

*Short Communication*

## **Intestinal Ischaemia-Reperfusion Injury and Semen Characteristics in West African Dwarf Bucks**

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**Summary:** Increasing production of goats takes their reproductive potential and fertility, into consideration. Gastrointestinal obstructive lesions can set up an intestinal ischaemia-reperfusion. Testicular torsion is an established cause of testicular damage and infertility and is a form of ischaemia-reperfusion injury. This study investigates the effect of intestinal ischaemia-reperfusion (IIR) injury on semen characteristics in WAD bucks. Six healthy adult male WAD goats were divided into two groups of three, a control and IIR group, one hour ischaemia and two hours of reperfusion were achieved in the intestinal ischaemia-reperfusion (IIR) group after all goats underwent a laparotomy. Semen collection was done using the electro-ejaculator method pre-operatively and weekly for four weeks post-operatively. The semen concentration, percentage of normal sperm cells, abnormal sperm cells and percentage abnormality were evaluated. In control animals, there was an increase in semen concentration postoperatively followed by a decrease whereas in IIR animals, a decrease was observed postoperatively till the 4th week. Total normal sperm cells decreased postoperatively and then increased to preoperative levels whereas a decrease was seen in IIR animals till the 3rd postoperative week. Abnormalities in sperm cells, normal head without tail, normal tail without head, bent mid-piece, curved mid-piece and rudimentary tail were all increased by the 4th week in IIR group though the total number of abnormal cells was observed to have decreased. The main effect of intestinal ischaemic-reperfusion injury on the semen characteristics of WAD goats is an increase in abnormalities with an adequate quantity of semen. Many of the abnormalities involved midpiece and tail abnormalities which are very vital to propulsion and may cause an inability of the sperm cells to fertilize. This hitherto silent phenomenon in farm animals may be the reason for iatrogenic causes of infertility.

**Keywords:** Intestinal Ischaemia-Reperfusion injury; semen characteristics; West African Dwarf bucks.

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### **INTRODUCTION**

The small ruminant industry in Nigeria represents a very important national resource contributing considerably to the national wealth, supply of protein, raw materials for industries and for festive and religious occasions (Remi-Adewumi et al., 2004). In Nigeria, goats are the most numerous of all types of livestock numbering about 27.6 million (Federal Office of Statistics, 1986). The animals are primarily for meat production (Hassan and Ciroma, 1992). Research attempts at improvement of reproductive potential of these small ruminants have made assessments of semen and semen characteristics necessary. 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 and Ubiogoro, 2005).

Increasing incidence of gastro-intestinal emergencies in sheep and goat are due to difficulties which include methods of husbandry, increased pressure of land utilization in urban areas, lack of enforcement of legislation on land use, the increasing

use and poor waste disposal of nylon bags used for food storage (Ghurashi *et al.*, 2009). Bowel obstructions, especially strangulating types are medical and surgical emergencies with grave consequences due to hypovolemia and septic shock secondary to devitalization of the intestinal wall (Eyarefe *et al.*, 2011). In Nigeria, intestinal conditions rank 8th of 11 categorized surgical conditions and made up 7% of surgical cases presented to Veterinary Teaching Hospitals and clinics most of which were strangulating obstructive conditions such as intussusception, volvulus and torsion which were recorded for large and small animals. Many of these surgical problems create ischaemia in the bowels and an attempt to correct the problems set up an ischaemia-reperfusion situation.

Intestinal Ischaemia-reperfusion injury generally stems from interruption of blood flow within the cranial mesenteric artery or vein and leads to small intestinal hypo-perfusion and a mortality rate of approximately 70% (Tendler, 2003). Testicular ischaemia-reperfusion is an established cause of testicular damage and infertility (Shalaby and Afifi, 2008). This study investigates the effects of intestinal

ischaemia-reperfusion on semen characteristics and fertility.

**MATERIALS AND METHODS**

Six male West African Dwarf goats aged one year and weighing  $13.5 \pm 0.71$ kg were used for this study. They were divided into two groups, A (control) and B (IIR). Goats were fasted overnight, sedated with Xylazine (0.05mg/kgbw) and an inverted L block of the right flank was done using 2% lignocaine (6mls). Control animals were sham operated by doing a laparotomy and closing up while the IIR animals had a laparotomy as well as intestinal ischaemia-reperfusion (IIR) injury. IIR was achieved by clamping the superior mesenteric artery for one hour and declamped to achieve reperfusion for two hours.

**Procedure**

Surgical site was prepared by shaving and washing with soap, water and methylated spirit. The skin and abdominal muscles were incised to expose the intestines which were exteriorized and the cranial mesenteric artery was located as it bifurcates from the aorta and clamped. Ischaemia was assessed as a paleness and bluish colouration of the intestines as well as loss of pulsation. Reperfusion was assessed as return of pulsation and change in colour of the intestines. Following reperfusion, the laparotomy was closed in three layers. Semen was collected by the electro-ejaculator method and examined macroscopically and microscopically according to Oyeyemi et al (2008).

**Statistical Analysis**

Data are presented as Mean  $\pm$  SEM. Analysis was done using ANOVA, Duncan multiple comparison test and Student t-test.  $p < 0.05$  is significant.

**RESULTS**

Sperm concentration decreased significantly from the 1st week following surgery and continued to decrease

till the 4th week in IIR group (Table 1) while in control animals, did not show any decrease till the 4th week postoperatively. The normal sperm cells decreased in both IIR and control group of animals from the 1st week till the 2nd week and then increased (Table 1). The total abnormal cells in IIR group were higher significantly at the beginning and the end of the study, ie preoperatively and at week 4 in comparison with control animals (Table 1). Preoperatively, there appeared to be higher numbers of all semen abnormalities except CM and CT in IIR group than control group (Table 2). The first postoperative week (Table 2), there appears to be an increase in sperm abnormalities in the control group while the IIR group abnormality remained unchanged. The second postoperative week, except for CT, all abnormalities in the control animals appear to be increased than the IIR group (Table 2). The third postoperative week, all sperm abnormalities except LT have increased in IIR group (Table 2) whereas in the control group, they have either decreased or remained unchanged. The sperm abnormalities in the 4th week in IIR group are all significantly increased except for LT while those of control animals have all returned to preoperative levels (Table 2).

**DISCUSSION**

Testicular injury following torsion includes nonspecific damage induced by ischemia per se in addition to damage caused by reperfusion, so it is called ischemic-reperfusion (I/R) injury (Shalaby and Afifi, 2008). Gastro-intestinal emergencies in WAD goats can usually set up an obstructive surgical condition leading to the establishment of an ischaemia-reperfusion injury and the remote effects of intestinal IR have also been attributed to the ability of the intestine to serve as a generator for ROS and its unique position in the gastro-intestinal system in

**Table 1:** Sperm Concentration, Normal Sperm count and Total Abnormal Sperm Count before and following IIR in WAD goats.

	Sperm Concentration ( $\times 10^9$ /ml)		Normal Sperm Count (%)		Total Abnormal Sperm Count (%)	
	Control	IIR	Control	IIR	Control	IIR
Preoperative	205 $\pm$ 3.5	215 $\pm$ 0.9	88.0	86.4	12.0	13.6
1week postop	215 $\pm$ 2.8	205 $\pm$ 1.2*	86.4	85.5	13.6	14.5
2weeks postop	200 $\pm$ 2.2	215 $\pm$ 6.5*	84.6	85.0	15.4	15.0
3weeks postop	200 $\pm$ 2.1	180 $\pm$ 3.5 *	86.4	85.2	13.6	14.8
4weeks postop	170 $\pm$ 2.3	150 $\pm$ 2.1*	88.0	86.6	12.0	13.4

\* $p < 0.05$

**Table 2:** Percentage Sperm Abnormality before and following IIR in WAD goats.

	NHWT		NTWH		BM		CM		RT		BT		CT		LT	
	C	IIR	C	IIR	C	IIR	C	IIR	C	IIR	C	IIR	C	IIR	C	IIR
Preoperative	0.7	1.2	0.7	1.2	2.7	2.8	2.9	1.8	0.3	0.7	1.7	2.6	2.6	2.4	0.3	0.7
1week postop	1.3	1.5	0.9	0.7	2.6	2.7	1.8	2.4	0.9	0.7	3.0	3.3	2.4	2.2	0.3	0.7
2weekspostop	1.5	1.0	1.2	1.5	2.7	2.4	2.7	2.3	1.2	1.5	3.5	2.5	2.2	2.8	0.2	0.6
3weekspostop	0.6	1.5	1.5	1.3	2.6	2.8	2.6	3.3	1.5	1.3	2.4	2.0	2.2	2.4	0.2	0.2
4weekspostop	0.7	1.3*	1.3	1.5	1.7*	2.4	2.2	2.2	1.3	1.5*	2.0	2.2*	2.0	2.0	0.7	0.3

NHWT- Normal Head without Tail; NTWH-Normal Tail without Head; BM-Bent Middle piece; CM-Curved Middle piece RT-Rudimentary Tail; BT-Bent Tail;CT- Curved Tail; LT-Looped Tail; C- Control group; IIR-Intestinal Ischaemia Group.

\* $p < 0.05$

distributing its metabolites around the entire body which lends credence to authors (Ogbuewu *et al.*, 2010) who found that excessive generation of ROS by abnormal spermatozoa & contaminated leucocytes is one of the few defined aetiologies for male infertility. The increased sperm abnormalities observed in this study around the fourth week may be due to pathophysiologic mechanisms that underlie testicular ischemic injury and germ cell death (Shalaby and Afifi, 2008) as due to a decrease in the blood flow which leads to reduction in oxygen supply relative to metabolic demands with subsequent depletion of the stored cellular energy and accumulation of toxic metabolites as lactic acid, hypoxanthine and lipid peroxides. There are reports (Franca *et al.*, 1999) of spermatogenic cycles of  $10.6 \pm 0.5$  days in goats and a total spermatogenic cycle of 4.5 cycles of seminiferous epithelium and lends credence to the effects of increased sperm abnormalities observed about the fourth postoperative week of this study.

The increased bent mid-piece and curved mid-piece abnormalities observed in the IIR group may be due to lipid peroxidation which is said to occur in IIR (Ogbuewu *et al.*, 2010). Decreased semen concentration observed in the control group is less than that observed in IIR group and since a critical level of semen concentration is desirable for fertility as reported (Oyeyemi and Ubiogoro, 2005), the effect of IIR may be seen as a decrease beyond acceptable levels. This may well be the challenge that IIR poses in animals used in production. The remote effects of IIR need to be considered in cases of iatrogenic causes of infertility to establish a robust health determination when acquiring animals for a breeding program where history of gastrointestinal incidents may carry little significance until breeding problems occur.

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