Effect of Warm Water Bathe on the Testes and sperm quality of Adult Wistar Rats

Abstract

Hot water bathe is both therapeutic and habitual. Studies have shown that the testis functions optimally at temperatures slightly below body temperature. This study is designed to evaluate the effect of regular warm water bath on the histoarchitecture of the testis and the sperm quality in Wistar rats. Twenty rats were divided into four groups of five rats each based on closeness of body weight. Rats were soaked fully (except head and neck) inside water of varying temperatures. The control group A was soaked in normal water at room temperature. The other rats in groups B, C and D were exposed to warm water at 30^{o}C, 35^{o}C and 40^{o}C respectively for 10 minutes every day for 14 days. Twenty four hours after the last exposure, the animals were weighed and sacrificed using chloroform sedation. The semen was extracted from the tail end of the epididymis for analysis of sperm quality while the testes were fixed in 10% formal saline for histological studies. The findings of the present study showed that warm water bath at 30^{O}C and 35^{O}C did not cause reduction in sperm count or rat weight. However, at 40^{O}C spermatogenesis was adversely affected.

Keywords: Warm water bath, sperm parameters, testes, sperm motility.

BACKGROUND OF STUDY

The testes are a paired organ within the scrotum surrounded by a strong capsule (Chaurasia, 2010). The testicular parenchyma is composed of 250 – 350 lobules, which drain through the mediastinum testis to the epididymis. A lobules of the testis consist of one or several seminiferous tubules, which end and start at the rete testis (Chaurasia, 2010). The testes are the

male gonads. Like the ovaries to which they are homologous, testes are components of both the reproductive system and the endocrine system. The primary function of the testes is to produce sperm (spermatogenesis) and produce androgens, primarily testosterone (Mahon, 2010). Both functions of the testicles are influenced by gonadotropic hormones produced from the anterior pituitary (Heptner*et al.*,2002). The testicles are located in the scrotal sac which hangs outside of the male's body. This is to ensure that they are maintained at a temperature 2-3 degrees lower than that of body temperature. It is true that high temperatures may affect spermiogenesis, however the level of temperature and the duration of exposure determines how much impact it has(Banks et al., 2005).

Supporting cells called sertolicells play important roles in spermatogenesis by nourishing of developing sperm cells. They also act as phagocyte consuming residual cytoplasm during spermatogenesis (Seco-Rovira et al., 2014), whereas the leydig cells are the main sources of androgen production (Zirkin and Papadopoulos, 2018). Both types of cells can readily be affected by high temperature (Pei et al., 2012). Alternation in functions of these cells may lead to a change in male hormone balance, hence disturbing the process of spermatozoa (Mieusset and Bujan, 1995).Investigators believe that excessive exposure to heat in the form of hot bathing water can significantly affect the testicular functions and may compromise spermatogenesis (Mohammed et al., 2012). This study was designed to verify these claims.

Comment [WPS_164741]: Spermatogenesis or sparmatozoa formation

Bathing is the washing of the body with a liquid, usually water or an aqueous solution or the immersion of the body in water (Reece, 2017). It is a means of achieving cleanliness by washing away dead skin cell dirt, and soil and as a preventative measure to reduce the incidence and

spread of disease (Clarke, 2008). It may also be practiced for religious ritual or therapeutic purposes (Reece, 2002). Any agent that increases the temperature inside the testicles (radiations, hot water, ultrasound waves etc.) can compromise the process of spermatogenesis and may lead to impaired fecundity and infertility.

Hydrotherapy has been practiced for centuries. Both the use of hot and cold water can have beneficial effects on the body (Prankel 2008). Showering is the most common form of bathing worldwide. Daily immersion of rat scrotum in warm water ($44^{O}C \pm 1^{O}C$) for a period of 8 weeks caused loss of testicular weight, atrophy of tubules and degeneration of the germinal epithelium. On reproductive performance, the rats were found to be infertile (Venkatachalamand Ramanathan, 1961).Although many authors believe that the effects of heat are fully reversible, there is now evidence of long-term effects, following either locally applied heat or temporary induced cryptorchidism. These long-term effects appear to be different from those seen followingirradiation, and may indicate a failure of sertoli cell function. Sperm produced by mice which had been exposed to a hot environment bind to ova normally butare less able to fertilize in vivo and in vitro, even when motile sperm are selected by a swim-up procedure, and many of the resultant embryos do not develop normally (Setchell1971).

MATERIALS AND METHODS

The materials used for this study include 20 adult male Wistar rats weighing 120g-180g, Metal mesh grid cages, Top feed growers mash (Premier feed mills co. LTD, Plateau state, Nigeria), gas burner, plastic basin of 10 liters deep, Weighing Balance, Dissecting kit, Measuring cylinder, Conical flask, Rotatory microtome (Leica products, MRM-1120), formal saline, hand

lens,Neuber counting chamber, (superior Marienfed, Germany), Light binocular microscope (Olympus, china with serial number X52-10TBN), Glass slide, Spatula, Freshly prepared bouin's fluid, Thermostat oven, HaierThermocool refrigerator.

ETHICAL CLEARANCE

The ethical approval was obtained from the ethical committee of Faculty of Basic Medical Sciences, NnamdiAzikiwe University, Nnewi, before the commencement of the research and was assigned the number NAU/CHS/NC/FBMS/230.

LOCATION AND DURATION OF STUDY

This study was carried out at the Animal House of the Department of Anatomy, Faculty of Basic Medical Science, NnamdiAzikiwe University, Nnewi, Anambra State.

Research Protocol

Twenty (20) adult male Wistar rats weighing between 120-180g were purchased from NnamdiAzikiweUniversity animal farm in Okofia, Nnewi south L.G.A, Anambra state. The animals were housed in well ventilated cages under normal temperature. They were fed with standard diet and water and were acclimatized for a period of fourteen days. The floors of the cages were overlaid with sawdust obtained from a nearby sawmill and the cages cleaned every two days to prevent infections. The weight of the rats were taken and documented after the acclimatization period. The Guide for the Care and Use of laboratory Animals was followed (Albus, 2012).

The twenty rats were divided into four groups (A, B, C, D)of five rats each. Rats in the control group were immersed in normal room temperature water for 10 minutes daily for 14 days. The immersion was such that the water was deep enough to contain the whole body of the rats but shallow enough to have them raise their head out of the water to avoid drowning. The other groups wereimmersed inwarm water at30^{o}C, 35^{o}C and 40^{o}C for rats in groups B, C and D respectively. After each day's exposure the rats were returned to their cages.

TERMINATION OF EXPERIMENT ANIMAL SACRIFICE

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Twenty-fourhours after the last exposure, the rats were weighed and the weight documented. Rats were then sacrificed by chloroform sedation according to the method reported by Aguwa*et al.,* (2020).Immediately after sedation, sperm was retrieved from the epididymis of each rat for semen analysis, after which the testes were harvested and fixed in 10% formal saline.

Sperm parameters

A thin smear of liquefied well-mixed semen was made on a slide. While still wet, the smear was fixed with diluted neutral buffered formalin (1:20) and with the aid of light microscope at x400 magnification the smear was examined for normal and abnormal spermatozoa using. A count of 100 was made and the percentage showing normal and abnormal morphology was recorded. Morphological changes were clear under the power of x400. In this study, a spermatozoon was considered abnormal morphologically if it had one of the following features: rudimentary tail,

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round head and detached head. Sperm count, sperm morphology and sperm motility were recorded for each rat.

Tissue Processing

After 48 hours, the testis was processed at the Histology Laboratory, Anatomy department, NnamdiAzikiweUniversity Nnewicampus using the routine H &E method according toDrury and Wallington (1980). The tissues were subjected todehydration, clearing, infiltration, sectioning and staining after which slides were examined under light microscope.

Data Analysis

Data was analyzed using the SPSS (version 21) software package. Results obtained were expressed as Mean value ± SD in each group. Tested parameters were subjected to one-way analysis of variance (ANOVA). Differences between means were regarded significant at $P<0.05$

RESULT OF PHYSICAL CHANGES

During the period of acclimatization, all the Wistar rats were healthy, with smoothly laid hairs on their skins, pinkish eyes, and normal skin color. After commencement of the experiment, all the groups showed no clinical sign.

TABLE 1: Result of rat body weight changes

GROUP	INITIAL WEIGHT	FINAL WEIGHT	P-VALUE

A	149 ± 1.15	183.5 ± 12.12	0.0006
B	148.5 ± 2.88	173 ± 5.77	0.0001
C	146.75 ± 0.5	168 ± 3.46	0.0000
D	166 ± 4.62	186 ± 6.93	0.0015

Results are presented as Mean ± S.D of 5 rats in each group.

Means of rat weight were compared using the student's t-test and values are considered statistically significant at $P < 0.05$.

The result of rat weight as presented in table 1 above shows that, animals in the control group had a significant weight gain at the end of the experimental period (FINAL) compared to the initial period (INITIAL). This was observed for rats in the experimental groups, B, C and D which all had significant weight gain at the FINAL compared with INITIAL.

TABLE 2: Showing percentage body weight gain

GROUP	INITIAL WEIGHT	FINAL WEIGHT	WEIGHT DIFFERENCE	PERCENTAGE WEIGHT GAIN
A	183.5	149	34.5	23.15
B	173	148.5	24.5	16.49

C	168	146.75	21.25	14.48
D	186	166	20	12.04

FORMULAE: % weight gain = weight difference x 100

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Initial weight

Table 3:**Effect of Warm water bath on Relative Testicular Weight**

Group	**Relative Weight of Testis (%)**	**p-value**
Group A	1.64±0.09	
Group B	1.64±0.13	0.98
Group C	1.53±0.23	0.69
Group D	1.66±0.26	0.95

Data were analyzed using One-way ANOVA and multiple comparison, and data were considered significant at $P<0.05$.

TABLE 4: Effect of warm water bath on sperm countinWistar Rats

GROUP	**SPERM COUNTP-VALVE**
A	225.00 ± 260.22

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B	152.50 ± 127.99	0.0823	
C	603.00 ± 179.610.1024		
D	560.50 ± 102	0.0935	

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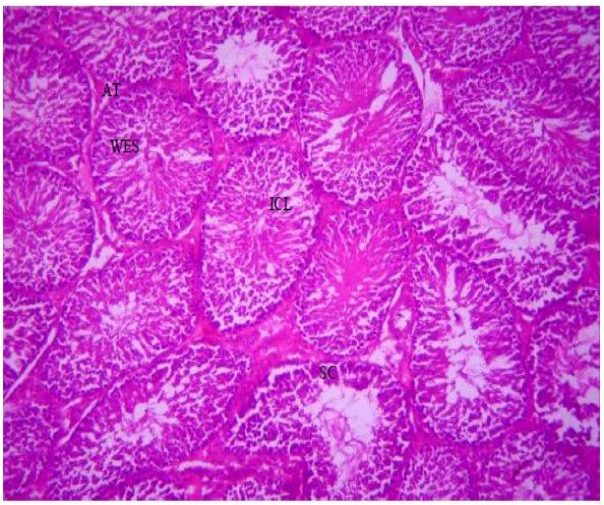
The result of semen analysis shows that there was no statistically significant different in the sperm count of rats in the experimental groups B, C and D compared to the control. Recall that rats in the experimental group B, C and D were immersed in warm, water at 30^{o}C, 35^{o}C and 40^{o}C respectively.

TABLE 5: Effect of warm water bath on Sperm Status inWistar Rats

GROUP	NORMAL	ABNORMAL
A	82.5 ± 3.54	17.5
B	77.5 ± 10.61	15
C	82.5 ± 3.54	17.5
D	80 ± 0.00	20

The result of the sperm status showed that the normal sperm motility for the experimental group are comparable and not significantly different from those of control group.

Result of histologicalstudies



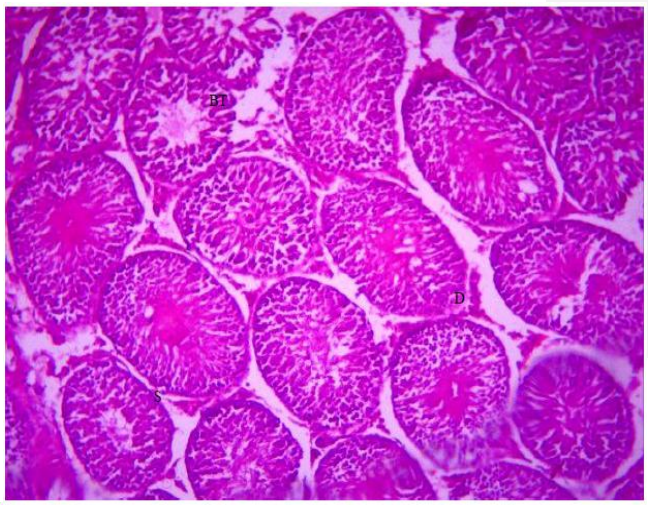


Figure 1 : Representative Photomicrograph of rat testis**: A (control)**shows normal testicular architecture with active seminiferous tubules that are lined with interstitial cells of leydig (ICL), sertoli cell (SC) and well enhanced spermatogenesis (ES);**B (rats** exposed to 30^{O}C warm water) shows mild distortion (D) of the seminiferous tubules otherwise normal with moderately enhanced spermatogenesis (S);**C (rats** exposed to 35^{O}C warm water shows normal seminiferous tubules with mild focal area hemorrhage (H) within the basal layer;**D (rats** exposed to 40^{O}C warm water) shows seminiferous tubules with moderate arrest of the spermatogenesis (AS) and areas of cellular distortion.(H/E) (x400).

Discussion

This study was principally carried out to investigate the effect of warm water bath on the testes and semen parameters of adult male Wistar rats. The result of this study reveals that immersion

of rats in warm water at 30^{O}C, 35^{O}C, and 40^{O}C respectively caused some characteristic physical changes as evident in the physical activities of the rats. The rats in the control group showed no observable changes in their physical appearance as they remained healthy and dry throughout, not immersed in water. Stool color remained normal in all groups. There was aggressiveness when exposed or immersed in water in group B, C and D, whichwere immersed in 30^{O}C, 35^{O}C, and 40^{O}C respectively. Group B rats however showed less reactiveness when immersed in water.

Observation showed that from the period of acclimatization to the end of administration, there was significant increase in body size in all groups. This could be physiological as the only substance they were exposed to was food and water. The increase in body weight of the rats indicated that the warm water bath had no adverse effect on the body weight of the rats. This agrees with the work done by Venkatachalam and Ramanathan, (1961), which reported a general increase in body weight following exposure to warm water ($44 \pm 1\ ^{O}$C).The relative organ weight result shows there was a non-significant difference in relative weight of testis in Groups B, C and D when compared to the control.This is in line with the work of Olurode *et al.,* (2018) on testicular histomorphometry and semen parameters of West African Dwarf bucks.

The result from the analysis of variance ANOVA obtained from the semen analysis carried out showed that there was anon-significant difference in sperm count in Group B, C and D which received 30^{O}C, 35^{O}C, and 45^{O}C respectively when compared to group A. This does not agree with the work done by Gbotolorun*et al.,* (2017) who reported that warm water caused morphological changes in the testis and significant reduction in motile sperm count and sperm motility. In the present study, it was observed that after exposure to warm water, there was no

statistically significant different in active sperm motility when compared with Group A (control). This does not agree with the work done by Gbotolorun*et al.,* (2017) which reported a significant increase in active sperm motility on short-term scrotal exposure to elevated temperature prior to mating.

The result from histological studies of the control group A showed normal testicular architecture with active seminiferous tubules that are lined with interstitial cells of the leydig, sertoli cell and well enhanced spermatogenesis.However, group B micrographs showmild distortion of the seminiferous tubules but otherwise normal testicular histoarchitecture.This agrees with the result of Venkatachalam and Ramanathan, (1961), who studied the effect of moderate heat on the testes of rats and monkeys and reported that there was a mild destruction of cells of the seminiferous tubules, though the mechanism was unclear.It is understood that the scrotal sac confers some level of protection on the testes.Group C which received $35^{O}C$ showed mild focal area hemorrhage within the basal layer of testis.This agrees with the result of Gbotolorun*et al.,* (2017), who reported disintegration in the arrangement of the cells of the spermatogenic series and slight widening and destruction of the interstitium and sperm cells following testicular exposure to heat.Group D which received $40^{O}C$ showed moderate arrest of the spermatogenesis and areas of cellular distortion. This is due to the adverse effect of heat on the testis.This agrees with the result of Venkatachalam and Ramanathan, (1961), who reported generalized atrophy of the tubular epithelium with complete absence of spermatogenic activity.

Submerging ourselves in water, whether in a bathing receptacle or in a natural body of water is something we do for personal hygiene, leisure and health,but the habitual practice of warm water bath may enhance the chances of testicular damages.(Venkatachalam and Ramanathan, 1961).

5.2 CONCLUSION

The findings of the present study showed that warm water bath at30^{O}C and 35^{O}C did not cause reduction in sperm count or rat weight. However, water at 40^{O}C spermatogenesis was adversely affected.

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5.3 RECOMMENDATION

Following the result of this study, it is imperative to suggest possibility of testicular damage that result from the habitual bathing of warm water at 40^{O}C. we therefore recommend that oxidative stress parameter of rats be examined in future studies.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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