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SYSTEMATIC REVIEW

Effects of nanotubes on semen quality and fertility in humans: A systematic review of literature

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Summary ***Background: In the medical field, it is increasingly common to observe the use of nanotubes, for example, in the administration of drugs. However, nanotubes raise concerns for male fertility due to potential effects on hormone levels and sperm quality observed in animal studies. In addition, animal exposure to multi-walled carbon nanotube models found alterations in hormone levels, sperm motility, and sperm count. Limited evidence in humans suggests no adverse effects, but further research is needed. This study aimed to perform a systematic review to assess the in vitro effects of nanotubes on semen and fertility in humans.***
Methods: We included all published in vitro studies about semen or sperm or male fertility and nanotubes in humans. A search was conducted in LILACS, PubMed, and SCOPUS as of May 2023. The risk of bias was assessed using the QUIN tool.
Results: Four studies using nanotubes on human sperm were included, nanotubes exposure appears not to affect sperm viability; however, some alterations to motility, velocity and production of reactive oxygen species were reported. Limited evidence is provided because of the small quantity of publications.
Conclusions: Nanotubes appear to have no adverse effects on human sperm.

KEY WORDS: Nanotubes; Semen; Fertility; Human; Nanoparticles; Reproduction.

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INTRODUCTION

Carbon nanotubes (CNTs) are nanomaterials that have garnered attention due to their unique properties and potential across various fields, including medicine (1). Composed of carbon atoms arranged in a hexagonal lattice, CNTs form cylindrical structures, akin to rolled-up graphene sheets. This structural arrangement imparts CNTs with remarkable mechanical strength, electrical conductivity, and chemical reactivity (2).

CNTs exhibit a wide range of dimensions, with typical diameters ranging from a few nanometers to tens of nanometers, and lengths varying from a few micrometers to millimeters. Based on their structural characteristics, CNTs can be categorized into two types: *single-walled nanotubes* (SWNTs) and *multi-walled nanotubes* (MWNTs). SWNTs consist of a single cylindrical graphene sheet, whereas MWNTs comprise multiple concentric layers of graphene sheets (3).

The remarkable physical properties of CNTs result from their nanoscale architecture. CNTs possess a high aspect ratio and extraordinary tensile strength, granting them unmatched mechanical resilience that exceeds traditional materials like steel and diamond in the strength-to-weight ratio (4). Additionally, CNTs exhibit excellent thermal conductivity, facilitating efficient dissipation of heat. These desirable characteristics, along with their electrical conductivity, make CNTs highly attractive for diverse applications, including electronics, energy storage, and biomedical devices (5).

In the field of medicine, integrating CNTs offers significant potential for revolutionary advancements in healthcare (6). The considerable surface area-to-volume ratio of CNTs, coupled with their molecular transport capabilities, positions them as promising candidates for drug delivery systems (7). Through the functionalization and loading of therapeutic agents onto CNTs, controlled and targeted release of drugs to specific tissues or cells becomes achievable (8). Additionally, the electrical properties of CNTs enable the development of biosensors and implantable devices for precise diagnostic and therapeutic applications, facilitating real-time monitoring and precise control. However, as the utilization of CNTs in medical applications continues to expand, thorough evaluation of their impact on human health becomes paramount (9). The interactions between CNTs and biological systems necessitate meticulous examination to ensure the safety and efficacy of these nanomaterials. Particularly, investigating the potential effects of CNTs on reproductive health assumes significance, as reproduction represents a fundamental process for the survival and perpetuation of the human species.

CNTs interaction with the male reproductive system raises concerns regarding health and fertility. Studies of exposure in animals to multi-walled carbon nanotubes models found alterations on hormone levels, sperm motility, sperm count (10). In humans, some studies found no adverse effects on

sperm quality (11, 12). Further research is needed.

Therefore, the objective of this study was to perform a systematic review to assess the in vitro effects of the nanotubes on semen and fertility in humans.

Materials and methods

The systematic review was performed following the *Preferred Items for Systematic Reviews and Meta-analysis* (PRISMA) reporting guidelines (13).

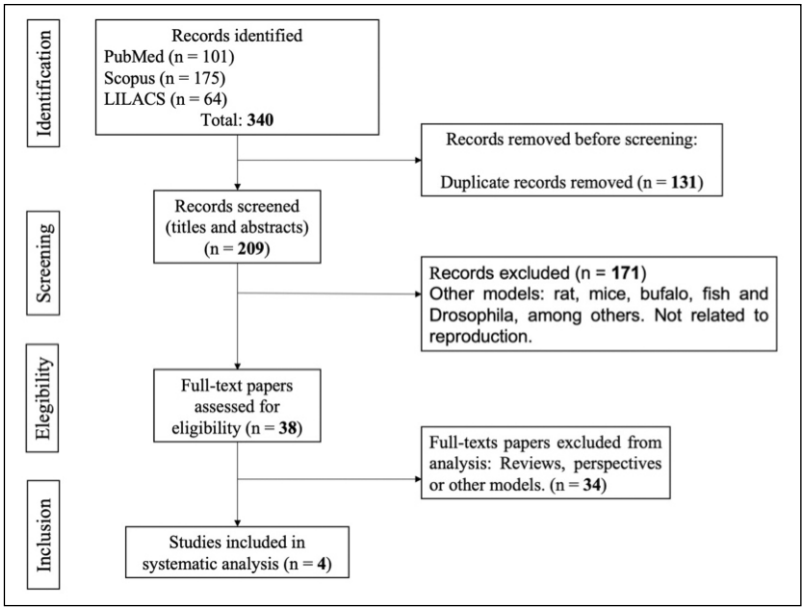
Eligibility criteria

All publication original investigation peer-reviewed articles in English and Spanish languages related to In vitro studies on human sperm in humans until may 2023 were included.

Exclusion criteria

All publications related to animal models investigation were excluded.

Figure 1.
Flow diagram of the study selection process.



Search methods

The following search strategy was used in *PubMed* (Fertility OR infertility OR semen OR Sperm*) AND Nanotubes); in *Scopus* (TITLE-ABS-KEY (nanotubes AND "*male infertility*") OR TITLE-ABS-KEY (nanotubes AND spermatozoa) OR TITLE-ABS-KEY (nanotubes AND semen)), and in LILACS ((Fertility) OR (Infertility) OR (Semen) OR (Sperm)) AND (Nanotubes).

Study records

The search results from electronic databases were imported into EndNote X8 software, and then duplicates were identified. Two independent authors (WFFZ and WDCM) screened the titles and abstracts of the identified studies to assess their eligibility based on predefined inclusion and exclusion criteria. Full-text articles of potentially relevant studies were retrieved for further evaluation. Any disagreements between the reviewers were resolved through discussion or consultation with a third reviewer to ensure a consensus-based selection process. The study protocol was registered with the Prospero International Prospective Register of Systematic Reviews (CRD42023435569).

During the data collection process, two independent authors (WFFZ and WDCM) extracted relevant information from the selected studies using a standardized data extraction form. This form captured the main characteristics of the study, the title of the study, the characteristics of the nanotubes, the details of the nanotubes, the outcome measures, and the main characteristics of the study. The data extraction process was conducted meticulously for accurate and consistent data collection. Any discrepancies or uncertainties were resolved by consensus or with a third reviewer's opinion (Figure 1).

Risk of bias in studies

The risk of bias was assessed using the QUIN tool (14). The present study conducted a risk of bias assessment, examining potential biases in twelve item criteria which wiere scored, and the scores were used to grade the in vitro study as high, medium or low risk of bias. A traffic light plot summarizing the risk of bias was generated using *Robvis* (15).

Results

Literature searches identified 340 documents, and 4 articles (11, 12, 16, 17) were finally included after excluding duplicate studies, irrelevant literature, and review articles adhering strictly to the inclusion and exclusion criteria (Figure 1).

Three of the studies used sperm from healthy male volunteers: *Aminzadeh et al.* (17) obtained samples from 30 nonsmoking males, *Cardona-Maya et al.* (12) obtained 12 samples from 24,6 ± 5,7 aged males; *Jha et al.* (16) used sperm from fertile males aged 25 to 40.

Ashgar et al. (11) used semen vials from a cryobank (as in Table 1).

These studies (11, 12, 16, 17) were conducted in the US, India, Iran and Colombia; all of them agreed that sperm viability was not affected by nanotubes, NO was not significantly produced compared to control in any of the two studies evaluating it (11, 17). *Aminzadeh et al.* (17) found a correlation between nanotubes concentrations added to sperm and ROS production similar to *Ashgar et al.* (11), which found an increase of ROS generation sperm.

Jha et al. (16) proved MWNTs can interact with and infiltrate sperm cells without causing cell damage. Their research reveals that MWNTs, compared to other nanoparticles, has markedly lower interaction rates with nucleic acids. Making it less likely to disrupt the cellular functions associated with these biological macromolecules. They also demonstrate the ability of CNTs to penetrate cell nuclei. This characteristic, combined with their

Table 1.
Summary of the studies included.

Study	Used nanotubes	Variables	Methodology	Conclusion
Ashgar et al. 2016 (11)	SWCNT	Viability, straight linear and curvilinear velocity, ROS generation, NO generation.	A solution of SWCNT was added to human sperm.	Exposing sperm to MWCNT did not lead to notable impacts on sperm viability, NO was not significantly produced. Nonetheless, there were observable alterations in sperm velocity and oxidative stress caused by ROS.
Jha et al. 2016 (14)	MWCNT	Suitability of drug delivery carrier.	MWCNT type 5 was added to human sperm.	MWCNT covers and enters sperm cells and produces no cell damage, produces less interaction with nucleic acids compared to other nanoparticles, CNT can penetrate nuclei and can be used as a marker, thus can be a candidate for drug delivery to the nucleus
Aminzadeh et al. 2016 (15)	SWCNT-COOH and MWCNT-COOH	Viability, motility ROS generation, NO generation.	Different concentrations (0, 1-100 µg) of a solution of acid-oxidized SWCNT and MWCNT were added to human sperm.	Viability was not altered, motility visible altered after 30 min exposure and decreased with CNT concentration, concentrations upper to 100 µg/ml at any time and exposures longer to 3 hours of SWCNT-COOH and MWCNT.COOH could induce ROS production and NO production similar to control.
Cardona-Maya et al. 2020 (12)	MWCNT	Sperm motility.	A solution of 1% MWCNT was added to human sperm.	MWCNT does not affect sperm motility.

MWCNT: Multi-wallerd carmbon nanotubes; ROS: Reactive oxigen species; NO: Nitric oxide.

Figure 2.
Risk of bias assessment with corresponding biases (D1 to D2) and risk indicators.

reduced interaction with nucleic acids, makes them an excellent candidate for use as intracellular markers. Furthermore, these attributes suggest that MWNTs could be a promising tool for targeted drug delivery to the nucleus, opening up an exciting new avenue in nanoparticle-based therapeutics.

In conducting our review, we made the decision to exclude the works of *Fan et al.* (18) and *Eyni et al.* (19). Although *Fan et al.* (18) provides essential insights into the utilization of nanotubes as part of an immunosensor for the follicle-stimulating hormone, their research unfortunately lacks any direct experimentation with human sperm. This absence limits the relevancy of their findings to our review, primarily focused on direct interactions and impacts of nanotubes on human sperm. Similarly, the study of *Eyni et al.* (19) while notable for its use of nanotubes in crafting a scaffold to induce differentiation of human stem cells into spermatogonial sperm cells, falls outside of our current review parameters. This study concentrates more on cellular differentiation and less on the interaction between nanotubes and human sperm. Therefore, despite their in the respective fields, these studies did not fit closely enough with the purpose of inclusion in our review.

None of the included studies specified the sample size calculation nor outcome assessor details; only one study specified operator details. However, it did not provide valuable details of the operator´s training, and overall, we conclude that the risk of bias in the included studies is low (Figure 2). There is no risk of bias standardized tool for in vitro studies in medicine, therefore, we used QUIN tool for reviewing risk of bias in the selected studies (14) (Figure 2).

Discussion

Our review aimed to assess the in vitro effects of nanotubes on human semen and fertility; four articles matched the requirements and were analyzed demonstrating that exposure of human sperm to CNT does not significantly affect its viability.

We did not find another systematic review addressing the research question. The absence of a previous systematic review in this field may be attributed to various factors, including the emerging nature of the research topic, limited research interest, or the absence of a consolidated body of evidence.

Our study represents a pioneering effort in systematically reviewing the impact of nanotubes on human sperm and male fertility. This is because most research has identified the effect of carbonaceous materials on other species such as rats and mice (20). Additionally, some research has

found that carbonaceous structures such as graphene, graphene oxide, and reduced graphene oxide have no significant effect on sperm viability. However, there were some significant changes in sperm velocity and oxidative stress due to reactive oxygen species (21). These changes were attributed to the exposure times. Likewise, some studies have investigated the viability of human sperm exposed to functionalized carbon nanotubes and have not found a significant effect. However, these studies reported that sperm motility decreased and oxidative stress increased, possibly associated with mitochondrial and DNA damage (17).

One limitation of our systematic review was omitting grey literature and unpublished works, potentially excluding relevant information.

Conclusions

In conclusion, we recommend that the scientific community advance studies employing human in vitro models to comprehend this phenomenon further. Such models can be used to comprehend the phenomenon better, resulting in more precise and valuable conclusions.

References

1. He H, Pham-Huy LA, Dramou P, et al. Carbon nanotubes: Applications in pharmacy and medicine. Biomed Res Int. 2013; 2013:578290.

2. Popov VN. Carbon nanotubes: Properties and application. Materials Science and Engineering: R: Reports. 2004; 43:61-102.

3. Anzar N, Hasan R, Tyagi M, et al. Carbon nanotube-a review on synthesis, properties and plethora of applications in the field of biomedical science. Sensors International. 2020; 1:100003.

4. Isaza M CA, Rudas JS, Cardona-Maya Y, et al. Interfacial phenomena in multiwalled carbon nanotube-reinforced magnesium nanocomposite synthesized by the sandwich technique. Metallography, Microstructure, and Analysis. 2023:1-9.

5. Atiq Ur Rehman M, Chen Q, Braem A, et al. Electrophoretic deposition of carbon nanotubes: Recent progress and remaining challenges. International Materials Reviews. 2021; 66:533-562.

6. Sharifi M, Pothu R, Boddula R, Bardajee GR. Trends of biofuel cells for smart biomedical devices. International Journal of Hydrogen Energy. 2021; 46:3220-3229.

7. Zare H, Ahmadi S, Ghasemi A, et al. Carbon nanotubes: Smart drug/gene delivery carriers. Int J Nanomedicine. 2021:1681-1706.

8. Saleemi MA, Kong YL, Yong PVC, Wong EH. An overview of recent development in therapeutic drug carrier system using carbon nanotubes. Journal of Drug Delivery Science and Technology. 2020; 59:101855.

9. Barbarino M, Giordano A. Assessment of the carcinogenicity of carbon nanotubes in the respiratory system. Cancers. 2021; 13:1318.

10. Farombi EO, Adedara IA, Forcados GE, et al. Responses of testis, epididymis, and sperm of pubertal rats exposed to functionalized multiwalled carbon nanotubes. Environ Toxicol 2016; 31:543-551.

11. Asghar W, Shafiee H, Velasco V, et al. Toxicology study of single-walled carbon nanotubes and reduced graphene oxide in human sperm. Sci Rep. 2016; 6:1-11.

12. Cardona Maya Y, Isaza Merino CA, Cardona Maya WD. [exposure of multi-walled carbon nanotubes to human sperm]. Revista Cubana de Obstetricia y Ginecología. 2020; 46:1-11.

13. Page MJ, McKenzie JE, Bossuyt PM, et al. The prisma 2020 statement: An updated guideline for reporting systematic reviews. Int J Surg. 2021; 88:105906.

14. Sheth VH, Shah NP, Jain R, Bhanushali N, Bhatnagar V. Development and validation of a risk-of-bias tool for assessing in vitro studies conducted in dentistry: The QUIN. J Prosthet Dent. 2022; S0022-3913(22)00345-6.

15. McGuinness LA, Higgins JPT. Risk-of-bias VISualization (robvis): An R package and Shiny web app for visualizing risk-of-bias assessments. Res Synth Methods. 2021; 12:55-61.

16. Jha R, Jha PK, Gupta S, et al. Probing suitable therapeutic nanoparticles for controlled drug delivery and diagnostic reproductive health biomarker development. Mater Sci Eng C Mater Biol Appl. 2016; 61:235-245.

17. Aminzadeh Z, Jamalan M, Chupani L, et al. In vitro reprotoxicity of carboxyl-functionalised single- and multi-walled carbon nanotubes on human spermatozoa. Andrologia. 2017; 49:e12741.

18. Fan Y, Guo Y, Shi S, Ma J. An electrochemical immunosensor based on reduced graphene oxide/multiwalled carbon nanotubes/thionine/gold nanoparticle nanocomposites for the sensitive testing of follicle-stimulating hormone. Anal Methods. 2021; 13:3821-3828.

19. Eyni H, Ghorbani S, Shirazi R, et al. Three-dimensional wet-electrospun poly (lactic acid)/multi-wall carbon nanotubes scaffold induces differentiation of human menstrual blood-derived stem cells into germ-like cells. J Biomater Appl 2017; 32:373-383.

20. Francis AP, Devasena T. Toxicity of carbon nanotubes: A review. Toxicol Ind Health. 2018; 34:200-210.

21. Hadizadeh N, Zeidi S, Khodabakhsh H, et al. An overview on the reproductive toxicity of graphene derivatives: Highlighting the importance. Nanotechnology Reviews. 2022; 11:1076-1100.

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