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Original Research

Long-Term Ice-Cold Water Drink Induced Testicular Damage and Altered Sperm Characteristics in Rats

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Abstract

An inappropriate lifestyle and nutritional habits such as high fat, high calorie food intake and quality of drinking water can change sperm parameters. Despite the fact that there are different and sometimes contradictory beliefs about the suitable temperature for drinking water, unfortunately, there is not much proven evidence. There are some long-term adverse effects of cold-water intake like liver, gastrointestinal and other internal organ damages and dysfunction. Regarding the high consumption of ice-cold water as a beverage and its probable role for infertility based on some trivial evidence and clinical senses, evaluation of the effects of ice-cold water drinking on reproductive system is noteworthy. Therefore, the main aim of this study is to assess the effect of long-term drinking of ice-cold water on the sperm count, morphology and motility and testis histology. Twenty-four male Wistar rats randomly divided into four groups (n = 6): Intervention groups that used ice-cold water (4°C) for two months (CW2M), three months (CW3M), two months ice-cold water and then one-month room-temperature water (CW2M + 1MRW), and control group that used room-temperature water. Spermatogram and histology of testis were assessed. Ice-cold water drink for the long run, reduced sperm count and sperm progressive motility; increased the percentage of non-motile sperm; changed normal morphology of sperm and destroyed Sertoli and Leydig cells. In conclusion, long-term ice-cold water drinking ought to be noxious for testis function and structure.

Keywords: Lifestyle; Ice-cold water; Testis; Spermatogram; Sertoli cells; Leydig cells

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Introduction

Infertility, by definition, is inability to conceive after 1 year of unprotected intercourse according to world health organization (WHO) [1]. It affects between 8 and 12% of reproductive-aged couples worldwide and has unpleasant family, social and economic consequences. Males are found to be solely responsible for 20-30% of infertility cases but contribute to 50% of cases overall [2-4].

There are many different reasons for male infertility such as physical, structural, hormonal, sexual, environmental and dietary factors [5-7]. An inappropriate lifestyle and nutritional habits such as high fat, high calorie food intake and quality of drinking water can change sperm parameters [8-12].

As a lifestyle index, both quality and quantity of drinking water may affect body health status. Despite the fact that there are different and sometimes contradictory beliefs about the suitable temperature for drinking water [13], unfortunately, in the search for reliable scientific sources, there is not much proven evidence. In the ancient medical sources there are detailed discussions about water type, its quality and the drinking conditions [14]. According to Avicenna and Rhazes, consumption of very cold water during and after a sporting event, after sleep, a hot bath, or sexual intercourse is harmful. It is mentioned that everything leading to the body's sudden cooling can damage the health. In addition to the acute adverse effects of cold-water intake, there are some long-term adverse effects like liver [15], gastrointestinal and other internal organ damages and dysfunction [13,16,17].

Regarding the high consumption of ice-cold water as a beverage and its probable role for infertility based on some trivial evidence and clinical senses, evaluation of the effects of ice-cold water drinking on reproductive system is noteworthy. Therefore, the main aim of this study is to assess the effect of long-term drinking of ice-cold water on the sperm count, morphology and motility and testis histology.

Methods

Animals

Twenty-four locally produced male Wistar rats weighing 180 ± 20 g kept at a constant temperature of 22 ± 2 °C with fixed 12:12 hour light-dark cycle. Animals were randomly divided into four groups (n = 6): Intervention groups that used ice-cold water (4°C) for two months (CW2M), three months (CW3M), two months ice-cold water and then one-month room-temperature water (CW2M + 1MRW), and control group that used room-temperature water. For preparing ice-cold water, animals' drink bottles were filled with cubes of ice twice a day in the morning and evening. The Ethics Committee of Tehran University of Medical Sciences approved all experimental procedures (IR.TUMS.VCR.REC.1398.189).

Preparation of the sperm sample

Before collecting sperms, the sperm washing media (8.5 ml Vita and 1.5 ml albumin) and slides were pre-warmed in 37 °C in the incubator for 5 minutes. The caudal part of epididymis was minced in 2 ml of the media with surgical scissors.

Sperm count

For sperm count, hemocytometer method was used. One ml of sperm suspension was diluted with 4 ml of formaldehyde. Then, 10 µl of diluted sample was placed on hemocytometer chambers. Sperm heads were counted in 5 microscopic fields using light microscopy at 400x magnification. The sperm count was expressed as106 sperm per milliliter [18].

Sperm motility

the motility of 200 sperms were assessed by placing 10 µl of sperm suspension on pre-warmed slides at 10 microscopic fields under 400x magnification phase-contrast microscope (Olympus, Germany). According to WHO 2010 recommendation, the percentages of progressive motile sperms (PMS), non-progressive motile sperms (NPMS) and non-motile sperms (NMS) were recorded [19].

Sperm morphology

To assess sperm morphology, eosin/nigrosine staining was used. Ten microliters of eosin and nigrosine was added to 50 µl of sperm suspension. Then, the smear was incubated for 45–60 minutes at room temperature. One hundred sperms were counted under a light microscope at 1000x magnification. Abnormal sperms with double heads, flattened and bent neck, bent tail and sperms with multiple abnormalities were recorded.

Tissue sampling

Testis separated from each rat immediately cleared of conjoining tissues and adhering fat, washed with normal saline and then kept in 10% formalin at once for ongoing histological examination. Animals were then killed by deep anesthesia in the end.

Histological staining of testicular tissue

Each fixed sample was embedded in paraffin, and then cut into 5-µm sections with a microtome and deparaffinized with xylene. Sections were then subjected to standard hematoxylin/eosin (H&E) staining and observed under a light microscope at magnification of 400x. An expert who was blind to the study design, qualitatively assessed the histological scoring. The observer evaluated five fields of view in each section, 5 sections for each animal.

Statistical analysis

Data presented as mean ± SEM. For statistical analysis, One-way ANOVA used to compare the mean differences among the groups followed by Tukey's post hoc test. Statistical analysis performed with GraphPad Prism software (Version 7) and $P < 0.05$ considered statistically significant.

Results

Sperm parameters

Sperm count significantly decreased ($p < 0.001$) in CW2M ($122.3 \pm 6.02 \times 10^6$) and CW3M ($116.5 \pm 51\% \times 10^6$) groups compared to the control group ($219.6 \pm 12.2 \times 10^6$/ml). Discontinuing ice-cold water for one month in CW2M + RTW1M group reversed the decrement change compared with control animals ($220.5 \pm 10.7 \times 10^6$/ml) (Fig. 1)

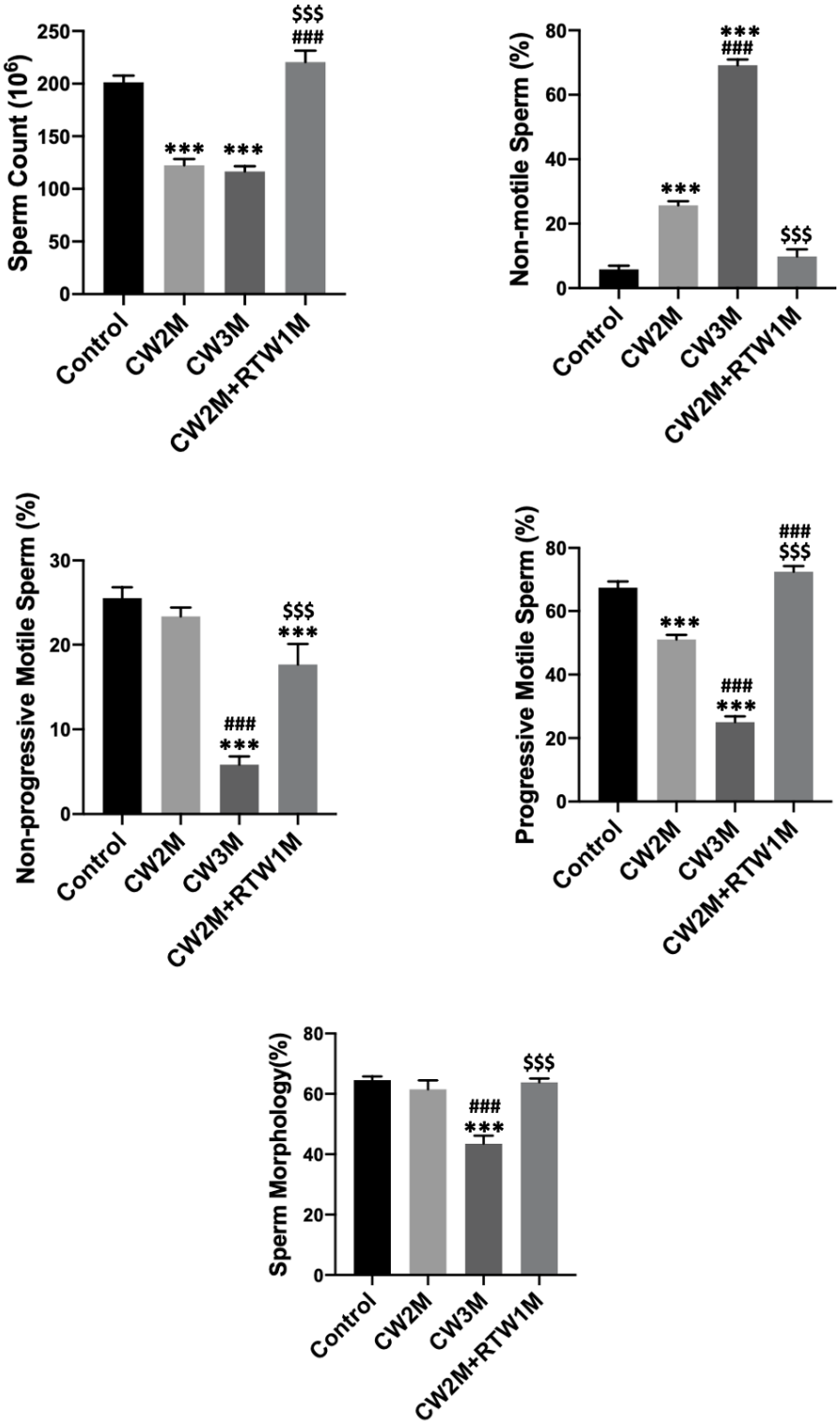

Fig. 1. Sperm parameters (sperm count, non-motile sperm, non-progressive motile sperm, progressive motile sperm and sperm morphology) in different groups. Data are presented as Mean ± SEM. *** $p < 0.001$ significant differences vs. control group, ### $p < 0.001$ significant differences vs. CW2M group, $$$ $P < 0.001$ significant differences vs. CW3M group.

Non-motile sperms percentage significantly increased ($p < 0.001$) in CW2M (25.6 ± 2.1%) and CW3M groups (69.1 ± 1.8%) compared to the control animals (7.5 ± 0.7%). In CW2M + RTW1M group this value nearly reached to the control animals (Fig.1).

Non-progressive motile sperms significantly decreased ($p < 0.001$) in CW3M group (5.83 ± 0.98 %) compared to control one (25.5 ± 1.3%). In CW2M + 1MRW animals, this parameter significantly dropped (17.6 ± 2.4%) ($p < 0.001$) compared to the control group (25.5 ± 1.3%), but this was still significantly higher than CW3M animals (5.83 ± 0.98 %) ($p < 0.001$) (Fig. 1).

The percentage of progressive motile sperms significantly decreased ($p < 0.001$) in CW2M (67.17 ± 0.9 %) and CW3M groups (67.5 ± 1.9%) compared to the control group (51 ± 1.5%). This percentage in CW2M + RTW1M group (72.5 ± 1.7%) did not statistically change (Fig.1).

Sperm morphology

The percentage of sperms with normal morphology significantly decreased ($p < 0.001$) in CW3M group (43.5 ± 2.64%) compared to the control animals, (65.17 ± 1.4%) while the morphological character was untouched in the other two interventional groups. (Fig. 1).

Testicular structure

In control group, normal seminiferous tubules with intact histological character are noted. Testes in all three interventional groups showed structural changes compared to the control animals. In CW2M and CW3M groups depletion of Leydig and Sertoli cells of seminiferous tubules was noted especially in CW3M animals. In CW3M group however, much cell detachment from the basement membrane inside the seminiferous tubules was seen. Irregular seminiferous tubules with disorganized epithelium and thick basement membrane together with some extent of architectural loss were noted in CW2M and CW3M groups. In CW2M + RTW1M group spaces between seminiferous tubules was observed but these changes were milder than two others intervention groups. (Fig. 2).

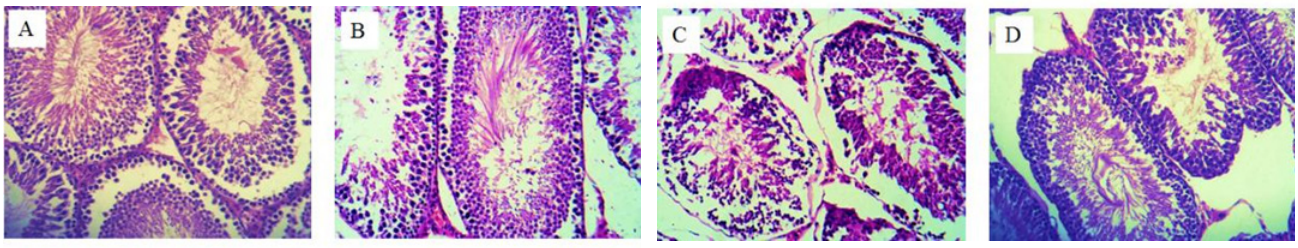

Fig. 2. Testis histopathology in different groups. A: Control, B: CW2M group (2 month ice-cold water), C: CW3M group (3 month ice-cold water), D: CW2M + RTW1M (2 month ice-cold water followed by one month room temperature water). (H&E staining 400X).

Discussion

In this study, we showed striking effects of long-term ice-cold water drink on sperm architectural and functional characters in such a way that, ice-cold water drink for the long run reduced sperm count and sperm progressive motility; increased the percentage of non-motile sperm; changed normal morphology of sperm and destroyed Sertoli and Leydig cells.

Prolonged ice-cold water drink could significantly reduce sperm count with a speedy action. There would be two potential reasons for the sperm reduction; spermatogenesis disruption and spermatozoid destruction. According to our findings, owing to Leydig and Sertoli cells destruction in the testis tissue, spermatogenesis disruption would be the main suspect.

Sperm count reduction has been a time independent process since the destructive effects were nearly the same both in two- and three-month ice-cold water drink groups, with a speedy resolution of one month post ice-cold water discontinuation.

The other spermatogram parameter that was amazingly affected during long-term drinking of ice-cold water is sperm motility which phenomenally dropped and reported as non-motile sperms percentage in intervention groups, in a time-dependent manner. The percentage is three times more in CW3M than CW2M group and reversed after one month of room water temperature discontinuation. Time dependent character of disturbed sperm motility indicates that this defect would be a direct testicular tissue destructive effect on sperm motility. Significant decrease in non-progressive motile in CW3M group is related to decrease in total motile sperms.

Sperm motility disturbance is one of the most striking findings in these results. According to our findings, in CW3M group, only 25% of sperms have normal motility compared with 75% normal motile sperms in control group which means that only one third of sperms have normal motility. Given that a 50% decrease of sperm count in intervention groups, the actual motile sperm count should only be one sixth of the control group.

The percentage of normal sperm morphology decreased in intervention groups, but it was only significant in CW3M group compared to control animals. This decrease is also time dependent and reversible, and is related to changes in normal process of spermatogenesis in seminiferous tubules. A plausible explanation for insignificant sperm morphology changes in CW2M animals is the ample epididymis sperm reserve that takes time to finish together with, spermatozoids renewal capacity. Yet, most studies have shown that sperm morphology is not as sensitive as motility to destructive factors [20]. The results obviously revealed that long-term drinking of ice-cold water can affect sperm count in a wider range than sperm motility, but the severity of sperm motility disturbance is more than sperm count reduction.

A standard human semen analysis should hold a sperm concentration of $> 20 \times 10^6$ sperm/ml with > 50% motility and > 30% morphology [21]. Accordingly, in a broader spectrum, our findings showed that long-term drinking of ice-cold water could probably be one life-style

related factor with male infertility potential. It is also noteworthy that ice-cold water drink in our study has comparable destructive effects as alcohol on motility and sperm count [22] and even much more destructive effects than nicotine [23,24].

Although the exact mechanism of long term cold water drinking effects on testis architecture and function is not clear and needs more investigations, one possible mechanism would be gastrointestinal (GI) thermoreceptor activation [25]. Stimulation of the upper GI tract thermoreceptors conduct via the vagus nerve to the nodose ganglion [25] whose neurons centrally synapse in the solitary nucleus. Outputs from the solitary nucleus project to many parts of the brain including paraventricular nucleus of the hypothalamus [26]. Activation of the gastrointestinal cold receptors through this pathway can conceivably inhibit vasopressin release [27]. Vasopressin is said to be a possible regulator for the testis circulation and can decrease its blood flow and prevent tissue hyperemia [28]. Spermatogenesis is a temperature sensitive process so that minor testicular temperature grow which is commonly seen during testicular hyperemia causes a significant drop in the sperm production [29]. Moreover, vasopressin can act on its receptors in Leydig cells and stimulate testosterone secretion [28]. It has also an insulin secretory effect and can activate insulin signaling cascade [30]. Insulin through hypothalamic-pituitary axis acts on Leydig cells and soars serum LH level for further spermatogenesis activation [31]. In brief, ice-cold water use in the long run can inhibit vasopressin secretion and insulin signaling cascade, impair spermatogenesis process and sperm quality, and bring about testis histological structure changes.

In conclusion, long-term ice-cold water drinking ought to be noxious for testis function and structure. More studies are suggested to be designed for further clarification, especially for human infertility state.

Declaration of competing interest

The authors declare that there was no conflict of interest.

Acknowledgment

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