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EFFECT OF DIRECT ICE-CONTACT INTERFERENCE ON SPERMATIC ACTIVITIES & INTEGRITY IN ALBINO WISTAR RATS.

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ABSTRACT

Aim/Background: The primary objective of this research was to evaluate the possible detrimental effect of ice on the epididymis architecture of scrotal testis, and its interactions with sperm parameters. **Method**: The study was done on adult wistar rats divided into four (4) groups within the weight range of 210-250g.The experimental groups were anaesthetized with 0.5mg/kg of thiopental in normal saline and each rat was administered with the drug, and just after the onset of sleep, the scrotal testis was positioned to lie directly in contact with ice for specific durations. Group I, was the control group that were not anaesthetized while Group II – IV were anaesthetized and ice directly in contact with the scrotal testis for one hour, 45 minutes, and 30 minutes respectively. After this duration of exposure the animals were castrated and semen collected. Spermatozoa were screened for percentage viability, percentage sperm motility, sperm count, percentage dead sperm cells, percentage sluggish sperm cells, and percentage active sperm cells ($P < 0.05$).Statistical analyses of the data were done using one – way ANOVA and comparisons among treatment groups were made using student t-test. **Result**: The result of the study indicates that application of ice significantly affected sperm parameters by reducing the percentage motility, sperm count, activeness and sluggishness of sperm cells and also increasing percentage in dead sperm cells and slight increase in percentage sperm viability ($P < 0.05$). **Conclusion**: The study suggested, cold and cold shock and their interactions are important sources of variation in sperm parameters and histological changes in the epididymis architecture of the scrotal testis. It is therefore recommended that the environment as it pertains to season should be given urgent attention in human and animal reproduction in order to obtain the best semen quality.

KEYWORDS: ice-contact, epididymis architecture, scrotal testis, spermatozoa, semen.

INTRODUCTION

The effect of temperature on spermatogenesis and on spermatozoa in mammals is very distinctive. Testicular temperature is a few degrees below body temperature, 1.5 – 2.5^{0}C, lower in rabbits, 2.0 – 3.5^{0}C in guinea pigs, 4-8^{0}C in rats.[1] Spermatogenesis is retarded or even completely stopped by increasing the normal temperature of the testis, as for example by natural and artificial cryptorchism or by applying heat to the testis.[2,3,4,5] An optimum temperature, a few degrees below that of the body, can therefore be postulated for spermatogenesis. Although the contraction and relaxation of the scrotum in response to the outside temperature can bring about thermo-regulation of the testes by both raising and lowering the temperature as suggested by [6], and this mechanism undoubtedly plays a part in reducing temperature, no ill effect on spermatogenesis of lowering temperature below the optimum has been found even when the testes of rats were subjected to a temperature as low as 6 -8^{0}C.[4] It may be that spermatogenesis is less susceptible to injury at a lower than at a higher than normal temperature.

Spermatozoa after ejaculation survive much better at a low temperature than a high a one.[7,8] Thus a temperature of 0 -10^{0}C is employed extensively for the storage of sperms in the practice of artificial insemination.[9,10] Nevertheless, spermatozoa do suffer from rapid cooling from body temperature to a low temperature[11] (temperature shock) and spermatozoa survive better when cooled slowly (acclimatization) as demonstrated previously.[12]

The epididymis is an ideal extra gonadal target site to inhibit fertility in the male.[13] Synthesis and secretion of constituents like sialic acids, protein and glyceryl phosphoryl choline by the epididymis epithelium under androgen control provide an ideal fluid environment for sperm maturation.

An optimal level of sialic acid secretion by the epididymis epithelium is needed to maintain functional integrity of sperm[14,15] the existence of specific androgen receptors in the epididymis and spermatozoa are related to their ability to metabolize androgen.[13]

Experimental Animals

Adult (200 – 250g) male wistar rats all of which had two palpably descended testicles were housed in a cage (cage made of wood and wire gauze) and the cage was partitioned into four partitions where each group were housed per partition with laboratory grade pine shavings as bedding. Food (Formulab; Ralston Purina Co., St. Louis, MO) and water were available and provided ad libitum. Animals were allowed to adapt and acclimatize for at least three (3) weeks before beginning treatment. Animals were maintained and handled according to protocols in Animal Care and Use by the Ethical committee of the University of Port Harcourt.

Semen collection/Procedure

After 3 – 4 weeks of acclimatization and adaptation, the rats in the four partitions were grouped into four groups (Group I – IV) where Group I were the experimental control while Group II – IV was the experimental treatment groups. Group II – IV were anaesthetized prior to exposure to ice with 0.5mg of thiopental (Rotex Medica, Trittau, Germany). 0.5mg of thiopental was diluted in normal saline (10ml) and each was administered with 0.5 – 0.7ml of diluted thiopental solution intraperitoneally. After administration of the sedative, followed by the onset of sleep, ice was placed in contact with the scrotal testis.

Group II were sedated for one hour (1hr) with the ice in contact with the scrotal testis while Group III and IV were also sedated with ice in contact with their scrotal testis for 45 minutes and 30 minutes respectively. After the period of sedation and placing of ice in contact with their scrotal testis, the testis and epididymis were isolated and collected following hemicastration prior to whole body perfusion where the semen was collected for semen analysis and thereafter the testis and epididymis was introduced into the universal bottle immersed with formalin for histological examination.

Sperm motility

A drop of the semen was introduced on a slide and was covered with a cover slide, after which it was examined under 10X objective lens for the motility. That is, active cells, sluggish cells, dead cells and motile cells. The active cells and the sluggish cells make up the motile cells.

Sperm morphology

1) A 1: 20 dilution of semen was made, after which a thin film was made on the slide.
2) The slide was fixed with alcohol for 3 – 5 minutes.
3) It was rinsed with a slow running tap water.
4) The slide was floored with carbon fuslin for 3 – 5 minutes.
5) The slide was rinsed under a slow running tap water and counterstained with methylene blue for 3 – 5 minutes.
6) The slide was again rinsed under a slow running tap water.
7) The slide was examined under 100X oil immersion for the head defect, tail defect and mid piece defect.
8) Defects were now ranged in percentage.

Sperm viability

A drop of semen was mixed with 1 drop of 0.5% eosin solution on the slide. The total number of head defect, mid-piece defect and tail defect will give the non-viability while the remaining will be the viability.

Total sperm cell count

1) A 1: 20 dilution of semen was also made.
2) Using a Pasteur pipette, an improved neubauer ruled chamber was filled with well mixed diluted semen and was allowed 3 – 5 minutes for the spermatozoa to settle.
3) The 10X objective lens with the condenser iris was closed sufficiently to give a good contrast.
4) The number of spermatozoa was counted in an area of 2sq.mm.
5) The number of spermatozoa was calculated by multiplying the number counted with the hand tally by 10^6.

Normal sperm count = 20 X 10^6 cells/ml – 200 X 10^6 cells/ml.

TESTICULAR HISTOLOGY

After sacrificing the animals, the testes were fixed in 10% formal saline for 24 – 48 hours, and sent to the laboratory for histological studies. In the laboratory, the tissues were identified and necessary parts were taking using surgical blade. The tissues were then put into the the processing/embedding cassette.

They were transferred into the tissue processor for the 24 hours cycle. Automatic processing, starting with 70% alcohol x2, 95% alcohol x2 and absolute alcohol x2, cleared in xylene x2 and impregnated in molten paraffin x2 of two hours each.

They were embedded, using the molds, cassette and molten paraffin wax. After embedding, trimming and sectioning were done in microtome equipment. Sections were picked as they floated out in a warm water bath, labelled and dried up in a hot plate.

STAINING USING HAEMATOXYLIN AND EOSIN (H&E)

The slides were dewaxed in xylene in two changes. They were then dehydrated through descending grades of alcohol starting from absolute alcohol down to water.

They were stained in hematoxylin solution for 20 minutes after which they washed in water and differentiated in 1% acid – alcohol briefly. The slides were further washed and blued in Scott's tap water for 8 minutes. Again, they were washed in water and counterstained in 1% eosin for 5 minutes. For the last time, they were washed in water and dehydrated in ascending grades of alcohol, starting from 70% alcohol to absolute and then cleared in xylene. Finally, they were covered with DPX mountant and cover slide, labelled and examined under the microscope.

STATISTICAL ANALYSIS

The experiment was carried out in the four groups consisting of three adult male wistar rats each. The effect of application of ice on the scrotal testes was analyzed using one-way ANOVA and comparisons among treatment groups were made using student t-test. Analysis of sperm quality parameters in response to duration of application of ice was significant and a level of significance of $P < 0.05$ was used to assess significance. All results were expressed as mean ± standard error of mean (SEM).

RESULTS

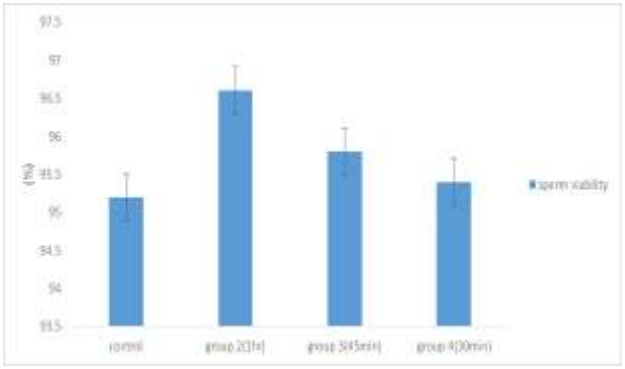


Figure 1: Evaluation of Sperm viability in the control and test groups.

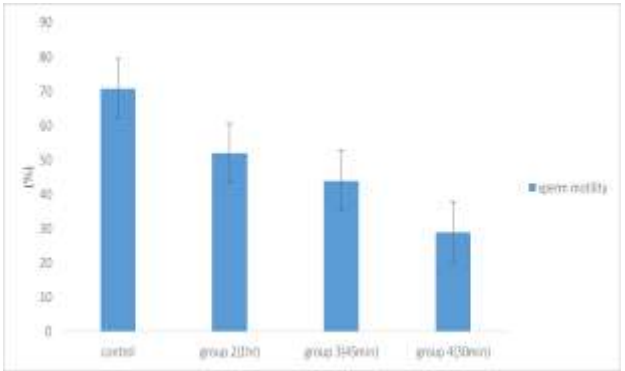


Figure 2: Evaluation of Sperm motility in the control and test groups.

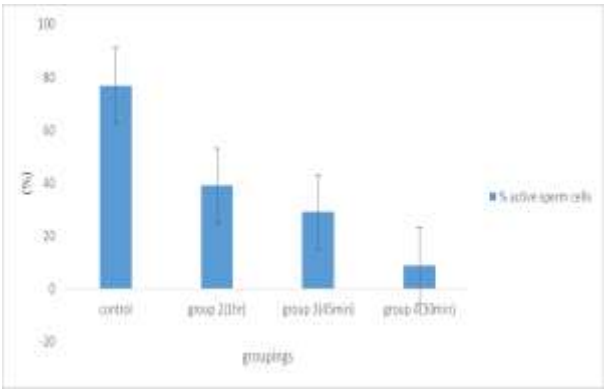


Figure 3: Evaluation of Percentage Sperm cell in the control and test groups.

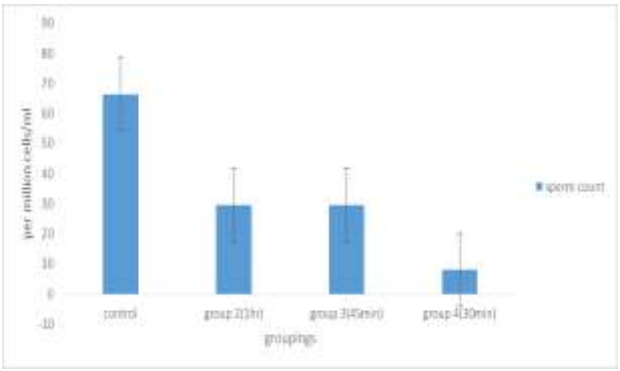


Figure 4: Evaluation of Sperm count in the control and test groups.

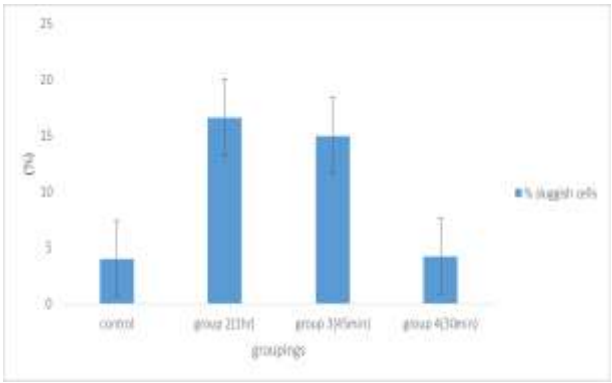


Figure 5: Evaluation of Percentage Sluggish cells in the control and test groups.

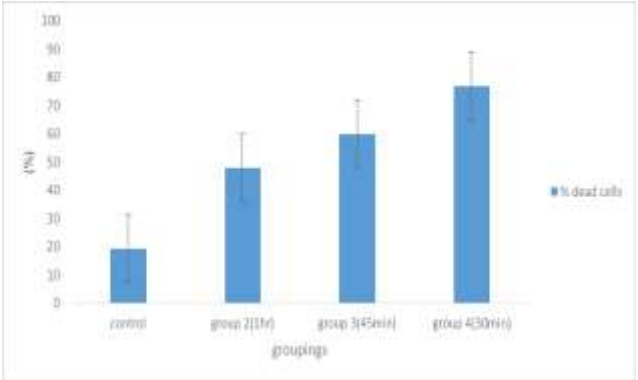
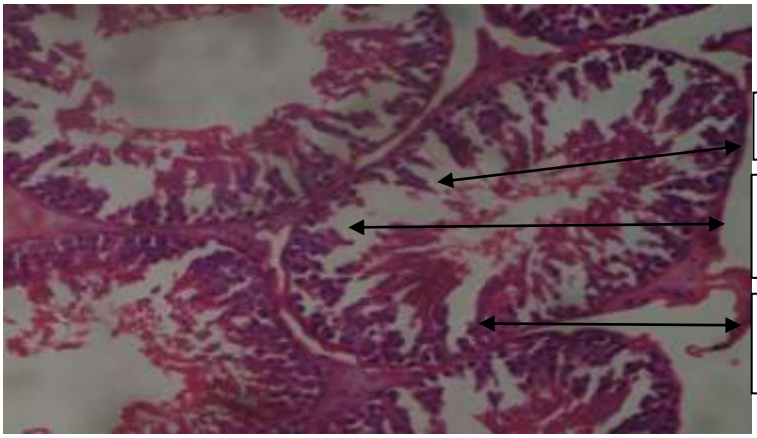


Figure 6: Evaluation of Percentage Dead cells in the control and test groups.

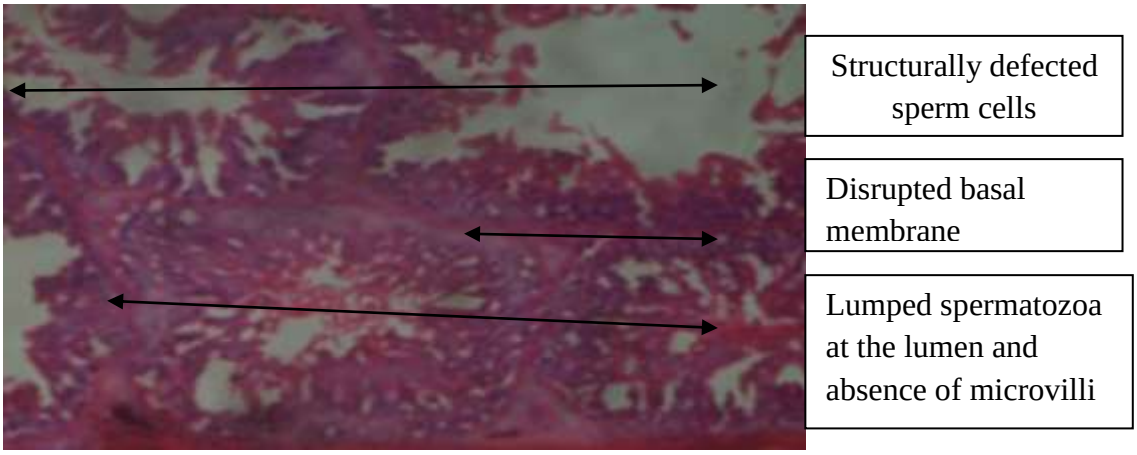
HISTOLOGICAL ANALYSIS

Group 1(control)



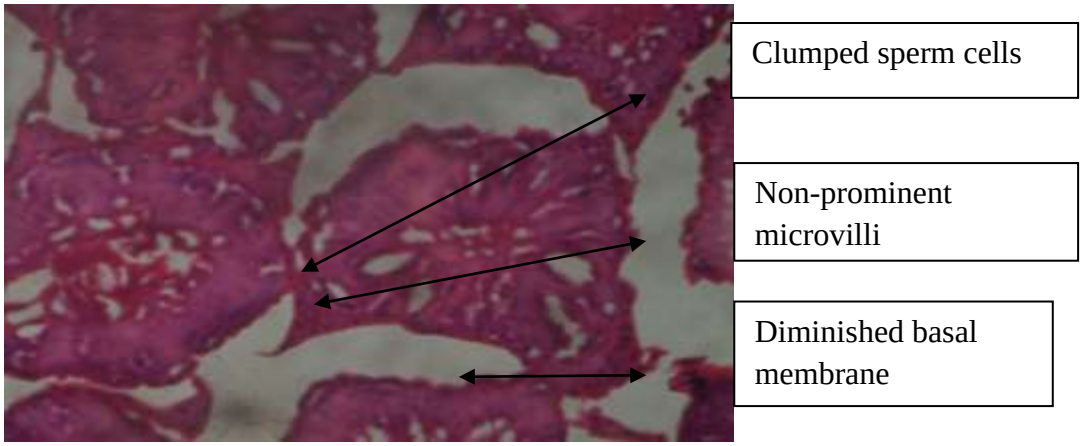
Slide plate 1.0: control group without administration of anesthetics and direct contact of ice with the scrotal testis. (H & E; X400).

Group2 (1 hour)



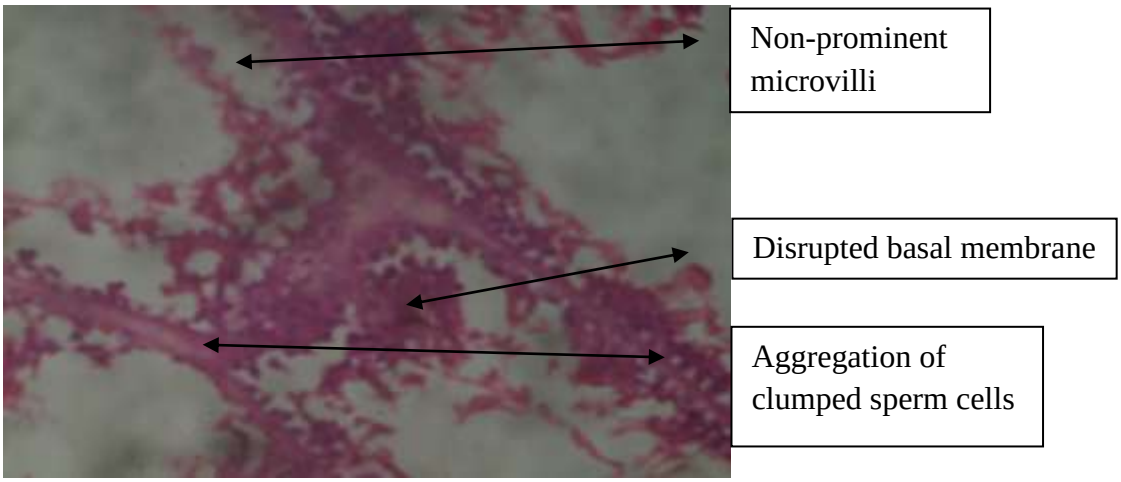
Slide 2.0: showing the effect of direct contact of ice on the scrotal testes (epididymis) for duration of 1 hour. (H & E; X400).

Group 3(45 minutes)



Slide 3.0: showing the effect of direct contact of ice on the scrotal testes (epididymis) for a duration of 45 minutes. (H & E; X400).

Group 4(30 minutes)



Slide 4.0: Showing the effect of direct contact of ice on the scrotal testes (epididymis) for a duration of 30 minutes. (H & E; x400).

DISCUSSION

In the study, we evaluated the effects of application of ice on the epididymis architecture of the scrotal testes as it pertains to histological changes as well as sperm parameters (sperm motility, sperm viability, percentage dead sperm cells, sperm count, percentage sluggish sperm cells and percentage active sperm cells). The result significantly confirmed the negative effects of application of ice on sperm parameters and indicates relationships of membrane integrity, motility and oxidative activity with cold-induced shock.

Various organelles have been known to be affected due to the detrimental effect of application of ice which affects sperm functions. Induction of premature acrosome reaction, altered mitochondrial function, reduction of motility and failure of chromatin decondensation, all of which influences the viability and fertility of the sperm cell have been reported by different authors.[16,17,18,19]

Cooling is a major stressor, and cooling process alone disturbs normal chemical and physical cellular conditions, therefore upsetting homeostasis and metabolism. More specifically, cellular injuries owing to low temperature are a result of cold shock-induced osmotic and oxidative changes, which are consequences of thermotropic phase transitions of membrane.[20,21,22] Osmotic and oxidative changes induce an upregulation of reactive oxygen species (ROS) and shift cellular oxidative balance in favor of pro-oxidant forces. As a result of cooling, membrane bound phospholipids reorient themselves into different configuration that disrupts membrane function and permeability.[23,24] Generally, cold shock damage manifested itself from the result of study in reduction in sperm motility, sperm count and percentage active sperm cell and an increase in the percentage sluggish sperm cell, dead sperm cells and viability and this occurred as a result of a decline in cell metabolism, altered membrane permeability and loss of intracellular components. The damage to cellular membrane is of most significance because it has a carry-over effect on other cellular structures and functions.

The severity of the cold shock depends upon the final temperature and the rate of temperature drop. The cellular damage resulting from cooling affecting both structure and function can be categorized as direct and indirect.[25,26] Direct damage is more definable and is the type usually associated with cold shock evidently after the drop in temperature and it's affected by the rate and duration of cooling, indirect or latent damage is more difficult to quantify and may not be initially apparent, it tends not to be dependent upon the rate of cooling.

A gradual reduction in the metabolic activity of spermatozoa at cold shock temperature could limit the production of detrimental by-products, which might compromise sperm function but metabolic activity altered in this way does also influence essential sperm function such as motility.

Among the different alteration of activity of the intracellular enzymes, glucose -6- phosphate dehydrogenase is the first enzyme which leaves the cell when the cellular membrane is damaged during cold shock. Generally, the intracellular concentration of ATP is decreased or lost and AMP/ADP – rate is increased.[27] The result from this study showed a decrease in sperm motility in the experimental group compared to the control group, result also showed that motility increases as duration of exposure to cold shock in increased and this is believed to occur as result of cellular adaptation and adjustment.

Sperm motility considered to be one of the most frequently used characteristics for evaluating the fertility potential of ejaculated spermatozoa, is known to be dependent on mitochondrial function. The ATP generated by oxidative phosphorylation in the inner mitochondrial membrane is transferred to the microtubules to drive motility. Hence, reduced sperm

motility induced by cold shock i.e. believed to be mainly associated with mitochondrial damage[28,29] in human spermatozoa mitochondrial enzymatic activities we`re shown to be correlated with spermatozoa motility.[29]

Also intracellular calcium ion (Ca^{2+}) accumulation has been reported and this influx can be attributed to modified plasma, mitochondria and nuclear membrane interactions that introduce leakage and ion pump dysfunction. Calcium is a major mediator of sperm function in which Ca^{2+} ATPase pumps maintains low ion concentration to allow correct signaling by calcium influxes, which are needed to initiate motility, hyper activation, capacitation, acrosome reaction and therefore fertilization.[30]

Lipid peroxidation is most significantly seen along the sperm mid-piece[31] and may be responsible for motility loss owing to proximity of the damage to energy-generating mitochondria. In addition to its negative effects on motility, ROS generation and peroxidation of sperm membrane can cause mid-piece abnormalities and reduce the ability of sperm-oocyte fusion.[32] Oxidative damage is caused by intra and extracellular ROS production and this cooling-induced oxidative balance, like osmotic stress, has been linked with reduced membrane integrity, motility.[33]

CONCLUSION

The result presented herein provides evidence for difference in oxidative activity and cell sensitivity between normal and cold shock environments and highlights the influence of low temperature, cooling rate and duration on sperm membrane oxidative damage and sperm cell quality. The proportion of effect in sperm parameters depends on the duration of treatment.

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