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Acute effect of temperature on gametocytes of *Rattus norvegicus*: focus on morphometric study

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Abstract

Study aimed to investigate the acute effect of temperature (at 39 ºC, 41 ºC and 43 ºC) on male gametocytes of the *Rattus norvegicus*. Experimental animals were divided into four sets as; control and remaining three were experimental groups. After completion of exposure period, all animals were sacrificed; semen sample was collected from epididymis. The sample was assessed for qualitative and quantitative analysis of sperm. The sperm count was carried out by using Neubauer's counting chamber. Results obtained were discussed in relation to sperm morphometry and fertility of experimental animal.

Key words : *Rattus norvegicus,* Temperature, Sperm morphometry, Fertility.

INTRODUCTION

Environmental factors has important role to run different physiological process in living animal. [1] and [2] reported that, fluctuations in environmental factors may be responsible for alterations in proliferation of germinal cells with at a large extent. Among environmental factors, temperature is considered as an important modulator of animal reproduction including aspects related to testicular function and spermatogenesis [3-7].

[8] reported that, mammal including human being where testicular temperature found lower than core body temperature and recorded scrotal temperature as 2 - 3°C lower than rectal temperature. In many mammals, including rats and man, the testes within the scrotum were maintained at a low temperature [9]. Optimum temperature required for spermatogenesis in human is 35°C ± 1. Increase in testicular or scrotal temperature has induced reduction in sperm count, total percentage and morphometry [10]. Temperature has also influenced and delayed rate of spermatogenesis [11-12].

From decades work has been carried out related to spermatogenesis in different animals including invertebrates. [13] studied the effect of high fluoride on quality of sperm in *Rattus norvegicus.* [14], studied the effects of *Phaleria macrocarpa* on male fertility by assessing its effect on the sperm characteristics including the sperm count, mortality, viability and its, morphometry. [15], studied the effects of lead poisoning on the testes in albino rats.

Available literature and information shows that scanty work has been carried out related to acute effect of temperature on spermatogenesis. So, present study was focused related to acute effect of varied temperature up to 96 h of exposure period. Data obtained was discussed in relation to morphometric analysis of sperm and reproductive physiology in experimental animal.

MATERIALS AND METHODS

1. Experimental Animals:

Experimental rat, ***Rattus norvegicus*** was selected for present study. Experimental animals were reared in Departmental animal house (CPC SEA / 233) of Department of Zoology, Shivaji University, Kolhapur. Under maintained laboratory conditions, experimental animals were acclimatized for two weeks before exposure. Normally animals were feed with pellets (Rat and mice feed. Pranav Agro Industries Ltd. Sangli) and supplied with drinking water.

2. Experimental Design:

Ten experimental animals of same body weight 250 ± 25 gm were selected and divided into two groups. First as - control and group second - experimental. Animals from group second was again classified into three sets as per their temperature differentiation as 39°C, 41°C and 43°C respectively. Animals from group second were subjected to abdominal heat shock for each temperature 39°C, 41°C and 43°C by the interval of 24 hrs, once in a day during morning hours in between 9.30 am to 10.00 am at about 20 minutes upto 96 hrs. After completion of each exposure periods animals were anesthetized with chloroform, sacrifised and cut opened ventrally so as to expose the testicular region (scrotum). Male reproductive tract was exposed to get semen sample from epidymal region. Epididymis was rinsed into 1% saline solution to maintain the sperm viability. Total experiment was repeated thrice for to obtained correct numerical data. Obtained data was subjected to stastical analysis.

3. Parameters under study:

I) Body and testicular weight:

Body weight and testicular weight of experimental animal was measured by standard electronic weight balance after 24 hrs of interval upto 96 hrs.

II) Sperm count:

Sperm count was analyzed as per method described by [14] and formula described by [16], as

Sperm count = total number of sperm in five square× 50000 ×100 (cells / ml)

III) Sperm morphology:

For morphological study, smear of semen sample was prepared and slide allowed to air dry upto 20 min. Smear stained with 10% of Eosin and observed under light microscope (L &M)

at 400x. The morphometric results were described as per [17].

VI) Statistical analysis:

Obtained data was statistically analyzed by graph pad In Stat application. The value of P > 0.05 was considered, as statically non-significant.

RESULTS

Body weight-

Comparatively mean body weight of treated animals before exposure was 207±2.257gm for 39˚C, 269±4.000 gm for 41˚C and 268± 2.887 gm for 43˚c respectively. After completion of acute exposure to the respective temperature Statistical it showed that the body weight of animal treated with 43˚C group was significantly decreased. The mean body weight of rat *Rattus norvegicus* treated with 39˚C and 41˚C was non significant as that of control. Data of body weight after exposure to heat shock and control group was shown in Table -1.

Testes weight

Alterations occurred in testes (left and right) in response to the heat treatments are shown in Table-2 Significant changes in weight of testes was found at 41˚C and 43˚C exposed heat shock group. Weight of testes was found to half of the normal value after 41˚C and 43˚C heat shock treated group. Numerical data is documented in Table 2.

Sperm morphology

In the present study sperm morphology was significantly affected from 41˚C and 43˚C heat shock treated group. After 96 h of exposure, some morphological abnormalities were observed in the all temperature group. 39˚C showed about 18.83% morphological abnormalities, where as 41˚C and 43˚C heat shock treated group showed significantly altered morphological features in sperm (37.33% and 46% respectively). Abnormalities includes head less, curved structure, tail less, double tailed and double headed (Fig- 1). The percentages of abnormal sperm from experimental group were graphically represented in Fig-2.

Sperm count analysis

The mean value of sperm count from control group is 1546 million cells/ml. The mean of sperms count from heat shock

Table-1 : Body weight (mean ± SD) of control and experimental rats. ns- p< 0.05 (non significant), * - p> 0.05 (partially significant), **- p< 0.01(significant), ***- p< 0.001(highly significant).

Body weight					
Temp.	Exposure period				
	Control	24 h	48 h	72 h	96h
39° c	207 ± 2.57	204 ± 9.644 ns	202 ± 7.00 ns	199 ± 8.888 ns	196± 8.505 ns
41° c	269 ± 4.000	267 ± 2.517 ns	266 ± 3.464 ns	264 ± 3.606 ns	258± 2.887 *
43° c	268 ± 2.887	264 ± 4.041 ns	249 ± 6.557 **	239 ± 6.429 ***	231 ± 2.887 ***

Table-2. Testes weight (mean ± SD) of control and experimental rats. ns- p< 0.05 (non significant), **- p< 0.01(significant), ***- p< 0.001(highly significant).

Testes weight		
Temp.	Testes Position	
	Left	Right
Control	6.113 ± 0.3529	6.2.48 ± 0.3.71
39° c	5.911 ± 0.2179 ns	6.010 ± 0.100 ns
41° c	4.1881 ± 0.1114 **	5.143 ± 0.4500 **
43° c	3.725 ± 0.4137 ***	3.7066 ± 0.3522 ***

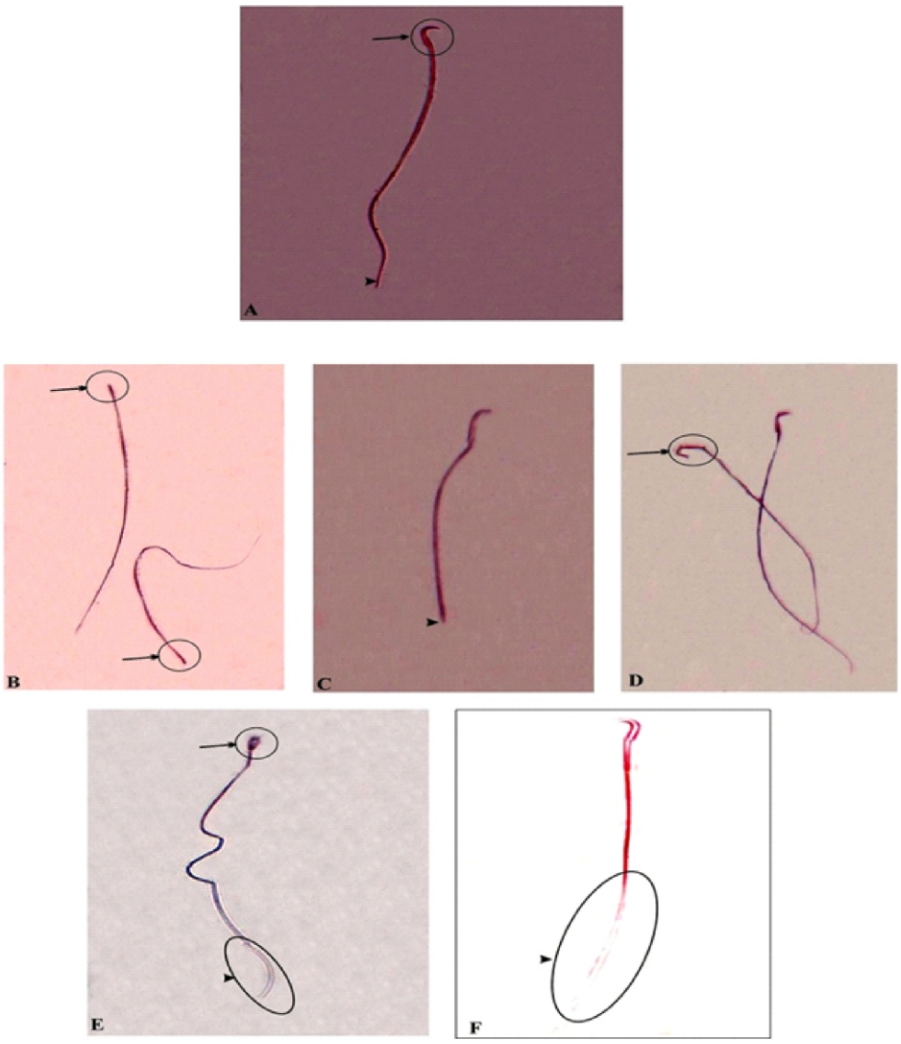

Fig 1. Normal and abnormalities sperms in the rat Rattus norvegicus. A- Normal sperm, B- Head less sperm, C- Tail less sperm, D- Curved sperm, E- Double headed sperm, F- Double tailed sperm

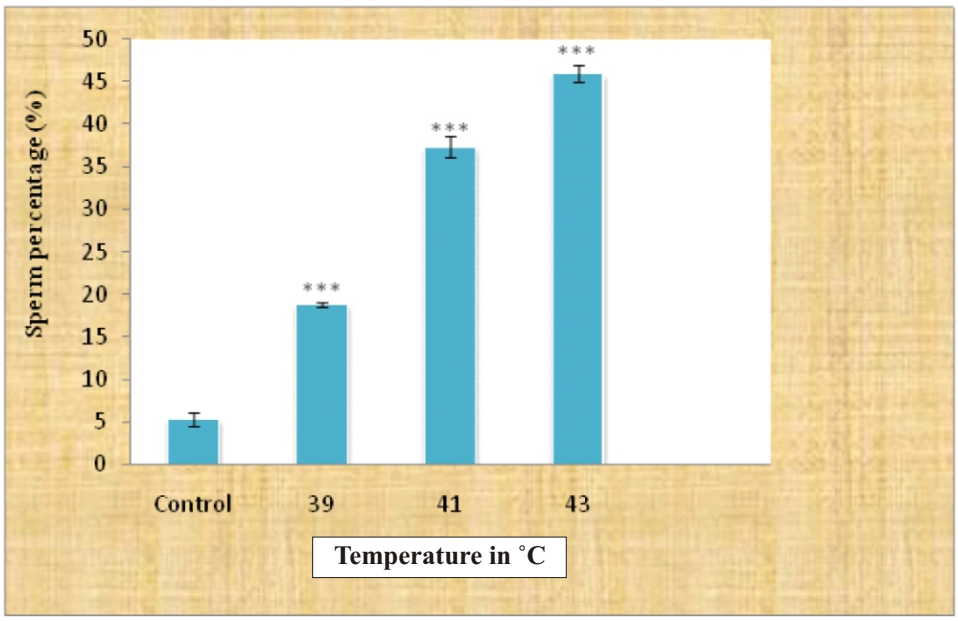

Fig 2. Percentage of abnormal sperm in rats Rattus norvegicus in different temperature. *** - $p < 0.001$ (highly significant).

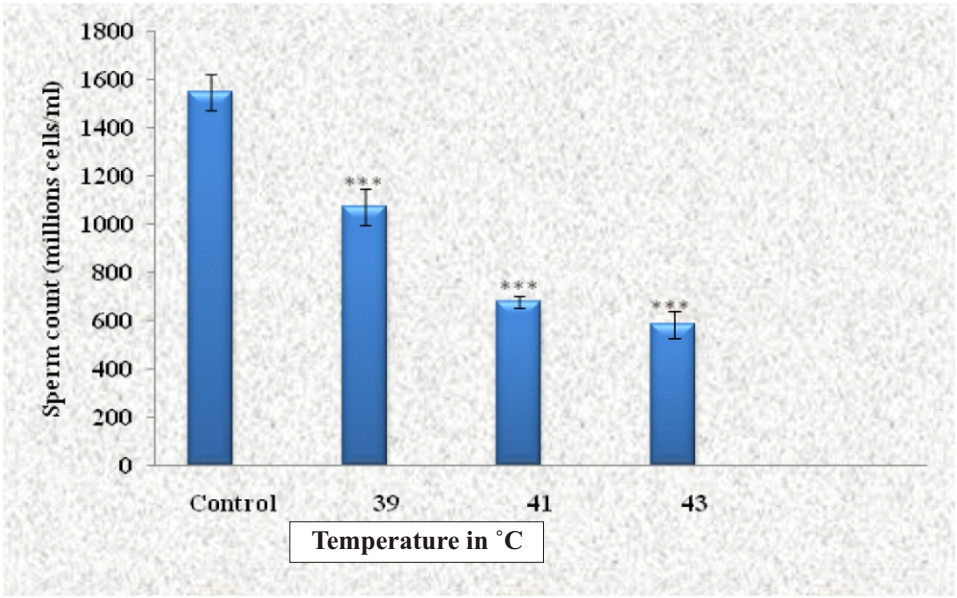

Fig 3. Alterations in rat sperm count in control and experimental group. *** - $p < 0.001$ (highly significant).

treated groups were significantly decreases after 96 h of exposure period. The mean sperm count from 39˚C as 1070 million cells/ml, whereas 678 million cells/ml from 41˚C and 583 million cells/ml from 43˚C after 96 h of exposure period. The result indicate that the there is highly significant difference between sperm count in experimental group as compared with control ($p < 0.001$). The detailed sperm count analysis was expressed graphically in Fig-3.

DISCUSSION

This study evaluated the effect of different temperature on sperm characteristics of adult rats *Rattus norvegicus* at 96 h of exposure period. The results of the present investigation have shown that, heat shock treatment to scrotum of albino rats in a warm water bath at different temperature (39˚C, 41˚C and 43˚C) for acute period produces severe changes in testes weight, sperm morphology and sperm count.

In the present investigation, body weight of rat *Rattus norvegicus* were showed non significant from 39˚C and 41˚C treated group, whereas 43˚C treated group showed significant reduced weight of rats. Similarly [18] reported there was no significant change in body weight of rat after seven days of heat treatment as compared with the control. [19] reported that, the treatment of *Phaleria macrocarpa* (Scheff. Boerl fruit) was significantly reduces the body weight gain and total cholesterol. The weight of testes showed significantly decreases in the entire heat shock group (39˚C, 41˚c and 43˚C) as compared with control after 96 h of exposure period. [20] reported the evaluated temperature reduces the weight of testes in rats. [18] also noted that there is significant decreased weight of testes at 43-5˚C heat treatment for 6 week. [21], reported the effect of phosphorothionates and parathion pesticides causes to decrease weight of testes in rat. [22] noted that, a dose dependent decrease in the weight of testes and assessory sex organs. The other parameters such as sperm count and its morphology were also assessed. The obtained result showed, mean sperm count of Rat *Rattus norvegicus* from all experimental groups (39˚C, 41˚C and 43˚C) found significantly decreased as compared with control ($p < 0.001$). The measure significant count of sperm found in 43˚C heat shock group than the 39˚C and 41˚C. [23] seen the significant decrease the sperm count in rat and monkey after moderate heat exposure. [24] reported reduced sperm count in wistar rats subjected to prolonged treatment of chloromphenicol . [14] studied reduction in sperm count of rats teated with *Phaleria mucrocarpa* can be due to increase in mounting frequency in rats of the same group.

Spermatocytes of rat *Rattus norvegicus* consist of a hook shaped head, neck, mid-pice and a long tail. · Similarly [24] noted that normal sperm cell of wister rats consist of a hook shaped head, a thin neck, mid-piece and long tail. [25] noted that only in mice and rat the heads of spermatozoa terminate in distinct hook shape. [26] suggested that normal sperm morphology is an essential characteristics for in vitero fecundity and in vitro fertilization. The different morphological abnormalities of sperm was found in the present study as head less sperm, tail less, double headed, double tailed. [27] reported increased morphological abnormalities in sperm of Wister rats due to toxic effect of endosulfan. Sperm head abnormalities after treatment of differentiating spermatogenesis are reported in mice [28]

CONCLUSION

Our preliminary finding conclude that acute heat shock treatment affects on testes weight, sperm count and sperm morphology in mature Rat *Ratttus norvegicus.* The present study provides experimental evidences that short term heat exposure impairs the testicular activity.. Our findings are important in future work if the quantitative changes supported by histopathological, immunocytochemical and biochemical findings in the testes of Rat *Rattus norvegicus*.

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REFERENCES

1. Chaves-Pozo E, Mulero V, Meseguer J, Ayala AG. An overview of cell renewal in the testis throughout the reproductive cycle of a seasonal breeding teleost, the gilthead seabream (Sparus aurata L.). Biol. Reprod. 2005: 72:593601.

2. McClusky LM. Stage and season effects on cell cycle and apoptotic activities of germ cells and Sertoli cells during spermatogenesis in thespiny dogfish (Squalus acanthias). Reproduction. 2005: 129:89102.

3. Borg B. Seasonal effects of photoperiod and temperature on spermatogenesisand male secondary sexual characters in the three-spined stickleback, Gasterosteus aculeatus. Can. J. Zool. 1982: 60:33773386.

4. Billard R. Spermatogenesis and spermatology of some teleost fish species. Reprod. Nutr. Dev. 1986: 26:877920.

5. Koger CS, Teh SJ, Hinton DE. Variations of light and temperature regimes temperature and gonad function in male tilapias 543 and resulting effects on reproductive parameters in medaka (Oryziaslatipes). Biol. Reprod. 1999: 61:12871293.

6. Shimizu A. Effect of photoperiod and temperature on gonadal activity and plasma steroid levels in a reared strain of mummichog (Fundulusheteroclitus) during different phases of annual reproductive cycle. Gen. Comp. Endocrinol. 2003:131:310324.

7. Quintana L, Silva A, Berois N, Macadar O. Temperature induces gonadalmaturation and affects electrophysiological sexual maturity indicators in *Brachyhypopomus pinnicaudatus* from temperature climate. J. Exp. Biol. 2004: 207:18431853.

8. Sikhun J, Kijiyanant Y, Vanadurongwan V, Pavasuthipaisit K. Effect of sauna on sperm movement characteristics of normal men measured by computer assisted sperm analysis. 1998: 21: 358-363.

9. Leo PK, Lee, Irving B. Fritz Studies on Spermatogenesis in Rats. The. J. Biol. Che. 1972: 247(24): 7956-7961.

10. Mieusset R, Bujan L. Testicular heating and its possible contribution to male infertility: a Review. Int. J. Androl. 1995 :18:169-184.

11. Egami N, Hyodo-Taguchi Y. An autoradiographic examination of rate ofspermatogenesis at different temperatures in the fish, Oryzias latipes. Exp. Cell. Res. 1967: 47:665667.

12. Vilela DAR, Silva SGB, Peixoto MTD, Godinho HP, Franc¸a LR. Spermatogenesis in teleost: insights from the Nile tilapia (Oreochromisniloticus) model. Fish. Physiol. Biochem. 2003: 28:187190.

13. Wan S , Zhang J, Wang J, Shanxi B. Effects of high fluoride on sperm quality and testicular histology in male rats. Fluoride. 2006: 39(1): 1721.

14. Parhizkar S, Yusoff MJ , Dollah MA. Effect of *Phaleria macrocarpa* on Sperm Characteristics in Adult Rats. Advan Pharmace Bullet 2013: 3(2): 345-352.

15. Ahmad I, Sabir M, Yasin KF. Study of the effects of lead poisoning on the testes in albino Rats. Pakistan. J. Med. Res. 2003: 42(3).

16. Rathje TA, Johnson RK, Lunstra DD. Sperm production in boars after nine generations of selection for increased weight of testis. J. Anim. Sci. 1995: 73(8):2177-85.

17. Soleimanzadeh A, Saberivand A. Effect of curcumin on rat sperm morphology after the freeze-thawing process*Veterinary* Research. Forum. 2013:4 (3): 185 189.

18. Bowler K. The effect of repeated applications of heat on spermatogenesis in the rat: a histological study. J. Rprod. Rert. 1972 : 28: 325-333.

19. Chong SC, Dollah MA, Chong PP, Maha A. Phaleria macrocarpa (Scheff.) Boerl fruit aqueous extract enhances LDL receptor and PCSK9 expression in vivo and in vitro. J. Ethnopharmacol. 2011:137 (1):817-27.

20. Gomes WR, Butler WR, Johnson AD. Effect og elevated ambient tempratuire on testes blood level and invitro bisysntheisis of testosterone in the Ram. J. Ani. Sci. 1971:33: 4-971.

21. Khan IA, Reddy BV. Acute and suacute effects of 2-butaneioc acids 3- (diethooxyphosphinothilo) methyleaster(RPR-I) on testes of albino rat. Indian. J. Expt. Biol. 2002:40: 162-8.

22. Ahmad I, Akhar K, Hussain T. Arsenic induced microscopic changes in rat testis. Profe. Medi. J. 2008:15: 287-291

23. Venkatachalam PS, Ramanathan KS. Effect of moderate heat on the testes of rats and monkeys. J. Reprod. Fert. 1962:4: 51.

24. Oyeyemi OM, Adeniji DA. Morphological Characteristics and Haematological Studies in Wistar Rats Subjected to Prolonged Treatment of Chloramphenicol. Int. J. Morphol. 2009 :27(1):7-11.

25. Garner DL, Hafez ESE. Spermatozoa and seminal plasma. In: Reproduction in Farm Animal.6th edn. Editedby: Hafez, E. S. E. Philadelphia, Lea and Febiger 1993: 165-87.

26. Nallella KP, Sharma RK, Aziz N, Agarwal A. Significance of sperm characteristics in the evaluation of male infertility. Fertil. Steril. 2006:85(3):629-34.

27. Dalsenter PR, de Araujo SL, de Assis HC, Andrade AJ, Dallegrave E. Pre and postnatal exposure to endosulfan in Wistar rats. Hum. Exp. Toxicol. 2003: 22(4): 171175.

28. Alventric D, Sujeraric I, Pecerski J. In vivo genotoxicity of nitatres and nitasrtes in germ cell of male mice 1. Evidances for gonadal exposure and lack of heritable effect. Mutat. Res. 1988:204(4): 689-95.