.00±0.94	0.24±0.05	256.77±9.54
Day 1	0.50±0.22	57.00±3.67	78.00±3.96	0.19±0.03	133.40±6.45
LSD $_{(0.05)}$	0.75	8.98	8.15	0.13	24.6
	*	*	*	Ns	*

LSD $_{(0.05)}$: Least significant difference at P=0.05, *: Significant, ns: not significant
Values are reported as Mean ± Standard Deviation

There is a decrease with significant difference (P≤0.05) in the mass activity at the pre insulation period 1.95±0.27 and day 1 0.50±0.22 after the removal of the insulating material. This is also the same for the percentage motility, live/dead ratio and the cell count. This confirms that testicular degeneration has taken place.

Table 2: Group treated for 5days.

	Mass Activity (5days)	Mass Activity(10 days)	Mass Activity (control)	motility % (5 days)	motility %(10 days)	Motility %(control)	Live/Dead ratio %(5 days)	Live/Dead ratio%(10 days)	Live/Dead ratio %(control)	Volume (ml)(5 days)	Volume (ml)(10 days)	Volume (ml)(control)	Count($x10^6$sperm/ml)(5 days)	Count ($x10^6$sperm/ml)(10 days)	Count ($x10^6$sperm/ml)
Day 5	0.00		0.33± 0.33	40.00± 11.55		56.67± 8.82	65.00± 9.57		65.00± 16.07	0.23±0.05		0.17± 0.03	105.75 ±3.71		107.00±5.86
Day 19	0.75± 0.25	1.33± 0.33	1.50± 0.50	55.00± 6.45	66.67± 6.67	75.00± 5.00	75.00± 6.12	83.33± 1.67	92.50± 2.50	0.15±0.05	0.17± 0.07	0.25± 0.05	165.00 ±22.28	143.00±10.97	144.00±10.00
Day 23	2.25± 0.48	1.00± 0.00	1.50± 1.50	83.75± 5.54	66.67± 3.33	70.00± 10.00	90.75± 3.94	85.00± 2.89	80.00± 10.00	0.15±0.03	0.17± 0.07	0.15± 0.05	174.75 ±16.18	146.33±13.09	155.00±13.00
Day 65	1.75± 0.75	2.00± 1.00	2.00± 0.71	75.00± 6.45	66.67± 23.33	82.50± 4.79	98.00± 0.00	86.67± 8.33	94.50± 1.66	0.08±0.01	0.18± 0.07	0.33± 0.05	162.75 ±6.52	143.33±24.94	142.00±16.09
LSD $_{(0.05)}$	1.47	1.72	2.26	23.25	40.03	21.48	17.48	14.64	28.11	0.12	0.24	0.18	39.33	56.41	2.61
	*	Ns	ns	*	ns	ns	*	Ns	ns	ns	ns	ns	*	ns	ns

LSD $_{(0.05)}$: Least significant difference at P=0.05, *: Significant, ns: not significant
Values are reported as Mean ± Standard Deviation

In the group treated for 5days (Table 3), at day 5 after the beginning of treatment, the mass activity had dropped to 0.00 despite having administered Clomiphene citrate for 5days. Percentage motility, live/dead ratio as well as the count still remained low. This signifies that action of the use of Clomiphene citrate is not seen immediately.

However, a progressive significant increase (P≤0.05) in all the parameters (except the volume) was observed from day 5 after the beginning of treatment to day 23. Day 65 showed otherwise except for the live dead/ratio.

In the group treated for 10 days (Table 3), although there was progressive increase (P≥0.05) in all parameters (except the volume) from day 19 after the beginning of treatment to day 23, it was not significant (P≥0.05)

In the untreated group (Table 3), although there were improvements in all the parameters (except the volume), the changes were not significant (P≥0.05).

4.0 Discussion:

4.1 Libido:

Scrotal insulation resulted in increased testicular temperature with alopecia of the scrotal skin and flabby testes. This is similar to the report of 12, 18.

All the WAD bucks displayed normal libido during scrotal insulation. This may indicate that the heat so generated during scrotal insulation had little or no effect on the Leydig cells which are the main source of testosterone [18, 19]. The testosterone from source other than the testes (adrenal glands) was enough to maintain the libido of the animals. Clomiphene citrate treated bucks, either for 5 or 10 days did not show any increased libido or aggressiveness. This may be due to the fact that clomiphene citrate has no androgenic activity. This is unlike the report by [12] which indicated that there was increased aggressiveness, vigour and libido in bucks with testicular degeneration treated with Proviron®. This disparity in result could be due to the fact that

Proviron® steroidal activity while Clomiphene citrate has non-steroidal activity and does not cause immunosuppression as compared to Proviron®.

4.2 Semen characteristics:

The highest level of abnormal sperm characteristics and morphology was recorded at day 5 after the beginning of treatment throughout the experiment. This shows the effect of clomiphene citrate is not immediate. Effect is usually seen after about 5days after the end of a 5-day treatment.

This study showed that clomiphene citrate, when used for 5 days in WAD bucks with testicular degeneration, causes a slow but progressive reversal of the effects of testicular degeneration on the sperm characteristics as shown in Table 1. This is contrary to the report by [12] which indicated that Proviron® causes a rapid reversal of the effects of testicular degeneration on the spermiogram. However, there was no significant reversal observed in the sperm morphological characteristics as shown in table 10. In bucks treated for 10 days, Clomiphene citrate did not cause any reversal in the sperm characteristics as shown in Table 2, but there was an improvement in the sperm morphology especially in the headless tail, curved mid piece and bent midpiece.

At 65 days after the beginning of treatment, in the group treated for 5 days, the spermiogram showed that the mass activity, motility and live/dead ratio did not return to the exact pre insulation values. However, the difference was not significant ($P \geq 0.05$). This showed that the treatment with clomiphene citrate for 5 days reversed the effect of heat induced testicular degeneration. The difference in the sperm count at the pre insulation phase and 65 days after the beginning of treatment was significant ($P \leq 0.05$). The treatment did not improve the sperm count.

For the group treated for 10 days and the untreated group, though the sperm count improved with significant difference ($P \leq 0.05$).

The percentage sperm morphological characteristics for the group treated for 5 days increased for some parameters and decreased for some. These changes however were not significant ($P \geq 0.05$). There was however a reduction in the percentage morphological sperm abnormalities when compared with day 1 of the treatment.

For the group treated for 10 days, there is an increase in the total percentage abnormality. The increase is significant ($P \leq 0.05$) for Bent tail, Curved tail, Curved mid piece and Bent mid piece. This implies that treatment with Clomiphene citrate for 10 days resulted in an increase in the percentage abnormalities. This will definitely reduce the fertility potential of the semen.

For the untreated (control) group the there was no significant change ($P \geq 0.05$) in all the parameters. This implies that WAD bucks with heat induced testicular degeneration, if left untreated will gradually recover, however, there is a point to which the degeneration gets to in which it becomes irreversible.

5.0 Conclusion:

The blood parameters remained significantly unchanged through out all the phases of the experiment. Hence testicular degeneration or its treatment with Clomiphene citrate does not alter the blood parameters of WAD bucks.

From this study, it can be concluded that scrotal insulation results in testicular degeneration and when testicular degeneration occurs, it takes a longer time for it to resolve than the duration of time of exposure to the cause. This mimicks a study by [20] in which severe scab formation on the scrotum of bull infected with dermatophilosis caused insulation severe enough to adversely affect the semen characteristics of such bulls.

Treatment with Clomiphene citrate for 5 days resulted in a progressive but gradual recovery of the condition while treatment with Clomiphene citrate for 10 days did not have any advantage over treatment with 5 days. It actually increased the percentage sperm morphology abnormalities.

At the end of the experiment, there was no significant difference ($P \geq 0.05$) in the spermiogram of the group treated for 5 days, the group treated for 10 days and the untreated (control) group.

When testicular degeneration is left untreated, it gradually resolves on its own if the source of the insult is removed and the degeneration was not severe enough to destroy spermatogonia A of the germinal epithelium.

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