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EFFECTS OF PREDATOR INDUCED PSYCHOSOCIAL STRESS ON SPERM PARAMETERS OF ALBINO WISTAR RATS

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ABSTRACT

Aim and objectives: This study evaluated the durational effects of predator-induced psychosocial stress on the sperm parameters using animal models. **Materials and Methods:** The rats were divided into four (4) equal groups (n=5). Groups A to B (short duration for 52 days) and groups C to D (long duration for 104 days). Groups A and C served as the controls, which were not stressed, while Group B and D were psychosocially stressed in the PTSD chamber at 3hr/exposure daily for 52 and 104 days respectively. The animals from Group A/B and C/D were sacrificed on the 53rd and 105th day respectively, and their total sperm count (×106 cells/ml), abnormal morphology (%), motility (%), immotility (%), progressive (%), and non-progressive (%) sperm determined. GraphPad Prism Version 8 (San Diego, USA) was used to analyse the data and the results described as mean ± Standard Error of Means (S.E.M.). Unpaired t-test (at 95% confidence level) was used to compare values of the control and experimental groups. **Results:** The result showed that the mean(S.E) sperm count (×106) for control A (39.00±3.32 cells/ml) and B (40.00±3.16 cells/ml) were significantly greater than their experimental groups (52 days [17.00±1.23 cells/ml] and 104 days [18.05±1.23 cells/ml]) respectively (P<0.001). On the other hand, the abnormal, immotile, and non-progressive sperm cells were significantly greater (P<0.01) in the experimental groups when compared to their respective controls; with better sperm parameters observed at day 104 when compared to day 52. **Conclusion:** The initial exposure of animals to chronic stress for 52 days was deleterious to sperm parameters; however, further exposure (twice the initial duration) produced results that suggested a kind of coping mechanism that reduced the damages.

KEYWORDS: Sperm parameters, Chronic stress, Psychosocial stress, Animal model.

1 INTRODUCTION

Psychosocial is a term that describes the words 'psychological' and 'social',[1] and the aetiology of psychosocial stress emanates from environmental occurrences and events of social origin which an individual undergoes, challenging his/her ability to cope.[1,2] Psychosocial stress is inevitable in life, as every human at a point in time in life will be exposed to it; either in the workplace, home or convivial environment. Stress can either be acute or chronic. The acute form of stress can be profitable, such as witnessed by athletics during training; however, when it becomes chronic, then it can lead to various detrimental effects.[3]

There are a lot of idiopathic simulants for the decline in male reproductive function;[4] However, studies have shown an inverse relationship between chronic stress and male reproduction.[3,5,6] Also, exercise and work-related stress have been documented to possess negative effect on the male reproductive system;[7-10] however, whether there was improvement or continuous decline based on the duration of stress exposure could not be resolved. The irreversibility of the deleterious effects of stress-induced from restraining and forced swimming has been investigated using animal model,[11] and it was found that the increased duration resulted in a significant increase in abnormal spermatozoa, steady degeneration of the histoarchitecture of the seminiferous tubules. However, MDA concentration, and sperm counts were highly depleted within one-month exposure and remained at that level for up to six months.

Adaptation plays a key role in sustaining the continuity of life, especially during unfavourable conditions. Studies have revealed that animals tend to adapt to unfavourable conditions to survive.[12,13] Duration of exposure, reoccurrence of a particular stressor, variation of the different stressor and animal perception play a significant role on the consequence of the stress on the individual.[14-16] In modelling chronic stress, psychosocial stress is induced in animals in a way that mimics the actual stimulus in human,[17,18] and this includes; stress caused by activities that elicit fear, death of a loved one, restrain (irresponsiveness) and helpless, and a

reoccurrence of the traumatization for short and long durations. This study, therefore, investigates the durational effect of psychosocial stress on the quality and quantity of sperm parameters.

2 MATERIALS AND METHODS

2.1 Research Design

This research was designed as an animal model (wistar rats) for mimicking chronic stress, using predator (cat)-induced psychosocial stress. This was done with the knowledge that rats exhibits strong innate fear of cat (Predator),[17,18] which will put the rat under a lot of emotional stress. Animal handling was carried out in line with the international, national, and institutional guidelines for the care and use of Laboratory Animals for Biomedical Research as documented by the Canadian Council of Animal Care (CCAC).[19]

2.2 Animal Care and Grouping

2.2.1 Animal (rats and cats) procurement and Care: Twenty (20) 170-250g male albino Wistar rats (*Rattus Norvegicus*) and the two cats (a male and a female) were used for this study. The procurement, and care have been described in previous study by the researchers.[18]

2.2.2 Grouping: The animals were divided into four (A, B, C and D) equal groups of 5 rats randomly placed into four different cages. To ensure close average body weight, the animals in the cases were weighed using a top loader weighing scale. The 5 rats in the four different were tagged on different parts of their body using indelible ink. This is to ensure that the rats were not mixed up during the research.

2.3 Procedure for psychosocial stress induction

The Wistar rats in Group B were kept in a 20×20×10cm metal cage restrainers (Post-Traumatic Stress disorder [PTSD] chamber) and taken to the cats housing room where they were placed on the floor of the room with the adult cats. The metal cage restrainer prevented any physical contact between the cats and the rats, but the rats were exposed to all sensory stimuli associated with the cat. Cat feed were given to the cats to increase cat motor activity. Each rat was placed in between the predator cats at certain duration and intervals according to Uwejigho, *et al*.[18] and Wilson *et al*.[20] The PTSD model has been reported to elicit systemic responses similar to those observed in humans under chronic stress in humans.[21-23]

2.4 Experimentation

The experiment lasted for 52 days and 104 days. Groups A, served as the control to Group B and was not psychosocially stressed. Group B animals were psychosocially stressed in the PTSD chamber for 3hr/exposure daily for 52 days. All rats in the groups A and B were sacrificed on the 53rd day. Group C, served as the control to Group D and was not psychosocially stressed. Group D animals were psychosocially stressed in the PTSD chamber for 3hr/exposure daily for 104 days. All rats in the groups C and D were sacrificed on the 105th day.

2.5 Animal sacrifice and sample collection

At the end of each experimental phase, all the rats were first weighed and then anaesthetised by placing them in a jar containing cotton wool soaked with chloroform anaesthesia. A midline incision was made through the ventral wall of the abdomen. Blood samples were obtained from the Inferior Vena Cava of each rat for Biochemical analysis. The testes and epididymis were excised, and spermatozoa collected from the caudal epididymis before fixing in Bouin's fluid prior to histological preparation.

2.5.1 Sperm analysis

Total sperm count was done using a light microscope with the aid of the improved Neubauer counting chamber (haemocytometer). The cauda epididymis was carefully dissected from the testis and 2 ml of normal saline was added to obtain the suspension. The obtained suspension was diluted with sodium bicarbonate-formalin in 1:20. The haemocytometer chamber was filled with the thoroughly mixed and diluted sperm before being placed under a binocular light microscope.[24] Using an adjustable light source, the ruled part was focused and the number of spermatozoa counted in five 16-celled squares. The total sperm cells were added and multiplied by 10^6 and expressed as (X) x 10^6/ml, where x is the total number of sperm cells in five 16-celled square.[25]

The progressive motility of sperm was tested immediately by obtaining Spermatozoa from the cauda epididymis onto microscope slide, two drops of normal saline was added and covered with a cover slip. The percentage motility was evaluated visually at a magnification of x400. The estimation of motility was done from the mean of three different fields in each sample, and stored at 35°C.[25]

Sperm morphology was evaluated by staining sperm smears on a light microscope slide. Caudal epididymis sperm obtained was diluted with 1:20 ratio with 10% neutral buffered formalin. scoring of morphological abnormalities was done using five hundred sperm from the sample, spermatozoon with round or detached head, rudimentary tails were considered abnormal and indicated as percentage of sperm with normal morphology.[25]

2.6 Statistical Analysis of the data

All statistical analysis was carried out using GraphPad Prism (version 8) software manufactured by GraphPad Software Inc (San Diego, USA). The analysed data were presented in tables and the values described as mean ± Standard Error of Means (S.E). The significance of difference in the means of all parameters was determined using unpaired student t-test. P values less than 0.05 was taken to be significant.

2.7 Ethical Consideration

Prior to the commencement of this research, ethical clearance with reference number UPH/CEREMAD/REC/MM55/012 was obtained from the University of Port Harcourt Ethics Committee, Rivers State, Nigeria.

3 RESULTS

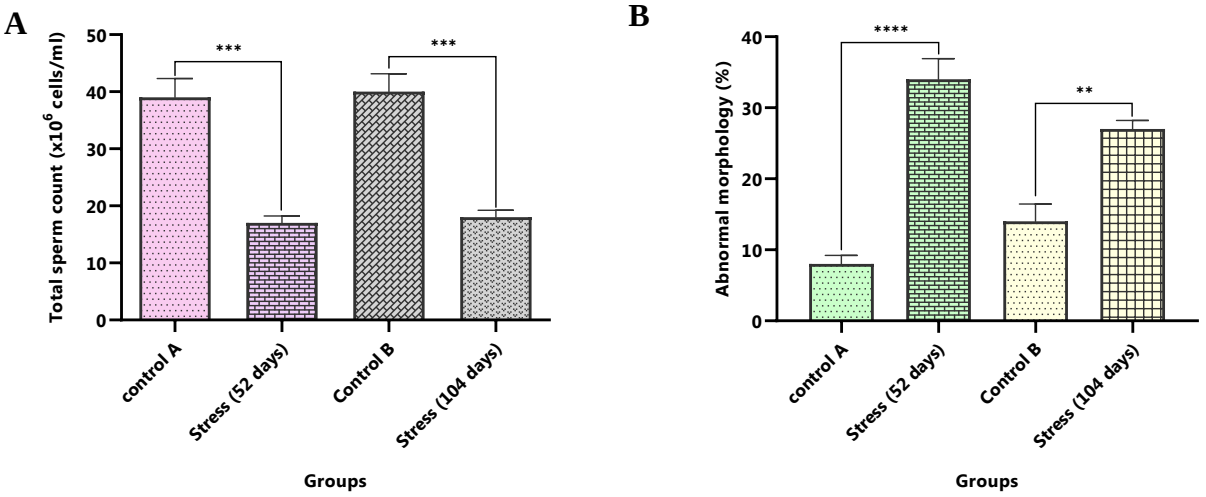
The descriptive statistics of the sperm parameters; total sperm count, motility, progressive motility, non-progressive motility, immotility and abnormal morphology of the different groups were described as mean (SE) in Table 1; with the unpaired t-test of mean difference between the control and experimental groups presented in Fig. 1A-F.

In Fig. 1, there mean(S.E) sperm count ($\times 10^6$) for control A (39.00±3.32 cells/ml) and B (40.00±3.16 cells/ml) were significantly greater than their experimental groups (52 days [17.00±1.23 cells/ml] and 104 days [18.05±1.23 cells/ml]) respectively (P<0.001) (Fig. A). The mean motile and progressive sperm cells were significantly greater in the control (motile; control A = 97.00±1.23% [P<0.01], control B = 92.00±3.39% [P<0.01] and progressive; control A = 90.00±3.16% [P<0.0001], control B = 84.00±2.45% [P<0.01]) when compared to the experimental groups (motile; stress 52 days = 46.00±6.78%, stress 104 days=76.00±5.01% and progressive; stress 52 days 20.00±4.47%, stress 104 days = 53.00±6.04%) (Fig. C & E).

On the other hand, the mean(S.E) abnormal, immotile, and non-progressive sperm cells were significantly greater in the experimental groups (abnormal morphology; stress 52 days = 34.00±2.92% [P<0.0001], stress 104 days = 27.00±1.23% [P<0.01], immotile; stress 52 days = 54.00±6.78% [P<0.0001], stress 104 days = 23.00±2.49 [P<0.001], and non-progressive; stress 52 days = 26.00±2.45% [P<0.0001], stress 104 days = 23.00±3.00 [P<0.01]) than control groups (abnormal morphology; control A = 8.00±1.23% [P<0.01], control B = 14.00±2.45% [P<0.01], immotile; control A = 3.00±1.23% [P<0.0001], control B = 6.00±2.45% [P<0.01], and non-progressive; control A = 7.00±1.23% [P<0.0001], control B = 10.00±1.58 [P<0.01]) (Fig. B, D & F).

Table 1: The mean (S.E) values of the sperm parameter in control and experimental groups.

Sperm Parameters	Control A	Stress (52 days)	Control B	Stress (104 days)
Total sperm count ($\times 10^6$ cells/ml)	39.00±3.32	17.00±1.23	40.00±3.16	18.05±1.23
Abnormal morphology (%)	8.00±1.23	34.00±2.92	14.00±2.45	27.00±1.23
Motility (%)	97.00±1.23	46.00±6.78	92.00±3.39	76.00±5.01
Immotility (%)	3.00±1.23	54.00±6.78	6.00±2.45	23.00±2.49
Progressive (%)	90.00±3.16	20.00±4.47	84.00±2.45	53.00±6.04
Non-progressive (%)	7.00±1.23	26.00±2.45	10.00±1.58	23.00±3.00



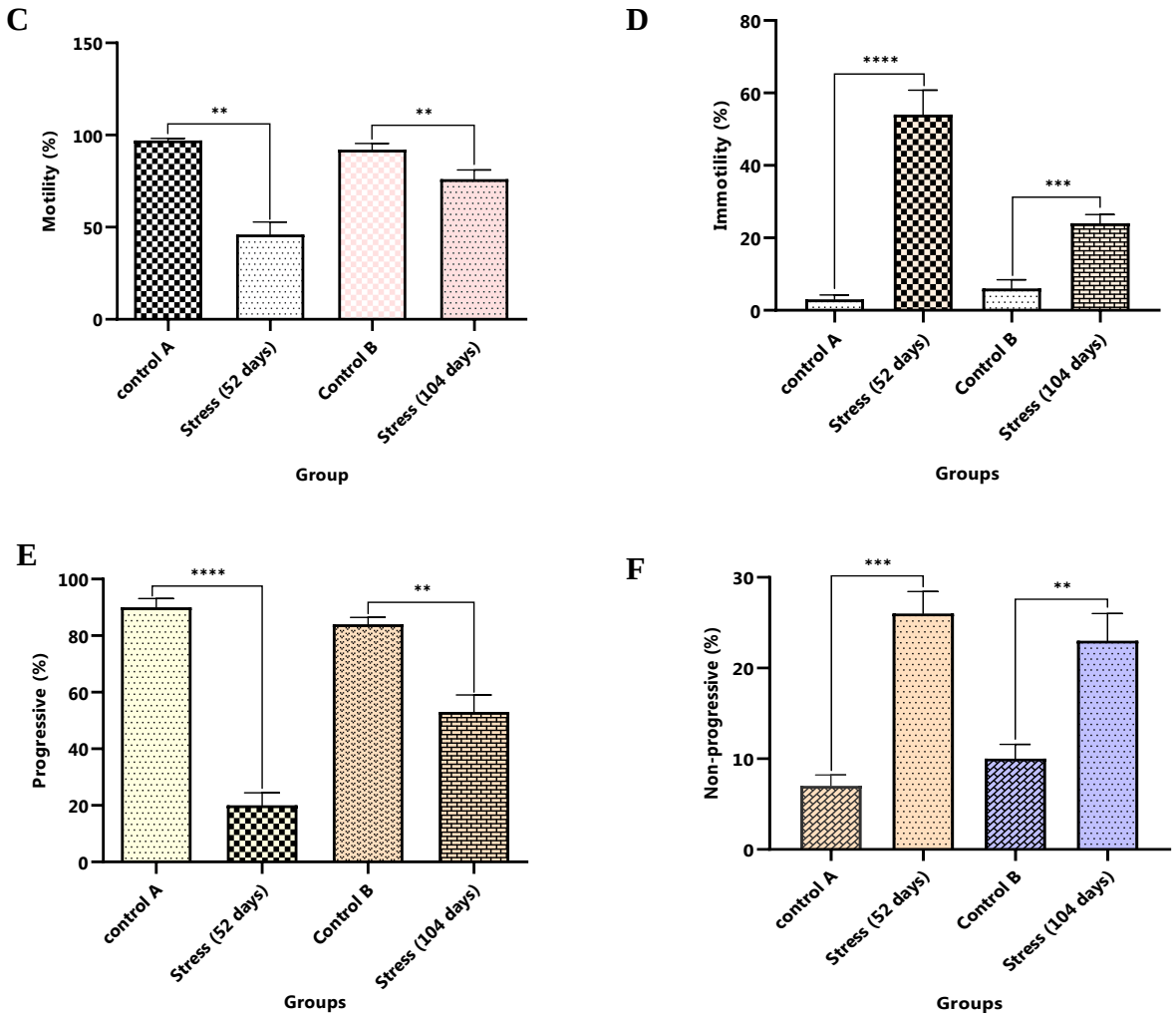


Figure 1: Mean (S.E) and unpaired t-test of the mean differences of the sperm parameters of the controls and experimental groups (n=5/group). ***(A) Sperm motility, (B) Sperm immotility, (C) Progressive sperm, (D) Non-progressive sperm, (E) Abnormal morphology, (F) Total Sperm count*** **(*P<0.05, **P<0.001, ****P<0.0001).**

4 DISCUSSION

This study evaluated the short and prolonged effect of predator-induced psychosocial stress on the sperm parameters of adult male Wistar rats. The present study reveals that chronic exposure to predator-induced psychosocial stress caused a significant reduction in total sperm count, motile and progressive sperm, which was more most noticeable in 52 days when compared to 104 days. Thus, indicating that chronic stress is deleterious to the reproductive system; as reported by earlier studies,[26-29] which found that stress causes generalized degenerative changes in the testis, suppression of spermatogenesis, and reduction in testosterone levels.[26-29] On the other hand, with prolonged exposure, the system developed some form of coping mechanism to reduce the further adverse effect, and this in some studies have been referred to as adaptation or resilience.[11,12,30]

The major factor responsible for the deterioration of spermatogenesis, fertility and male sexual behaviour observed in males during stress is the inhibition of testosterone due to steroidogenic restraint and programmable cell death of Leydig and testicular germ cells.[2,26] Males exposed to stressful conditions can develop erectile dysfunction, reduced sexual motivation, and infertility. However, stress is not the only causative agent of infertility, but it is treated as an important risk factor for idiopathic infertility.[16] Reduced semen quality and concentration has been observed in males exposed to emotional stress at work and even on the depressive reaction to infertility or its remedy.[15] Psychological stress has a diverse negative outcome on the volume of semen, motility, concentration,[31,32] and sperm morphology.[27-29] It is also known to heighten abnormalities of sperm in healthy men.[2]

These decline in male fertility associated with chronic stress is brought about by the reduction in the testosterone level as a result of the stress, however, stress hormones can have a direct negative impact on the seminiferous epithelium, crippling spermatogenesis.[33] This is because the process of spermatogenesis is dependent on testosterone which is arrested during severe chronic stress.[2] In animal models, research has

shown that chronic stress can lead to a decline in the area of seminiferous epithelial, decreased spermatogonia type A, spermatocytes and spermatids.[34] Additionally, reduced sperm viability, motility and concentration have been observed in rats exposed to chronic stress.[2,28-30]

5 CONCLUSION

The initial exposure of animals to chronic stress for 52 days was deleterious to sperm parameters; however, with longer exposure, the adverse effects of stress tend to wane, suggesting that the possibility of adaptation even when the animals were still under the same circumstance of the stressful situation.

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