ORIGINAL ARTICLE

EXOGENOUS OXYTOCIN IMPAIRS CAUDAL EPIDIDYMAL SPERM CHARACTERISTICS IN SPRAGUE-DAWLEY RATS

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ABSTRACT

Background: Oxytocin is a neuropeptide hormone that is released from posterior pituitary gland and has been shown to increase cervical contractions, modulates sperm production, transport and ejaculation. **Objectives:** This study aimed at determining the effect of exogenous oxytocin on caudal epididymal sperm characteristics in Sprague-Dawley rats. **Method:** Forty adult male Sprague-Dawley rats weighing between 180-300 g were randomly distributed into 4 groups A, B, C and D of 10 rats each. Group A served as the control group while groups B to D were the treated groups. Oxytocin was administered intramuscularly to groups B, C and D at the doses of 1 IU/kg/body weight(b.w.), 2 IU/kg/b.w. and 3 IU/kg/b.w. two days of three days interval per week respectively while 0.5ml of 0.9% physiologic saline was administered to group A. The treatment was carried out for a period of 8 weeks. **Results:** The result showed a significant increase in the mean caudal epididymal sperm volume among the groups that received 2 IU/kg/b.w. and 3 IU/kg/b.w. C and D at 4weeks and 8weeks when compared with control ($p<0.05$). The caudal epididymal sperm concentration of the treated groups showed a significant decrease compared to the control at 4 and 8weeks ($p<0.001$). Groups that were administered with 2 IU/kg/b.w. and 3 IU/kg/b.w. oxytocin showed a significant decrease in sperm concentration compared to control and the lowest dose oxytocin treated group ($p<0.001$) at 8weeks. **Conclusion:** Chronic intramuscular administration of oxytocin has significant deleterious effect on caudal epididymal sperm concentration.

Keywords: Oxytocin, Epididymal Sperm Characteristics, Sprague-Dawley Rats, Intramuscular, Neuropeptide Hormone

INTRODUCTION

Oxytocin is a neurohypophysial hormone secreted by the hypothalamic magnocellular neurons and stored in the posterior pituitary until its release into the blood stream.[1] It modulates mammalian sexual behaviour.[2-4] It has also been shown to increase cervical contractions, modulate sperm production and transport.[5] It also effects secretion of sex steroids.[6,7] An experiment in mice showed that intra-testicular injection of oxytocin increased basal testosterone level.[7] Plasma oxytocin levels increase around the time of ejaculation in rams,[8] rabbit[9,10] and man.[11,12] The oxytocin rise during ejaculation may be important for spermatogenesis as oxytocin has been shown peripherally to increase sperm volume and concentration in ejaculate in a number of species.[13] In man, oxytocin doubles the number of ejaculated sperms in oligozoospermic patients.[14] The degree of sexual excitation before collection is important in determining the volume and quality of semen ejaculated by bulls.[15] High levels of oxytocin have been found in samples of blood taken from bulls immediately before and immediately after 'service' compared with those from bulls which were not sexually excited.[16] Injection of oxytocin

immediately before service increased the volume of semen from both bulls and rabbits.[17]

Sperm production is among the most important determinants of the reproductive capacity of an individual male. Though oxytocin has been shown to increase the sperm concentration per ejaculate immediately following administration in many studies, no study to the best of author knowledge has been conducted over a complete spermatogenic cycle (56days) to demonstrate the effect of oxytocin on sperm seminal fluid characteristics. This study focuses on the effect of varying doses of exogenous oxytocin on caudal epididymal sperm characteristics (sperm volume, concentration, pH, percentage motility and morphological abnormality) in Sprague-Dawley rats.

MATERIALS AND METHOD

Materials used in this study include: adult male Sprague-Dawley rat (180-300g), plastic rat cage, oxytocin injection, eosin-nigrosin, 96% ethanol, needle and syringe, light microscope, Improved Neubauer Counting Chamber, Dissecting set etc.

Procurement of Animals

Forty adult male Sprague-Dawley rats weighing between 180-300g were used for this experiment. They were purchased from commercial farms in Ogbomoso, Oyo State. The animals were housed in well-ventilated plastic cages under standard room temperature, in the Animal house of the Department of Anatomy, College of Medicine of the University of Lagos. The rats were fed with Livestock Balanced Rations Growers Mash which was purchased at the general Mushin market, Lagos and clean tap water was provided ad libitum. The rats were acclimatized for two weeks before the commencement of the experiment.

Experimental and Control Drugs/Solution

Oxytocin(Hubei Tianyao Pharmaceutical, China, marketed by Pemason Pharmaceutical, Agege Road, Alakara, Lagos, Nigeria) was administered intramuscularly at the doses of 1 IU/Kg/bw, 2 IU/Kg/bw and 3 IU/Kg/bw,two days of three days interval in a week for the treatment groups while 0.5ml of 0.9% physiologic saline was administered to the control group. The treatment was carried out for the period of 8 weeks.

Grouping of the Animals

Forty adult male Sprague-Dawley rats weighing between 180 and 300 g were, randomly distributed into 4 groups (A, B, C and D)of 10 rats each.

i. Group A: (Control) administered 0.5ml of 0.9% N/S (Physiologic saline) biweekly

ii. Group B: Oxytocin administered at the dose of 1 IU/Kg/bw

iii. Group C: Oxytocin administered at the dose of 2 IU/Kg/bw

iv. Group D: Oxytocin administered at the dose of 3 IU/Kg/bw.

Weighing: The rats were weighed prior to the commencement of experimentation and thereafter weekly using a top loading digital scale (NV2101 model, OHAUS Corporation USA).

Experimental protocol: Oxytocin was administered at the doses of 1 IU/Kg/bw, 2 IU/Kg/bw and 3 IU/Kg/bw two days of three days interval in a week to the treatment groups (B, C and D) while physiologic saline 0.5ml was administered to the control group A. The administration was done for the period of 8 weeks. Four weeks into the experiment 5 rats per group were sacrificed while the remaining 5 rats in each group were sacrificed at the end of 8 weeks. The rats were sacrificed by cervical subluxation followed by laparotomy to harvest the testes.

One of the testes was blotted dry and the caudal epididymis neatly excised and the epididymal fluid obtained by the "swim up" technique[18]for immediate sperm motility assessment while the other was used for the assessment of the epididymal sperm volume, pH, concentration and total sperm count.

Immediately after cervical subluxation blood was collected by cardiac puncture, centrifuged at 1000 g, for 10 min in an angle head centrifuge. Serum collected was immediately assayed for hormonal assay (testosterone levels).

Animal ethics

This study was approved by the Department of Anatomy Ethical Committee on the Use and Care of Animals in conformity with international

acceptable standards and conformed to the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals[19]

Seminal Fluid Analysis (Seminogram): The volume of the samples was taken by direct method using a 1ml graduated test tube. Liquefaction was complete within 30mins of collection of samples.Appearance was observed macroscopically while the samples were in plain universal bottles.Sperm motility was assessed within an hour of collecting the samples by preparing a wet preparation on a slide covered with a cover slip. The slide was then viewed using a phase-contrast microscope and observed under x40 objective lens. The percentage of progressive motile spermatozoa was assessed according to the WHO guidelines 2010.[20]

Sperm count was done by diluting the semen sample using 1:20 dilution which was loaded on improved Neubauer counting chamber. The chamber was mounted on microscope stage and the number of spermatozoa observed on all the grids was counted.

The morphology of the spermatozoa was assessed by preparing a smear of semen on a slide, air-drying, fixed in 96% ethanol and stained with eosin-nigrosin dye which was then mounted on the microscope stage and examined with oil immersion objective lens.

Testosterone assay

Blood sera collected were evaluated in groups from a standardized curve using the enzyme linked immunosorbent assay (ELISA) method.[18,21] The microwell kits used were from Syntro Bioresearch Inc., California USA. Using 10 µl of the standard, the samples and control were dispensed into coated wells. 100 µl T conjugate reagent was added followed by 50 µl of anti-T reagent. The contents of the microwell were meticulously mixed and then incubated for 90 min at room temperature. The mixture was washed in distilled water and further incubated for 20 min. The reaction was stopped with 100 µl of 1N hydrochloric acid. Absorbance was measured with an automatic spectrophotometer at 450 nm.

Statistical Analysis

Statistical evaluation was done by randomized complete block analysis of variance (ANOVA). Significant difference was acknowledged if $p < 0.05$. The hypothesis tested was that no change in the progressive motility of spermatozoa or the percentage of morphologically normal spermatozoa, but an increase in total number of spermatozoa, in the epididymal sperm, when Sprague-Dawley rats were treated with oxytocin compared with sterile physiological saline.

RESULTS

The results of this study generally showed the effect of varying doses of administered exogenous oxytocin on seminal fluid characteristics. All semen samples had normal alkaline pH, which was within the normal limit (7.2 – 8.0). There was no significant difference in the appearance, liquefaction and viscosity between the oxytocin treated groups and the control. The major observed morphological abnormality of the spermatozoon was double tail. However, worthy of note was a significant increase in the number of abnormal sperms in a dose dependent manner among the treated groups at 8 weeks with highest level of abnormality (double tails) found in group D that received the highest dose of oxytocin.

The various results for the investigated parameters are depicted in Tables 1-4. Table 1 showed the effect of exogenous oxytocin on epididymal sperm volume. It revealed that exogenous oxytocin at the doses of 2 and 3 IU/kg/bw increased the epididymal sperm volume (0.94±0.20) and (1.08±0.25) respectively at 4 weeks and 3 IU/kg/bw at 8 weeks significantly increased the sperm volume (2.67±0.33). The group that received 3 IU/Kg/bw of oxytocin maintained a larger volume of caudal epididymal sperm.

The effect of exogenous oxytocin on epididymal sperm concentration was shown on table 2 which revealed that 3 IU/Kg/bw oxytocin significantly decreased epididymal sperm concentration ($47.08\pm3.63^{***}$) at 8 weeks.

There was significant decrease in sperm motility among the group that received 2 IU/kg/bw. Of oxytocin at 4 and 8 weeks with $p < 0.05$ and $p < 0.01$ respectively as shown in Table 3.

Table 4 showed the effect of exogenous oxytocin on serum testosterone which was increased in groups that were administered with 2 and 3 IU/kg/bw. Of oxytocin at 4 and 8 weeks ($p < 0.05$)

Table 1: Effect of Exogenous Oxytocin on Epididymal Sperm Volume (ml)

		Seminal Fluid Volume at	
Groups	**Doses**	**4 weeks**	**8 weeks**
A	0.5ml of 0.9% N/S	0.21±0.03	0.50±0.29
B	1 IU/Kg/bw of Oxytocin	0.62±0.05	1.25±0.48
C	2 IU/Kg/bw of Oxytocin	0.94±0.20*	2.00±0.41
D	3 IU/Kg/bw of Oxytocin	1.08±0.25*	2.67±0.33*

Values are expressed as mean ± SD; N/S =Normal Saline, *Indicates significance from control at $p < 0.05$

Table 2: Effect of Exogenous Oxytocin on Epididymal Sperm Concentration ($x10^6$/ml)

		Epididymal Sperm Concentration at	
Groups	**Doses**	**4 weeks**	**8 weeks**
A	0.5ml of 0.9% N/S	31.60±9.90	97.50±4.62
B	1 IU/Kg/bw of Oxytocin	2.16±0.69	88.44±3.90
C	2 IU/Kg/bw of Oxytocin	8.74±2.41	50.31±1.39***
D	3 IU/Kg/bw of Oxytocin	25.55±12.56	47.08±3.63***

Values are expressed as mean ± SD; N/S =Normal Saline, ***Indicates significance from control at $p < 0.001$

Table 3: Effect of Exogenous Oxytocin on Sperm Motility (%)

		Sperm Motility at	
Groups	**Doses**	**4 weeks**	**8 weeks**
A	0.5ml of 0.9% N/S	66.0±6.00	62.50±6.29
B	1 IU/Kg/bw of Oxytocin	60.0±4.47	57.50±5.95
C	2 IU/Kg/bw of Oxytocin	31.0±6,21**	30.0±7.91*
D	3 IU/Kg/bw of Oxytocin	52.0±4.06	48.33±6.01

Values are expressed as mean ± SD; N/S =Normal Saline, *Indicates significance from control at $p < 0.05$, ** indicates significance from control at $p < 0.01$

Table 4: Effect of Exogenous Oxytocin on Serum Testosterone

		Serum Testosterone at	
Groups	**Doses**	**4 weeks**	**8 weeks**
A	0.5ml of 0.9% N/S	0.20±0.15	0.23±0.15
B	1 IU/Kg/bw of Oxytocin	0.60±0.21	0.83±0.43
C	2 IU/Kg/bw of Oxytocin	3.10±0.97*	0.43±0.09
D	3 IU/Kg/bw of Oxytocin	3.17±0.49*	2.23±0.96*

Values are expressed as mean ± SD; N/S =Normal Saline, *Indicates significance from control at $p < 0.05$

DISCUSSION

Presented in this work is the consequence on the reproductive integrity of male Sprague-Dawley rats after oxytocin injection at doses 1, 2 and 3 IU/kg/b.w. for 4 and 8 weeks.

At 4 and 8 weeks, when compared to the control, oxytocin significantly increased the caudal epididymal semen volume among the treated groups (which were more marked in groups that received 2 and 3 IU/kg/b.w.). Also noted was a

significant increase in the number of doubled tailed abnormal spermatozoa among the rats that received the highest dose (3 IU/kg/b.w.) of oxytocin at 8 weeks. In addition, caudal epididymal sperm concentration and motility of the treated groups correspondingly showed a statistically significant decline, with the reduced values more striking in Groups that were administered with 2 and 3 IU/kg/b.w. of oxytocin. This increase in caudal epididymal seminal volume agreed with the results of Nicholson et al,[22] and Milovanov et al.[23] However, the findings of decrease in concentration of spermatozoa per caudal epididymal semen were contrary to those of Ratnasooriya et al,[24] Nicholson et al,[22] and Milovanov et al,[23] in bulls and in rabbits. Some researchers working with rabbits, obtained indirect evidence that oxytocin also stimulated the accessory sex glands.[25,26]

The study also presents confirmation of some degree of perturbation of the central regulatory conduit for testicular function which is the hypothalamic-pituitary-testicular axis that culminates in production of spermatozoa.[18,27] This is evidenced by the evaluated serum hormonal testosterone levels in the experimental rats. It was observed that in the 4- and 8-weeks oxytocin treated groups compared to control, the peripheral serum testosterone levels increased in value (more marked in groups that received 2 and 3 IU/kg/bw. at 4 weeks as well as 1 and 3 IU/kg/bw. at 8 weeks). These results were largely not in tandem with similar study by Nicholson *et al*,[28]that showed a significant reduction of testicular and plasma testosterone levels throughout the 4 weeks study period in an *in-vivo* long-term administration of oxytocin to the adult rat testis. This decline in testosterone concentrations were also accompanied by alterations in spermatogenesis or epididymal sperm counts.

The expected outcome for an increased plasma testosterone level is a concomitant increase in total sperm concentration although when testosterone is in excess it may result in a negative feedback on the hypothalamic-pituitary-testicular axis compromising spermatogenesis.[29]Exogenous oxytocin caused a significant elevated peripheral testosterone levels with a decrease in ***spermatogenesis*** (sperm concentration and volume). The exact mechanism is subject to further studies, but it may not be unconnected with perturbation of the hypothalamic-***pituitary-gonadal axis***.

CONCLUSION

Testosterone and epididymal sperm volume were increased by administration of oxytocin, it also had deleterious effect on sperm morphology, motility and concentration. It is therefore recommended that clinicians should avoid chronic administration of oxytocin to men with decrease in the sperm count/number (oligospermia) and motility (asthernozospermia). More research is needed to elucidate the possibilities of reversal of these effects.

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