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Specific sperm abnormalities observed in rams (*Ovis aries*) following cypermethrin treatment

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This work was designed to make further observations on the specific types of sperm abnormalities present in Cypermethrin treated rams and contribute information towards understanding how Cypermethrin affects male's reproduction. Sixteen sexually-mature, healthy Yankasa rams aged 18 to 30 months and weighing between 21.5 to 46.5 kg were used. The 16 rams were divided equally into two groups (A and B). Group A served as the treatment group, while B served as the control. The rams in Group A were given Cypermethrin (3%) at the dose rate of 3 mg/kg (0.1 ml/kg) body weight, topically as pour-on. The control rams were given distilled water at the same dose rate and route. These treatments were repeated every two weeks for a period of 12 weeks. Semen was collected on weekly basis from each ram during the experimental period by means of a hand held electro-ejaculator (EE). The morphological sperm abnormalities/defects were determined by examining semen smears stained with eosin-nigrosin on a glass slide. Results showed that sperm abnormalities recorded were detached head, mid piece droplet, free tail, coiled tail and bent tail. There was no significant difference in the percentage of these abnormalities recorded between the two groups (p>0.05) from week 1 to 12. It is concluded that Cypermethrin did not induce any specific sperm abnormalities in rams treated with 3 mg/kg body weight as pour-on. Sperm death in Cypermethrin label dose treated rams may be mediated through oxidative stress. It was recommended that more studies be directed towards stress biomarkers in Cypermethrin treated rams.

Key words: Sperm, abnormalities, cypermethrin, rams, oxidative stress.

INTRODUCTION

Spermatozoa are divided into three main segments: the head, mid-piece and tail. The head consists of a condensed nucleus (Morton, 1977). During the acrosome reaction, the outer acrosomal membrane fuses with the plasmalemma of the sperm head, under the control of intra-and extracellular calcium, where exocytosis of the

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contents of the acrosome occurs (Harrison and Roland, 1990). Sperm metabolize simple molecules, principally sugars and their derivatives (e.g. fructose, glucose, mannose and pyruvate), by both aerobic and anaerobic pathways to provide energy for motility and the maintenance of ionic gradients across membranes (Harrison, 1977). Forward motility of sperm results from coordinated waves of flagella bending progressing from the neck along the length of the tail. Bending of the tail occurs as a result of forces generated between adjacent peripheral doublets of the axoneme (Satir et al., 1981). The dynein arms of the doublet, which in the resting state are bound to the adjacent doublet, unbind, elongate and then bind to a new site further along the filament.

Oxidative stress is defined as disruption of the prooxidant-antioxidant balance in favour of the former, leading to potential damage (Sies, 1997; Halliwell, 2007). Costatini and Verhulst (2009) and Lykkesfeldt and Svendsen (2007) defined it as an imbalance between oxidants and reductants (antioxidants) at the cellular or individual level. Oxidative stress was also defined as a redox imbalance with an excess of oxidants or a deficiency in antioxidants. When oxidative stress occurs, cells function to counteract the oxidant effect and restore redox balance by resetting critical homeostatic parameters. Such cellular activity leads to activation or silencing of genes encoding defensive enzymes, transcription factors and structural proteins (Dalton et al., 1999).

The cellular macromolecules are natural targets of oxidation. Numerous oxidative modifications to DNA can lead to base misincorporation, mutation, single or double DNA strand breaks and eventual cell death (Poulsen, 2005) by apoptosis or necrosis, and structural tissue damage (Lykkesfeldt and Svendsen, 2007). All the major biomolecules (like lipids, proteins and nucleic acids) may be attacked by free radicals, but lipids are probably the most susceptible. Protein oxidation leads to malfunction of enzymes, which become incapable of performing their cellular task. Lipids are easily oxidised and may initiate chain reactions resulting in further oxidative damage, which can compromise the cell integrity (Lykkesfeldt and Svendsen, 2007). Because, the body is not necessarily fully protected against oxidative damage, some of its constituents may be injured by free radicals, and the resultant oxidative products have been used as markers (Yoshikawa and Naito, 2002). Free radicals are molecules possessing one or more unpaired electron in their outer orbit that can be considered as fragments of molecules, and which are generally very reactive (Cheeseman and Slater, 1993; Halliwell, 1996; Menviell-Bourg, 2005). The OH^- and alkoxy free radicals are very reactive and they attack the macromolecule in cells. OH^- is the most reactive species known (Buettner, 1993). However, O_2^-; lipid hydroperoxide and NO^- are comparatively less reactive (Singh et al., 2004). In general, O_2^-; H_2O_2 and HO collectively, are called reactive oxygen species, but only O_2^- and HO are free radicals, while H_2O_2 is not. Various active oxygen species are generated in the body during the process of utilizing oxygen. Since the body is furnished with elaborate mechanisms to remove active oxygen species and free radicals, these by-products of oxygen metabolism are not necessarily a threat to the body under physiological conditions. However, if active oxygen species or free radicals are generated excessively or at abnormal sites, the balance between formation and removal is lost resulting in oxidative stress (Yoshikawa and Naito, 2002). The balance between production and disposal of oxidants is essential for tissue homeostasis. Increased rate of free radical production or decreased rate of removal leads to free radical accumulation and cellular damage (Hammadeh et al., 2009). Environmental stressors like some pesticides are known to induce oxidative stress and alteration in cellular redox balance. Oxidative stress has been widely shown to induce apoptosis. Studies suggest that long exposure to oxidative stress leads to cell death, whereas low or transient exposure leads to survival/differentiation (Franco et al., 2009). Oxidative DNA damage and oxidative-stress induced cell membrane damage are other important apoptotic signaling cascades activated by oxidative stress.

Cypermethrin is a pesticide, and it is able to influence some reproductive and fertility parameters as exposure to this chemical can cause significant increase in the production of non-viable or abnormal sperm in mice (Jalal et al., 2010; Caroline, 1996; Bhunya and Pati, 1988). Experiment on the effect on biochemical parameters, of testes after administration of 250 mg/kg, P.O of α-Cypermethrin in albino mice was reported to cause histologic changes in spermatogenic cells, like rupture of cell membrane, shrinkage in the nucleus, presence of stages of apoptosis, condensation of chromatin and decrease or absence of cytoplasmic organelles, as revealed by transmission electron microscopy (TEM) (Prakash et al., 2010). Oral administration of two synthetic pyrethyroid insecticide on male mature rats at doses of 200, 100, 1 and 5 mg/kg body weight respectively, for 65 successive days showed a significant increase in the percentage of sperm head and tail abnormalities (Hassan et al., 1993). A similar report following oral administration at a dose of 55.1 mg/kg body weight (1/5 LD_{50}) for 60 successive days have also been made (Assayed et al., 2008). Pesticide exposure is associated with infertility, there is much concern that exposure to environmental contaminants causes decreased sperm counts, impairment of sperm motility, reduced fertilization ability, producing abnormal sperm in men and wildlife (Elbetieha et al., 2001). The mechanism by which Cypermethrin affects male reproduction is unclear (Wang et al., 2009). This work was designed to make further observations on the specific types of sperm abnormalities present in label dose Cypermethrin treated rams and contribute information towards understanding

Table 1. Mean ± SEM base line data for specific sperm abnormalities in both the treated and control rams (n=8).

Parameters	Treated (n = 8)	Control (n = 8)
DH (%)	2.25± 0.68	1.75 ± 0.50
MPD (%)	0.00 ± 0.00	0.50 ± 0.19
Free tail (%)	2.50 ± 0.62	3.00 ± 0.66
Bent tail (%)	4.87 ± 0.77	2.75 ± 0.31
Coiled tail (%)	4.38 ± 0.73	5.13 ± 0.72

DH = detached head; MPD = mid piece defects.

how Cypermethrin affects male's reproduction.

MATERIALS AND METHODS

Study location

The research was carried out at the National Animal Production Research Institute (NAPRI) Shika, Ahmadu Bello University Zaria, which is situated in the Northern Guinea Savannah and lying between latitudes 11° and 12°N and longitude 7° and 8°E, at an elevation of 650 m above sea level. The area has an annual rainfall of 1100 mm (Igono et al., 1982). There are two seasons {rainy season (May-October) and dry season (November-April)}.

Experimental animals

The animal experiments followed the principles of the Laboratory Animal Care (CACC, 1993). Sixteen sexually-mature, healthy Yankasa rams aged 18 to 30 months and weighing between 21.5 and 46.5 kg with clinically normal genitalia were used. The rams were purchased from the open market in Sabua Local Government Area of Katsina State. They were housed at the Small Ruminant Research Programme Experimental Unit of NAPRI. The house was made of brick concrete pens with concrete floors. They were given concentrate feed *ad libitum* (cotton seed, maize offal, maize, wheat offal, bone meal and salt) in the morning and later in the evening; hay was made available during the day at intervals. The hay used was *Digitaria simuthii*, and water was given *ad libitum*.

Experimental design and treatment

The 16 rams were divided equally into two groups (A and B). Group A served as the treatment group, while group B served as the control. The animals were acclimatized for two weeks during which blood and faecal samples were collected and analyzed for haemoparasites and helminths and treatments given where necessary.

Administration of 3% Cypermethrin

The rams in Group A were given Cypermethrin (3%) at the dose rate of 3 mg/kg (0.1 ml/kg) body weight, topically as pour-on. The control Group B rams were given distilled water at the same dose rate of 0.1 ml/kg body weight topically as pour-on. These treatments were repeated every two weeks for a period of 12 weeks. Semen was collected before the administration of 3% Cypermethrin to establish base line data. This was done by getting the average values for the eight animals in each group before the experiment started.

Semen collection

Semen was collected on weekly basis from each ram during the experimental period by means of a hand held electro-ejaculator (EE). Semen collections were done in the morning between 8:00 and 10:00 am once every week. Before semen collection, the animals were adequately restrained and the prepuce disinfected using 4.8% chloroxynol (Detol®) diluted with water prior to the rectal insertion of the probe. The electrode was lubricated with petroleum (KY®) jelly to ease insertion. The lubricated probe of the electro-ejaculator (Electrojet®, Electrovet, Sao Paulo, Brazil) was then inserted into the rectum and switched on to produce an erection with subsequent ejaculation. By using the manual button of the instrument, the power output was switched on by pressing and holding for 2 to 3 s. The power output was again pressed in to cut off the output. This procedure was repeated after a rest period, equal to the duration of electrical stimulation, by increasing the duration of power output gradually on every attempt until ejaculation takes place. The urethral process and end of the penis was held in a semen collection tube for the ejaculate to be collected. Semen was collected in graduated plastic tubes for processing.

Preparation of semen smears for morphological defects (%)

Sperm morphology was determined by methods described by Zemjanis (1970) and Sekoni et al. (1981). The morphological sperm abnormalities/defects were determined by examining semen smears stained with eosin-nigrosin on a glass slide (Vilakazi and Webb, 2004; Michael et al., 2008). Sperm abnormalities were classified as described by Zemjanis (1970) and Sekoni (1992). The staining mixture consisted of 1% eosin B and 5% of nigrosin in 3% sodium citrate dehydrate solution. One drop of raw semen was added to one drop of the stain, it was mixed thoroughly and a fresh smear was made from it. At least, 100 cells (both stained and unstained) were counted and a percentage of each abnormality was estimated. Morphological sperms were determined by counting abnormal spermatozoa which included head, mid-piece and tail abnormalities. This was done by viewing the stained slides through the light microscope using oil emersion x100.

Statistical analysis of data

Data were expressed as means and standard error of mean (SEM). Data were analyzed using paired student's t-test with SPSS/PC computer program (Version 20.0, SPSS®, Chicago IL, USA). Differences with confidence values of $p < 0.05$ were considered statistically significant (Daniel, 1991).

RESULTS

The mean ± SEM base line data for specific sperm abnormalities in both the treated and control rams is presented in Table 1. The mean ± SEM weekly detached head and mid piece abnormalities (%) of Yankasa rams during the treatment period is presented (Table 2). There was no statistically significant difference between the two groups (P>0.05). The mean ± SEM weekly free tail, coiled tail and bent tail abnormalities (%) of Yankasa rams during the treatment period is presented in Table 3.

Table 2. Mean ± SEM weekly head and mid piece defects (%) of Yankasa rams during the treatment period (n=8).

Weeks	Detached head		Mid piece defects	
	Treated	Control	Treated	Control
1	2.50 ± 0.78	2.25 ± 0.90	0.13 ± 0.13	0.23 ± 0.16
2	2.13 ± 0.48	2.50 ± 0.66	0.00 ± 0.00	0.23 ± 0.16
3	1.75 ± 0.37	2.75 ± 0.31	0.00 ± 0.00	0.23 ± 0.16
4	2.13 ± 0.48	2.50 ± 0.42	0.13 ± 0.13	0.38 ± 0.19
5	6.38 ± 0.71	3.63 ± 0.96	0.00 ± 0.00	0.25 ± 0.16
6	6.13 ± 0.88	5.43 ± 1.46	0.13 ± 0.13	0.29 ± 0.18
7	4.43 ± 0.37	4.13 ± 0.74	0.43 ± 0.20	0.75 ± 0.25
8	4.00 ± 0.95	4.50 ± 0.91	1.14 ± 0.34	1.00 ± 0.38
9	4.29 ± 0.47	2.88 ± 0.58	0.43 ± 0.20	0.63 ± 0.18
10	4.83 ± 1.09	5.17 ± 1.35	1.00 ± 0.52	0.67 ± 0.21
11	2.25 ± 0.63	0.50 ± 0.29	0.50 ± 0.29	0.50 ± 0.50
12	3.29 ± 0.97	1.50 ± 0.42	0.43 ± 0.20	0.50 ± 0.19

There was no statistically significant difference between the two groups (P>0.05).

Table 3. Mean ± SEM weekly tail abnormalities (%) of Yankasa rams during the treatment period (n=8).

Weeks	Free tail		Coiled tail		Bent tail	
	Treated	Control	Treated	Control	Treated	Control
1	2.38 ± 0.42	2.50 ± 0.68	5.50 ± 0.98	4.63 ± 0.91	4.38 ± 0.60	2.88 ± 0.55
2	1.25 ± 0.37	2.38 ± 0.50	4.75 ± 0.62	5.13 ± 0.85	6.13 ± 0.81	3.13 ± 0.69
3	2.00 ± 0.57	1.38 ± 0.26	5.63 ± 0.42	5.38 ± 0.57	5.88 ± 0.52	2.25 ± 0.90
4	1.75 ± 0.70	1.75 ± 0.41	4.75 ± 0.59	4.50 ± 0.68	6.63 ± 0.76	6.75 ± 0.65
5	0.63 ± 0.26	3.13 ± 0.88	4.38 ± 1.03	6.88 ± 0.83	2.88 ± 0.52	3.00 ± 0.82
6	0.75 ± 0.25	2.43 ± 0.75	4.50 ± 1.41	6.43 ± 0.81	2.63 ± 0.42	3.00 ± 0.87
7	1.71 ± 0.29	2.50 ± 0.54	2.57 ± 0.57	3.38 ± 0.71	1.57 ± 0.83	2.88 ± 0.23
8	1.71 ± 0.29	2.88 ± 0.69	2.71 ± 0.61	3.00 ± 0.73	2.29 ± 0.64	2.63 ± 0.46
9	2.00 ± 0.76	1.63 ± 0.32	5.57 ± 0.53	6.13 ± 0.79	2.86 ± 0.51	3.75 ± 0.65
10	2.50 ± 0.56	2.50 ± 0.56	1.67 ± 0.33	2.00 ± 0.37	4.00 ± 0.52	3.83 ± 0.31
11	0.50 ± 0.29	1.25 ± 0.63	5.00 ± 0.41	4.75 ± 0.75	2.25 ± 0.48	2.00 ± 0.82
12	3.14 ± 0.34	5.13 ± 0.79	4.29 ± 0.68	4.75 ± 0.53	4.71 ± 1.34	6.63 ± 1.27

There was no statistically significant difference between the two groups (P>0.05).

There was no statistically significant difference between the two groups (P>0.05).

DISCUSSION

The results of the present study showed that Cypermethrin treatment at 3 mg/kg as pour-on in rams did not induce any specific abnormalities. This is evident because there was no statistically significant difference between the treated and the control groups in the sperm abnormalities recorded. The present study contradicts reports in laboratory animals. Previous studies showed that Cypermethrin was able to influence some reproductive and fertility parameters as exposure to this chemical can cause significant increase in the production of nonviable or abnormal sperm in mice (Jalal et al., 2010). The disagreement here is in terms of sperm abnormalities. All doses of 1, 10 and high dose 20 mg/kg beta Cypermethrin used in a 36 day experiment decreased sperm count, viability and intact acrosome rate (Wang et al., 2009).

A significant (P<0.05) increase in the proportion of dead and abnormal sperm cells in mice exposed to Cypermethrin have been reported (Bhunya and Pati, 1988). After 4 and 8 weeks, beside that sperm motility

percentage was affected sperm concentration were found to be significantly decreased after 2, 4 and 8 weeks of exposure (El-Ashmawy et al., 1993). Studies on the effects of oral administration of two synthetic pyrethroid insecticide has shown significant increase in the percentage of sperm head and tail abnormalities (Hassan et al., 1993). The above cited reports disagree with the findings of the current studies in relation to sperm abnormalities. There is much concern that exposure to environmental contaminants causes decreased sperm counts, impairment of sperm motility, reduced fertilization ability, producing abnormal sperm in men and wildlife (Elbetieha et al., 2001). The contrary report on sperm abnormalities in rams against many laboratory animals may be due to the species differences, route of administration and the dose of Cypermethrin. The present study indicates that Cypermethrin may not have a direct effect on sperm morphology of treated rams at the dose of 3 mg/kg. The widely reported effect of Cypermethrin on sperm viability may be mediated through direct effect of oxidative stress and apoptotic cell death. Acidic pH of semen in Cypermethrin treated rams may be responsible for the oxidative process. Semen of Cypermethrin treated rams have been reported to tend towards acidic pH (Ubah et al., 2016). All the major biomolecules (like lipids, proteins and nucleic acids) may be attacked by free radicals, but lipids are probably the most susceptible. Protein oxidation leads to malfunction of enzymes, which become incapable of performing their cellular task. Lipids are easily oxidized and may initiate chain reactions resulting in further oxidative damage, which can compromise the cell integrity (Lykkesfeldt and Svendsen, 2007). Sperm cell membrane is composed of lipoproteins, therefore loss of cell membrane integrity may contribute to sperm cell death in Cypermethrin treated rams. Oxidative DNA damage and oxidative-stress induced cell membrane damage are other important apoptotic signaling cascades activated by oxidative stress. It is generally accepted that oxidative stress and ROS cause DNA damage, if unrepaired or with faulty cellular repair mechanism, contribute to apoptosis (Barzilal and Yamamoto, 2004). ROS produced during oxidative stress initiate signaling cascades, ultimately leading to apoptosis. Apoptosis is a form of programmed cell death, which have been linked to exposure to environmental toxicants, including some pesticides (Caughlan, 2003). Apoptotic cell death differ from necrosis morphologically (cell shrinkage and DNA fragmentation) and biologically (requirement of ATP) (Tomei and Cope, 1991).

Conclusion

Cypermethrin did not induce any specific sperm abnormalities in the treated rams at the dose of 3 mg/kg body weight as pour-on. The contrary report in rams against many laboratory animals may be due to the species differences, route of administration and the dose of Cypermethrin. Sperm death in Cypermethrin treated animals may be mediated through oxidative stress. It was recommended that more studies be directed towards stress biomarkers in Cypermethrin treated rams.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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