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Spermatology in Squamata reptiles: A systematic review protocol

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ADMINISTRATIVE INFORMATION

Support - COMECYT 2024-0034.

Review Stage at time of this submission - Completed but not published.

Conflicts of interest - None declared.

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Amendments - This protocol was registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) on 27 February 2025 and was last updated on 27 February 2025.

INTRODUCTION

Review question / Objective What is the state-of-the-art knowledge about the studies in Squamata that consider sperm and seminal parameters?
The research sub-questions are:
What are the spatial-temporal trends of sperm-related studies in Squamata?
Which Squamata groups are the most studied, and which are their IUCN category?
What is the most common approach (ecological, physiological, morphological, molecular, etc.) used in the study of sperm?
What are the most used methods for semen obtention and their associated approaches (population, euthanasia)?
What is the state-of-the-art knowledge about the studies in Squamata that consider sperm and seminal parameters?
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What are the most used methods for semen obtention and their associated collection sources (field population, euthanasia)?

Rationale Spermatology aims to understand the functional and structural diversity of the spermatozoa in diverse species [1]. Numerous studies in fish, birds, and mammals provide a broad conceptual framework for understanding the mechanisms involved in gamete formation[2,3]. It is generally assumed that in male vertebrates, sperm development involves a process of morphological changes and subsequent maturation to interact with the oocyte [4]. However, compared to other groups the Squamata are less studied. The above is despite the possibility of using them as models to study ecotoxicology [5], sperm competition [6,7], and cryopreservation

[8,9]. There is currently no comprehensive review or Scientometric analysis of Squamata spermatology, particularly regarding reproductive biology, sperm traits, and seminal parameters. This study addresses that gap by conducting a bibliometric analysis to reveal research trends and biases, offering a foundation for future studies. Additionally, we provide a systematic review to identify key methodologies and patterns in the field of sperm research and seminal parameters in Squamata.

Condition being studied This study examines the current state of research on Squamata spermatology, focusing on reproductive biology, sperm traits, and seminal parameters. It identifies trends, biases, and methodological approaches through a bibliometric analysis, providing a foundation for future studies and a systematic review of key research practices in the field.

METHODS

Search strategy A previous scoping exercise was conducted on the WoS database to build up the search string. This systematic review will follow the Preferred Reporting Items for Systematic Review and Meta-analysis of Protocol (PRISMA-P) guidelines.
The terms related to sperm, spermatid, spermatic, spermatozoo, spermatozoa, ejaculate, semen, seminal and the taxa squamata, lizard, snake and amphisbaenid were combined until the best performance was observed. Within Web of Science, we employed the following research string: (TS=(Spermatozo* OR semen OR seminal OR sperm) AND TS=(lizard OR squamata OR snake OR amphisbaenid*)) AND DT=(Article). The combination of words for Scopus was used as follows: TITLE-ABS-KEY (spermatozo* OR semen OR seminal OR sperm) AND (lizard OR squamata OR snake OR amphisbaenid*). Only English terms were included in the search strings. However, those hits either in English, French, Italian, or Spanish were revised, due to limited languages understood by the map team.

Participant or population Studies related to Squamata's sperm (working with ejaculates or spermatozoa).

Intervention Related studies of Squamata spermatozoa that provide a detailed description or function of the cell.

Comparator Comparison of methodologies commonly used in sperm analysis to evaluate differences in seminal parameters among studies.

1. Sperm Morphology Assessment – Studies using stains to measure sperm morphometry or electronic microscopy.
2. Sperm Motility Analysis – Comparisons will be made between studies employing manual microscopic assessments (subjective evaluation) and those using computer-assisted sperm analysis (CASA) (objective evaluation).
3. Sperm Viability Tests – Studies using staining techniques (e.g., eosin-nigrosin, Diff-Quik, sperm viability kit) or phase-contrast microscopy for the normal vs. abnormal sperm morphology assessment.
4. Sperm Concentration Quantification – Comparisons will include studies using Neubauer chambers, spectrophotometry, or CASA-based quantification.
5. Membrane Integrity and Acrosome Reaction – Evaluations will compare the different methodologies such as fluorescein isothiocyanate–conjugated peanut agglutinin (PNA-FITC) staining, or Pisum sativum agglutinin (PSA-FITC) based assays.

Study designs to be included Articles published in peer reviewed journals. The studies that include only bright field histology of the male reproductive system and gonadal-somatic indexes will be excluded.

Eligibility criteria This systematic review will include studies conducted in any country, with no restrictions on the year of publication. Only English, Spanish, French, or Italian-written articles will be considered. Eligible studies must focus on the sperm of Squamata, specifically on ejaculates or spermatozoa, and analyze spermatozoa or seminal parameters. Additionally, studies must specify the section or tissue from which the spermatozoa were obtained. To ensure accessibility and reproducibility, only full-text articles will be included in the review.
The review will exclude gray literature, books, reviews, meta-analyses, meeting abstracts, and conference presentations. Reviews and meta-analyses will be excluded as they may represent duplicates and analyze data from original articles rather than providing new primary data. Studies that solely focus on bright-field histology of the male reproductive system and gonadal-somatic indexes will not be considered. Additionally, research involving taxa that do not belong to Squamata will be excluded from this review.

Information sources We performed a literature search using the National Autonomous University of Mexico institutional subscriptions. The databases employed up to December 2024 were

Web of Science (WoS) (Clarivate), and Scopus (Elsevier). The search was limited to these databases because of their renowned relevance as databases for scientific literature with a peer-review process.

Main outcome(s) The following data were extracted from the eligible papers: Country of the studied population, families, genus and species and studied, parity mode, sample obtention, methodological approach, IUCN category (Data Deficient, Least Concern, Near Threatened, Vulnerable, Endangered, Critically Endangered, Extinct in the Wildlife, Extinct). Bibliometric indicators such as article title, source title, list of authors, their affiliations and countries, abstracts, keywords, and the citations they receive.
Describe the outcomes of the review including all relevant details such as timing and effect measures.

Additional outcome(s) Not applicable.

Data management The studies selected after the full-text screen and the articles that met the inclusion criteria were analyzed to extract information that was categorized. An Excel sheet was developed to record the data from the manuscripts retrieved. The extraction was performed by at least three members (KVAC, NBCC, UASR, RICC) of the team to ensure homogeneity. The discrepancies were reviewed and resolved by a fourth reviewer and if necessary, a consensus discussion. The data items extracted for the Excel datasheet were a) Bibliographic: (article title, publication year, journal name, authors, cites, keywords, institution, and country); b) Study intervention (spermatozoa, semen, ejaculates), outcome, suborder, family, specie, use of euthanasia, method of euthanasia, methodological approach, wildlife or colony-breeders. A search in the IUCN red list (https://www.iucnredlist.org/) was made to include the category of each species (Data Deficient, Least Concern, Near Threatened, Vulnerable, Endangered, Critically Endangered, Extinct in the Wildlife, Extinct). A detailed explanation of the variables is listed above.
1.- Year: Year of paper publication
2.- First author's country: Country of the first author's adscription
3.- Suborder: Taxonomic rank of the species studied that falls below an order and above family
4.- Specie: The species studied
5.- Euthanasia: Studies where the authors used a painless method of ending the life of the organisms
6.- Method of euthanasia: The technique or procedure used to end the life of the animals humanely and painlessly (decapitation, lethal injection, cervical dislocation, inhalation, freezing)
7.- Method of sample obtention: Refers to the procedure employed to obtain spermatozoa or semen from individuals for analysis (massage, electroejaculation, pressure, tissue extraction, washing, etc.)
8.- Population studied: Considers if the study was performed in a wildlife population or a breeding colony
9.- IUCN status: Conservation status or risk of extinction reported by the IUCN.

Quality assessment / Risk of bias analysis To reduce the risk of bias and errors, the manuscripts retrieved were screened by at least two authors in each phase. The manuscripts listed as uncertain were resolved via group discussion or a third independent reviewer. If there was detected at least one exclusion criterion they were not considered for the mapping. The selected articles are available in an Excel datasheet, where the review team included the full record obtained from WoS and listed the reasons for the exclusion. Then the articles that met the inclusion criteria were imported into the reference management software (Mendeley, Elsevier), via RIS file where all the reviewers accessed the results. Describe the method of quality assessment in primary studies.

Strategy of data synthesis Before the screening stage, the whole team was capacitated to perform an adequate review of the papers considering the inclusion and exclusion criteria established in the PCC-PICO framework. The screening strategy was performed in a two-stage manner: In the first, we focused on a screen of the title, abstract, and keywords; in this step, the inclusion and exclusion criteria were refined iteratively to confirm their reproducibility. The second stage was performed by reading the full text. To reduce the risk of bias and errors, the manuscripts retrieved were screened by at least two authors in each phase. The manuscripts listed as uncertain were resolved via group discussion or a third independent reviewer. If there was detected at least one exclusion criterion they were not considered for the mapping. The selected articles are available in an Excel datasheet (Suppl. 1) where the review team included the full record obtained from WoS and listed the reasons for the exclusion. Then the articles that met the inclusion criteria were imported into the reference management software (Mendeley, Elsevier), via RIS file where all the reviewers accessed the results.

Subgroup analysis Taxonomic groups: Differences in sperm parameters between lizards, snakes, and amphisbaenids.
Tissue source: Sperm collected from the epididymis vs. directly from the testes or ejaculate.
Reproductive season: Variations in sperm quality between breeding and non-breeding periods.
Preservation method: Fresh vs. cryopreserved sperm samples.

Sensitivity analysis Not applicable.

Language restriction Only English terms will be included in the search strings. However, those hits either in English, French, Italian or Spanish were revised, due to limited languages understood by the team.

Country(ies) involved Mexico.

Keywords Semen, sperm, squamata, reproductive, systematic review, bibliometrics.

Dissemination plans The systematic review findings will be published in a peer-reviewed journal and/or presented at conferences.

Contributions of each author
Author 1 - Norma Berenice Cruz-Cano - Conception, Methodology, Data extraction, Writing the initial draft, Formal analysis, Development of the selection criteria, Visualization, Drafting the work, Investigation, Writing (review & editing), and Approval of the final manuscript.
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Author 3 - Karla Viviana Aranda-Carrillo - Methodology, Data extraction, Formal analysis, Development of the selection criteria, Visualization, Investigation, Writing (review), and Approval of the final manuscript.
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