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M A G A Z I N E

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NATURE

Editor's choice: two systematic reviews in this issue.

Deep in the dense forest, the wolf searches for a mate to hybridise with and the rat is deeply anaesthetised.

1234567890



Table of contents

List of participants	2
Research topics proposed by participants.....	3
Topic 1 - The scientific evidence on hybridisation events in wild canids and its effect on species fitness, dispersion and genetic variation.....	4
Group members	4
First version.....	5
Reviews	13
Final version.....	28
Topic 2 - The effects of maternal exposure to anaesthesia during pregnancy on the offspring neurodevelopment – a systematic review of studies in small mammal models	36
Group members	36
First version.....	37
Reviews	47
Final version.....	60

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Research topics proposed by participants

1. The effect of bilingualism on developing dementia.
2. Systematic review on sleep and memory consolidation.
3. Systematic review on systematic reviews in biology.
4. Wildlife intra- and interspecific interactions – a systematic review.
5. The effects of ambient temperature on circadian rhythms of birds and mammals: a systematic review.
6. The influence of microplastic on the nervous system.
7. Are female rodent models more included in studies of autism disorders over the last decade?
8. Work-life balance and PhD students.
9. Systematic review of plant-animal interactions in urban and suburban environments.
10. General idea: systematic review of life and career outcomes of PhD graduates in (life and environmental) sciences.
11. The effect of high-fat diet in adulthood on the development of mood disorders.
12. The influence of global warming on antibiotic resistance in bacteria.
- 13. The effects of maternal exposure to anaesthesia on offspring neurodevelopment.**
14. The influence of taurine consumption (energy drinks) on children and teenagers health- analysis of data from the past decade.
15. A systematic review of methods of scientific communication and combating misinformation in public spaces.
16. Systematic review of the reactions of local residents on living near national parks.
17. Systematic review of the effects of climate change on Arctic Tundra communities.
18. The relevance of vitamin D to the COVID-19 pandemic.
19. A systematic review of neurotoxicity of persistent organic pollutants (POPs) in urban areas and implications for human health.
20. Neurobiological topic: Involvement of non-classical neurotransmitters in the pathophysiology of neurodegenerative diseases.
- 21. Systematic review on wolf-dog hybridization.**
22. The effect of microplastic on plant/soil interaction in croplands.
23. The impact of microplastic on the reproductive system and fertility.
24. The use of induced pluripotent stem cells in the treatment of neurodegenerative diseases.
25. The effects of fertilizers on oxidative stress in animals and plants.

Topic 1 - The scientific evidence on hybridisation events in wild canids and its effect on species fitness, dispersion and genetic variation

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First version

The scientific evidence on hybridisation events in wild canids and its effect on species fitness, dispersion and genetic variation

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Summary

Interspecific hybridisation, i.e. interbreeding between distinct species, has become an increasingly important factor carrying major implications for population genetics, species ecology and conservation. This review focuses on evaluating existing research on hybridisation in wild canids, particularly its impact on species fitness, genetic variation, and dispersion. By analysing 133 relevant publications from an initial pool of 3.466 records, we gained valuable information on the prevalence and consequences of hybridisation events. The studies used various methods to detect hybridisation and its effects, with an emphasis on genetic analysis (61% of studies) or its combination with morphology (23%). The majority of publications (89%, n=118) detected hybridisation between canid species. Researchers put an emphasis on measuring genetic variation (n=81) and animal dispersion (n=49), providing methodological (n=78) and conservation (n=75) recommendations. This review revealed a scientific gap in the assessment of recent hybridisation events from the perspective of canid reproduction and survivability, which are the key factors in the conservation of numerous species. In addition, despite the increasing number of human-wildlife conflicts, only 12 papers mentioned any societal recommendation regarding canid hybridisation.

Introduction

Interspecific hybridisation is a complex phenomenon and a subject of study in many areas of biology, such as evolutionary biology, population genetics, ecology, and conservation. It occurs when two distinct, but not entirely reproductively isolated species come into contact and interbreed (Schwenk, K., Brede, N., & Streit, B., 2008). This may result in the creation of offspring that can also be partially or completely fertile and interbreed with one or both of its parent species. In such cases, through repeated backcrossing, introgression – or the flow of genes between different species – may occur (Aguillon, S. M et al, 2022).

Hybridisation and introgression can have varying consequences for the hybridising populations (Adavoudi, R., & Pilot, M., 2021). It may have negative impacts, such as the creation of infertile offspring and a consequent drastic reduction in fitness (Phadnis, N., & Orr, H. A., 2009), introduction through introgression of harmful alleles (Rinker, D. C et al, 2020), or a reduction in the genetic diversity of the population (Fraïsse, C. et al, 2014). On the other hand, introgression sometimes introduces favourable gene variants and can improve the fitness of the population, in which case it is referred to as adaptive introgression (Hedrick, P. W., 2013).

Hybridisation is of particular interest in wild canids. Dogs (*Canis lupus familiaris*), one of the most common pets and an invasive species, share their ancestry with the grey wolf (*C. lupus*), and are able to interbreed, producing fertile offspring, with many canid species, including wolves, coyotes (*C. latrans*), dingoes (*C. dingo*), and golden jackals (*C. aureus*) (Leonard, J. A. et al, 2013). This may result in the introgression of undesirable traits, such as a lowered apprehension towards humans, which can increase human-wildlife conflicts and threaten the conservation of the species (Pilot, M. et al, 2021; Lescureux, N., & Linnell, J. D., 2014).

Foxes are another species cultivated by humans, which include red fox (*Vulpes vulpes*) and arctic fox (*V. lagopus*). Both are known to escape fur farms and hybridise with several wild fox species, which can threaten their conservation (Noren, K. et al, 2005; Wierzbicki, H. et al, 2021). Furthermore, wild canids can interbreed with each other – for example, coyotes and wolves (Bohling, J. H., & Waits, L. P., 2015) forming so-called coywolves, or red wolf (*C. rufus*) and grey wolf (Jenks, S. M., & Wayne, R. K., 1992), which can have positive or negative impacts on their genetic diversity, fitness, and conservation status.

In this review, we investigated the scope of research on wild canid hybridisation, whether with human-cultivated species or with each other, occurring since the year 1900. We aimed to create an overview of the field and examine whether studies assessing hybridisation in wild canids report its effects on fitness, dispersion, and genetic variation of the examined species. We also examined whether the studies provide recommendations on methodology of research or wildlife conservation policy and societal action. This study can improve our understanding of the research gap on canid hybrids and provide the context for ecological, genetic, and conservation researchers.

Materials and Methods

The systematic review started from formulating a research question: “Do studies assess species fitness, dispersion and genetic variation when interspecific hybridisation occurs?”. To capture the comprehensive range of relevant studies, a search string was created, incorporating key terms and synonyms related to the topic of interest (Fig. 1). On 04.06.2024, abstracts were collected from two databases, Web of Science and Scopus. Those abstracts were deduplicated, then systematically screened

using Rayyan software (M. Ouzzani et al, 2016), with every abstract screened by at least two people. Following abstract screening, all the articles included according to the criteria (Table 1) passed into full-text screening. The selected studies were reviewed to confirm their relevance and adherence to the research question and subsequent coding of these studies was conducted using Google Forms. On each stage, the exclusion and inclusion criteria were used (Table 1). We decided to only include papers that were readily available in the open access or by university subscriptions.

Table 1. Criteria used to determine inclusion/exclusion of articles for the review.

INCLUSION CRITERIA	
Hybridisation study	The article must be a hybridisation study with the usage of any genetic, morphological, both genetic and morphological or other methods in order to detect hybrids and access its effect on wild canid populations
Species	Any wild-living canid species as one of the parents of a specimen that is a suspect of hybridisation
Time of event	The study includes hybridisation or introgression event that occurred since the last century (1900)
Language	The study must be published in English
Publication	It must be a research article (may be a preprint) with analysis of data for a new study
EXCLUSION CRITERIA	
Irrelevant	Articles that do not study hybridisation or their method is not applicable for hybridisation detection (for e.g., only mtDNA genetic analysis)
Phylogenetic	Article studies species on time scale not including hybridisation events happened after year 1900
Species	Articles in which both parents come from non-wild (captive) population
Misclassified	Articles that studied other than canid species in terms of hybridisation or hybridisation is inter-, not intraspecific (for e.g., between different populations or subspecies)
Accessibility	The full text was unavailable and we could not check if the article met the criteria
Publication	Any review, conference paper, book chapter was excluded since they did perform an original experiment or the data may be duplicated

Upon inclusion, data on species studied (genus and species name); country; analytical method of assessing hybridisation (genetic, morphological, both genetic and morphological, and others); parameters accessed (reproduction, survivability, genetic variation, dispersion); and if methodological, conservation or societal recommendations provided were extracted. This information is available as metadata attachment (SM1). The construction of graphs and visual representations of the data was performed using R environment (R Core Team, 2023) and assorted packages (“network3D”, “webshot2”, “rworldmap”) to illustrate key findings and trends within the literature.

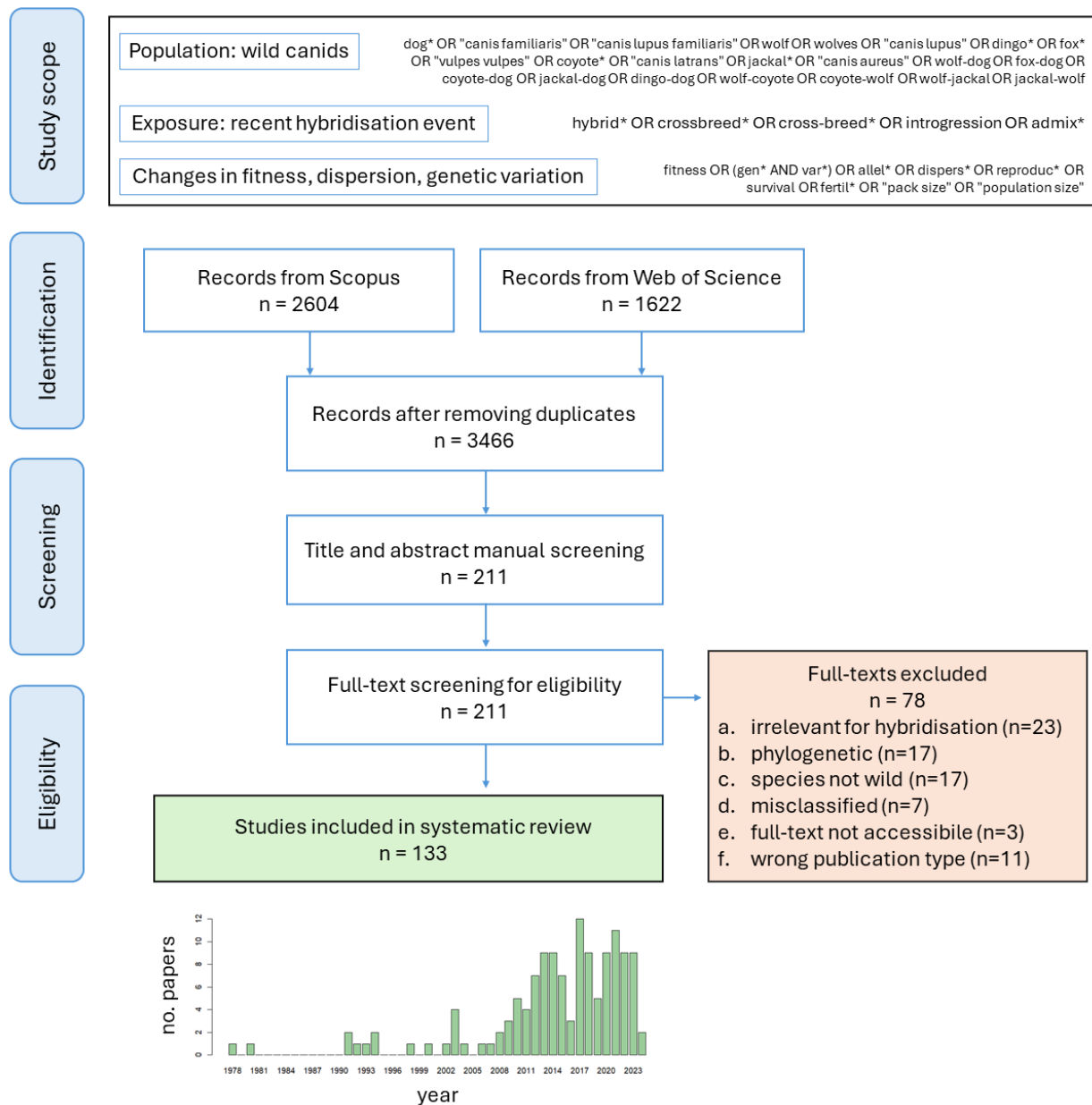


Fig. 1. PRISMA flow diagram showing the procedure of literature search and selection according to inclusion/exclusion criteria summarised in Table 1.

Results and discussion

Our review focused on 133 articles, out of which in 89% of studies (n=118) hybridisation was detected in the results of the analysis. Out of 15 studies where hybridisation was not detected, 11 studies measured genetic variation and 8 studies provided methodological recommendations.

The main methods used in the studies were: genetic analysis (n=81, 61% of the studies), followed by both genetic and morphological analyses (n=31, 23%) and morphological (n=13, 10%). Other methods included: acoustic (n=1); semen analysis (n=1) and mathematical model (n=1); both genetic

and radio-tracking (n=2); both genetic and mathematical model (n=2); genetic, morphological and histological (n=1).

Research on hybridisation in wild canids is conducted globally, indicating widespread interest in this topic on every continent; we counted 53 countries in which these studies have been done. The percentage breakdown shows the highest interest in hybridisation research in wild canids in North America, which accounts for 49.6% (n=66) of all the articles reviewed. Europe is the second most active region with 42.9 % of the research. Asia, South America, and Australia have smaller but still significant shares in the global study of hybridisation in wild canids (Fig. 2A). These intensive studies highlight the importance of hybridisation for conservation and population genetics. The majority of research originates from North America, where hybridisation between wolves, coyotes, and domestic dogs is extensively documented. These studies frequently focus on genetic diversity and the ecological and evolutionary impacts of these interactions. While North American countries – USA (n=44) and Canada (n=22) – dominate in the number of papers, in Europe Italy is leading in the hybridisation research (n=21). This is largely connected to strong wolf research by one of the pioneers of wolf conservation in Europe Luigi Boitani and his team (for e.g., Boggiano et al., 2013; Ciucci et al., 2003). Other notable research states are Australia (n=10), Croatia (n=8), Russia (n=8), Spain (n=7), Bulgaria (n=7) (Fig. 2A).

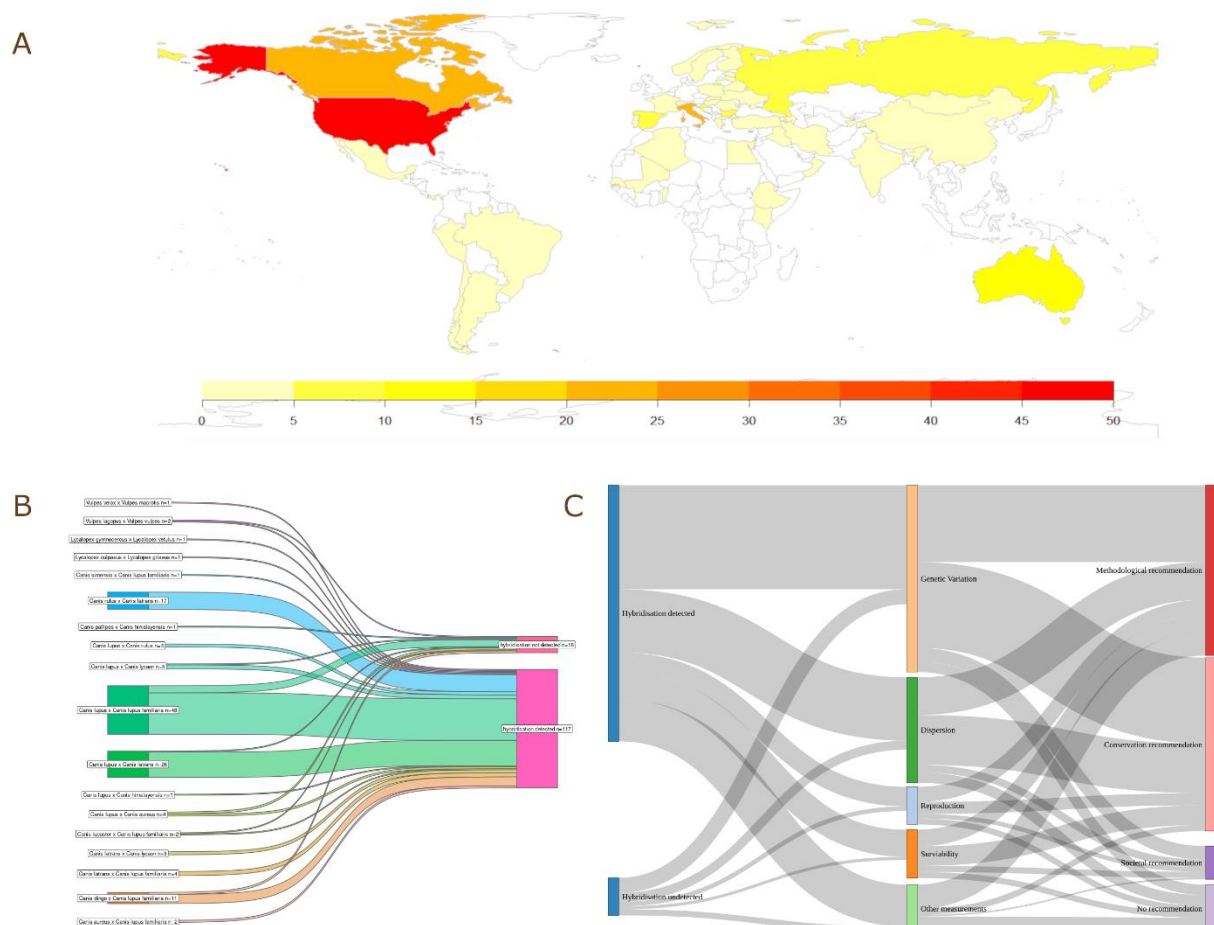


Fig. 2. The distribution of the studies by: (A) the countries where wild populations were sampled, (B) the species whose hybridisation was studied, and (C) measured fitness parameters and recommendations provided.

From the parameters accessed 61% of all studies (n=81) measured genetic variation in context of hybridisation in wild populations. The next by popularity was dispersion, measured in 49 studies (37%), followed by survivability and reproduction parameters accessed in 20 and 17 studies respectively. Out of 133 studies, majority was methodological- and/or conservation-oriented, with 59% (n=78) and 56% (n=75) of studies providing methodological and conservation recommendations respectively. Only 12 studies provided societal recommendations in context of hybridisation.

Out of 13 Canidae genera, our research string provided us with hybridisation studies focusing on three of them: wolves *Canis* (n=128); followed by *Vulpes* genus (n=3); and South American foxes *Lycalopex* (n=2). The domestic dog appeared as a source of introgression in almost half of the studies (n=67). Moreover, *C. lupus* x *C. l. familiaris* were the focus in 48 studies, while 10 other Canidae genera were not detected within our research scope.

This review revealed the problem of distinct species recognition on the span of continuing evolution. As an example, a *C. lycaon* species used to be a subspecies *C. lupus lycaon* (Sears et al., 2003), but currently is a species in the span of a “Canis hybrid swarm” in North America (Rutledge et al., 2010). In addition, there was observed a recent distinction of two more separate species: *C. pallipes* (formerly *C. lupus pallipes*) and *C. himalayensis* (formerly *C. lupus chanco*) (Srinivas and Jhala, 2023). According to this knowledge, we tried to unify how we address the species in Fig. 2B.

The findings from this review underline the complexity and prevalence of hybridisation events among wild canid populations. The detection of hybridisation in a significant portion of the studies suggests that hybridisation is a widespread phenomenon with potential implications for genetic diversity and species conservation.

The predominance of genetic analysis as a method reflects the importance of molecular tools in identifying hybridisation events and assessing their impact on genetic variation. The relatively high number of studies focusing on dispersion indicates a growing interest in understanding how hybrid individuals spread and integrate into wild populations (Fig. 2C).

Our study of hybridisation events in wild canids suggests that hybridisation is a widespread phenomenon with major implications for species fitness, genetic variation, and distribution. Most studies have focused on genetic analyses, highlighting the importance of molecular tools for understanding hybridisation. Although many studies have provided valuable methodological and conservation recommendations, major gaps remain in assessing the impacts of recent hybridisation events on reproduction and viability, key factors for effective species conservation. Closing these gaps and incorporating societal

recommendations into future studies will be critical for mitigating human-wildlife conflict and protecting wild canid populations.

Author contributions

All authors contributed to data identification, screening of abstracts and full-texts and preparation of this paper. J.P. and M.C. organised the work division, E.R. and R.M. organised data processing and compilation, R.M. and J.P. provided statistics visualisation, M.C., P.D., E.R. and M.S. worked the most on the review text.

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Reviews

1. Dr Agnieszka Olszańska

The manuscript “The scientific evidence on hybridization events in wild canids and its effect on species fitness, dispersion and genetic variation” presents an interesting study on the extremely important (especially for the wildlife management and conservation) issue of wild canids hybridization. It was really ambitious and probably requiring lots of work and personal involvement to accomplish the study. Considering the fact that the Authors are young researchers, I congratulate them. I am positive that the results might provide valuable material for the scientific community, as well as nature conservation and management institutions. Yet still, I have impression that Authors should reconsider, improve or rewrite some sections of the manuscript. As a general remark (repeated numerous times), I strongly suggest to be as specific as possible, avoid unnecessary adjectives, read and read again all the sentences used, especially avoid general and uninformative statements, repetitions and overinterpretation.

I believe that after a throughout revision, the manuscript would be worth publishing and dissemination. Thus, I would recommend careful revision of the manuscript.

Below I present some additional comments and remarks:

I am not sure about the form of the citations in the main text are provided. Please reconsider and correct the form according to the requirements or examples provided by the editors and Publisher.

INTRODUCTION

Lines 31-33: There is something missing in this sentence. Please rewrite.

Line 42: Why Authors consider dogs as invasive species. Are there any references supporting such statement or classification? Please refer or rewrite. I am also not sure if the dogs here are understood as companying animals or free-ranging animals released into the wild.

Line 43, line 49: I would recommend avoiding unsupported adjectives, like “many” in this case or “several”. The Authors suggest there are four species of wild canids that may hybridize with the dogs, that is not “many”. I am also not sure how the red fox (*Vulpes vulpes*) may hybridize with the wild red fox (*Vulpes vulpes*), as they are the same species.

Line 48, line 56: “Cultivated” is a bit awkward term in relation to animals. Maybe “bred”? Please rewrite.

Lines 52-54: Please be more specific. Rather than repeat “which can have positive or negative impacts on their genetic diversity, fitness, and conservation status”, please give explicit examples and references.

Line 56: It is unclear what do Authors do, whether they analyze the phenomenon of hybridization documented since 1900 or the articles published since 1900.

Lines 56-58: Maybe instead of “We aimed to create an overview of the field and examine whether studies assessing hybridisation in wild canids report its effects on fitness, dispersion, and genetic variation of the examined species”, just “We aimed to analyse the phenomenon of hybridisation in wild canids in the research published since XXXX till XXXX and its effects on fitness, dispersion, and genetic variation of xxxx, xxxx, xxxx, and xxxx species”. Please try to be as specific as possible, it helps the Reader follow your research.

Lines 60-61: Again, please avoid general statements like “This study can improve our understanding of the research gap”. Maybe something like “We provide the analysis of the hybridization effects on the wild canid species fitness, genetic variation, ecology and thus conservation”.

MATERIALS AND METHODS

In this section I do not find any information on the crucial part of the “Material and methods” part and also necessary for the reader to fully appreciate the work done. I have several questions to this part:

- 1) What was the time scope (articles published since XXXX and XXXX)?
- 2) What was the language of the published articles (I understand from the Table 1 that “The study must be published in English”, yet does it refer to the abstract or to the full text of the research paper?)?
- 3) Did the search string changed between the first (title and abstract screening) and second (full-text screening) selection of manuscripts to be analysed?
- 4) What species the Authors concentrated on? Here I have some doubts. Trying to understand Fig. 1 I see that there were six species: domestic dog *Canis familiaris*/*Canis lupus familiaris*, wolf *Canis lupus*, dingo *Canis dingo*, coyote *Canis latrans*, golden jackal *Canis aureus*, and red fox *Vulpes vulpes*. Why not any other fox species were searched, especially that Authors mention (and that is a known fact) that foxes may hybridize between the genus *Vulpes* and between foxes and dogs (the Pampas fox and dogs for example). Please explain why such selection of search terms was used for the analyses.

Please consider referring to the PRISMA guidelines (especially that the term is mentioned in the Fig. 1 title) specified in:

Page M J, McKenzie J E, Bossuyt P M, Boutron I, Hoffmann T C, Mulrow C D et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews BMJ 2021; 372 :n71 doi:10.1136/bmj.n71

Line 66: The exact date when your research started is not necessary.

69: maybe “at least two researchers”?

Lines 71-72: I am not sure what Authors meant by “was conducted using Google Forms”. Please explain.

Line 73: which University? Please be specific.

Lines 75-78: Be a bit more specific. Maybe: “The analyze units / criteria were as follows: ...”. Be also specific in terms like “country” – is it the country where the study was performed?

RESULTS AND DISCUSSION

Line 90: Maybe “The main analytical methods to assess hybridization were: solely genetic analysis (n=81, 61% of the studies), both genetic and morphological analyses (n=31, 23%) and solely morphological”.

Line 97: I am not sure about the statement “the highest interest in hybridization research”. Maybe just “the highest number of research on hybridization”? Do not assume and be specific. Same for lines 98-99, rather than “Europe is the second most active region”, maybe just “the number of research studies performed in Europe”.

Lines 100-101. It is an overstatement. Authors (till this part of the presented manuscript) did not provide information about the reference of the analyzed studies to the species conservation and population genetics, why then assume that “These intensive studies highlight the importance of hybridization for conservation and population genetics”?

Lines 101-104: Repetition, please remove. And again, try to be specific (which exactly “ecological and evolutionary impacts”?), avoid using adjectives (like “frequently”).

Lines 104-106: Again, repetition. Maybe instead of for the third time mentioning that the majority pf the papers on hybridization are done by the North American authors on the North American species, a table with exact numbers, references and aspects of the research would be clearer?

Line 107: I would avoid statements like “This is largely connected to strong wolf research by one of the pioneers of wolf conservation in Europe Luigi Boitani and his team”? Maybe yes, maybe no. Maybe Italy has more money for the research or maybe they were just more fluent in English or maybe there is another reason. Try to avoid overstatements, assumptions and overinterpretation.

Lines 111-113: The figure 2 is not clear and unreadable for the reader. I am unable to understand what is there (Fig. 2B and 2C are totally unreadable, thus useless for the reader....). When describing the figures or tables, please be as specific as possible. The present title of Fig. 2 is not fully informative. Here you might add info on how many papers, which were the studies species, which were the measured parameters and recommendations.

Line 114: maybe “assessed” rather than “accessed”? And also repetition – this information was already given in line 90.

Line 115: Avoid statements like “The next by popularity”. The research is not about popularity ;-). Maybe “The analysed research papers provided also results on dispersion,”

Lines 121-123: The information on species should be given in the “Materials and methods” section. Also the *Lycalopex* genus did not appear in the search strings presented in Fig. 1.

Lines 126-132: This section should rather be moved to the “Materials and methods” section. And also should be somehow addressed in the research strings presented in the Fig. 1 or described precisely in a form of “Supporting information”.

2. Joanna Roszkowska

The manuscript presents a systematic review focusing on the impact of hybridisation in wild canids. The study is novel and highly relevant, given an increasing recognition of interspecific hybridisation as a significant factor in population genetics as well as ecology. The authors gathered and analysed data from 133 publications out of an initial pool of 3 466 records, which gives a reasonable success rate of 3.8%.

The manuscript follows the guidelines and structure recommended by Biology Letters, maintaining an organisation into sections: Summary, Introduction, Materials and Methods, Results and Discussion. The word count does not exceed the 2 500-word limit, there are 2 figures and one table included, which also meets the criteria. The methodology is very well-documented – all inclusion and exclusion criteria are explained in a table, although the “phylogenetic” criterion is a bit unclear. The authors formulated a precise research question and used a comprehensive search string to include relevant studies from two databases. The use of R environment for data visualisation further enhance the robustness of methodology. The PRISMA diagram is very informative, indicating the number of articles included after each step of the selection process, however the font on the inserted bar chart could be larger. Figure 2. is very aesthetic, however, similarly, the font in charts B and C is too small to read without zooming in. The world map showing the geographical distribution of analysed articles is particularly relevant in the case of this review. The included articles are relevant to the topic and were mainly published during the last two decades. As the oldest one was published in 1978, the timespan of included studies could be specified more strictly than dating back to 1900.

The results section is well organised together with the discussion, which is a common practise in this type of reviews. The authors highlight that the detection of hybridisation was reported by majority of the studies, with emphasis on genetic analysis as primary method used. This reflects the scientific trend towards molecular tools in studying hybridisation among other fields. A significant research gap is recognised by the authors, stating that the effects of hybridisation on the reproductive aspects of canids populations are understudied with respect to recent events. Also, as highlighted by the authors, only a few studies provided societal recommendations, which is a critical aspect given the increasing number of human-wildlife conflicts including canids. Identification of such research gaps is crucial and should be considered by the scientists from the field. At the same time the authors are aware of potential difficulties in recognising and separating distinct species, as the evolution is a continuous process and often results in inconsistencies. This aspect is taken account of and, as a non-expert in the field, I agree that it was the best way to assess the problem. The review underscores the fact, that the research of hybridisation is very complex, which is why such summarising articles are very helpful and necessary.

In summary, the review provides a comprehensive overview of the prevalence and consequences of hybridisation in wild canids. The list of references is particularly extensive and the authors acquire a broad knowledge regarding the scope of the study. Due to the identification of research gaps, the review provides a valuable insight into the topic of hybridisation and can serve as a guideline for the scientists planning future studies and analyses.

3. Martyna Gorkowska

The systematic review entitled ‘The scientific evidence on hybridisation events in wild canids and its effect on species fitness, dispersion and genetic variation’ meticulously evaluates existing research on hybridisation in wild canids, focusing on its impact on species fitness, genetic variation, and dispersion. The title and abstract are highly informative and appropriately reflect the paper’s content.

In the introduction, the authors clearly explain the hybridisation process and its positive and negative influences on the ecosystem, supported by extensive literature references. However, the explanation of the hybridisation process and its potential drawbacks felt somewhat redundant and too long, repeated unnecessarily. The necessity for a systematic review on this topic is well justified. The authors designed a comprehensive search string for two databases, yielding 3466 records after deduplication for abstract screening. The inclusion and exclusion criteria are well-defined and suitable for the intended outcomes. From these, 211 publications were selected for full-text screening, and 133 were included in the review. The reasons for excluding the remaining 78 are clearly stated in the PRISMA diagram.

The methodology is detailed enough to allow replication, with understandable search strings and inclusion/exclusion criteria. The authors adhere to the guidelines regarding the number of figures and tables for reviews in *Biology Letters*. While the figure descriptions are appropriate, the font size in the figures should be increased for readability.

The review effectively highlights the countries where studies were conducted, identifying both overstudied and understudied regions. This is one of the research gaps identified by the authors. The results are detailed, presenting the number of papers and proportions related to the chosen categories based on the inclusion criteria. The discussion is well-grounded in the results and the knowledge gaps are outlined in the conclusions.

Overall, the review is written in high-quality English, with correct grammar and no errors. The figures and tables are well-prepared and appropriately placed within the article. In conclusion, this systematic review is of high quality, and its conclusions may be valuable for scientists focusing on hybridisation in canids.

4. Szymon Kantor

The review by 6 authors from Jagiellonian University titled “The scientific evidence on hybridisation events in wild canids and its effect on species fitness, dispersion and genetic variation” focuses on, as the title itself says, evaluating existing scientific literature on hybridisation in wild canids, particularly its impact on species fitness, genetic variation, and dispersion. The review identifies significant gaps in the existing literature, particularly in the assessment of recent hybridisation events concerning reproduction and survivability. While the introduction does not explicitly state hypotheses, the study is structured around two questions: (i) whether studies in wild canids assess species fitness, dispersion, and genetic variation when interspecific hybridisation occurs, and (ii) whether studies provide methodological, conservation, or societal recommendations related to hybridisation in wild canids.

The article contains 10 pages and follows the modified scheme of a “Biology Letters” article. Its content includes: (i) the aforementioned title, (ii) a list of authors with affiliations, (iii) a summary, (iv) an introduction, (v) a materials and methods section, (vi) results and discussion combined into one section, (vii) an author contributions section, and (viii) references (21 records). However, the paper lacks an acknowledgment section. The review adheres to the adopted text formatting conditions (<2500 words in total, 2 figures, 1 additional displayed element, i.e., table, line spacing of 1.5, line numbering). The text is written in British English, as identified by certain spellings and word choices, such as “organised” in line 151 (American English: “organized”) and “methodological-oriented” in line 117 (American English: “methodologically oriented”). Additionally, phrases like “human-wildlife conflicts” from line 148 are probably more common in British English. From an editorial point of view, the consistency in maintaining a uniform dialect throughout the work is commendable, especially considering the collaborative nature of the text. However, there are a few punctuation errors regarding the use of commas, mainly in constructions with the word *and*.

The review is based on a literature search, analysing 133 publications out of 3,466 initial records obtained by screening two databases: (i) Web of Science and (ii) Scopus. Thus, considering the short time the authors had to prepare the work, the authors made an effort to detect as many articles as possible that were potentially worth considering. On the other hand, covering studies since 1900 might be too broad, potentially including outdated methodologies and findings. A more focused temporal scope might yield more relevant and current insights. A strong point of this review is its coverage of research from various regions globally, spanning 53 countries (as shown in Fig. 2). This geographic diversity helps provide a comprehensive understanding of hybridisation events in different environmental and ecological contexts.

What is questionable is the not-fully-specified database search process. Unfortunately, the search string was presented by the authors as an unselectable fragment of Fig. 1, making it difficult for the reviewer to verify its effectiveness. There are also doubts about the discrepancy between the number of literature

records retrieved using the search string provided in the text and the number checked by the reviewer. For example, manually rewriting the presented string from Fig. 1 into the Web of Science database with the "All fields" option selected resulted in 4,356 records. Changing the option to "Abstract" gave a value of 1,603 literature records, which was similar to that presented by the authors (1,622). Providing a full string would certainly help avoid similar misunderstandings.

The authors used rational and justified criteria for the inclusion or exclusion of scientific articles for analysis. However, it is noteworthy that a large number of initial records translates into a relatively small percentage of articles qualified for systematic review after abstract review and full text screening (approximately 3.84%).

The tools used in the process of preparing the article (including Rayyan software for abstract screening, Google Forms for evaluation of full texts, and R packages for data visualisation) were adequate for the purposes for which they were used. However, the authors did not specify the details regarding the deduplication process of literature records (whether it was done in the literature manager, and if so, which one; during manual browsing, automatic detection, or a dedicated plug-in was used).

The data were visualised by the authors in a clear and visually attractive way. The type of charts used, and the information presented in them corresponded well with what would be expected in similar work. The authors exercised reasonable minimalism and did not overload the work with uninformative and unnecessary charts, which should be considered a great advantage as it does not distract from the content of the article.

The description of the results is quite comprehensive and intellectually satisfying for the reader. The flow of information and the order in which it is presented are logical and smooth. Finally, as a result of a proper discussion, the review effectively identifies research gaps, particularly the lack of studies on recent hybridisation events from the perspective of reproduction and survivability.

To sum up, it is a good work, well thought out and organized, identifying important research gaps, although it is not free from some errors and omissions: technical (mainly in terms of punctuation) and methodological (imprecisely described database search process).

4. Martyna Marzec

The paper under review is a systematic review that focuses on interspecific hybridisation among wild canids, exploring its impacts on species fitness, genetic variation, and dispersion. The review synthesizes data from 133 publications selected from an initial pool of 3,466 records. The scope provides a comprehensive dataset for understanding the prevalence and consequences of hybridisation in wild canids, highlighting its significance for population genetics, species ecology, and conservation.

The title and the abstract accurately reflect the content of the manuscript. The introduction clearly states the aims of the studied topic. The methodology is accurate and well-structured. The authors formulated a clear research question: “Do studies assess species fitness, dispersion, and genetic variation when interspecific hybridisation occurs?” They utilized a search string related to hybridisation and the species of interest, and screened abstracts from two major databases, Web of Science and Scopus. The authors used Rayyan software for abstract screening and Google Forms for data extraction from the full-texts. The inclusion and exclusion criteria (included in Table 1) are well-defined, ensuring that only relevant studies were considered. The criteria focused on the type of study (hybridisation studies with wild-living canids since 1900), language (English), and publication type (original research articles), which enhances the reliability and relevance of the selected studies. The exclusion criteria further refined the dataset by eliminating irrelevant, phylogenetic, or inaccessible studies. The methods are described in sufficient detail for the study to be easily repeated. The review provide significant findings and valuable insights into the hybridisation of wild canids. Majority of the reviewed studies (89%) focused on hybridisation, what indicates that this phenomenon is common among wild canids. A significant number of studies (61%) focused on genetic variation, highlighting the importance of molecular tools in understanding hybridisation. However, the relatively low emphasis on morphological and other methods (e.g., acoustic, semen analysis) may limit the understanding of hybridisation's phenotypic and behavioural impacts. A broader approach that incorporates diverse methodologies could offer a more comprehensive understanding. The manuscript includes studies from 53 countries, providing a global perspective on the issue of hybridisation in wild canids. This geographical diversity enhances the relevance and applicability of the findings across different regions and is definitely a strength of the review. The identification of research gaps, particularly in the assessment of recent hybridisation events on reproductive and viability parameters, is a significant contribution, indicating areas that require further investigation. Additionally, the lack of societal recommendations points (mentioned only in 12 studies) to the need for integrating social aspects into future research to better manage human-wildlife interactions.

The manuscript is structured in a logical manner, guiding the reader through each section with ease. The figures and graphs are thoughtfully designed, effectively illustrating described results, however the small font size on the graphs may reduce accessibility for readers. Table 1 and PRISMA flow diagram are presented in a format that is straightforward to interpret, which helps to better understand the procedure of literature search and selection that authors utilized to create the review.

In summary, this systematic review offers a comprehensive and well-organized synthesis of the existing literature on hybridisation in wild canids, with clear methodology and valuable insights into research gaps. It may be a source of valid information for specialists in the field.

5. Anbarieh Saadat

This review highlights the complex nature and common occurrence of hybridisation events within wild canid populations which holds a significant interest for behavioral ecologists. It presents a novel idea that builds upon previous research in this area. The authors have effectively described the content of the articles that they used for this review, and the title and abstract convey the purpose of the review. In the abstract they explained the aim of this review well and what is the importance of collecting and analysing literature for the hybridisation. They also proposed how this will help to fill the knowledge gap for canid reproduction and survivability. In the introduction, the authors provided a comprehensive explanation for the need to study the topic, highlighting its importance and the potential effects of inbreed hybridization on both human and other species' lives. They very well described the consequences of hybridisation, both positive and negative, and its significant implications for various aspects of species fitness, genetic variation, and distribution. They explained the implication of this review for species conservation and the importance of this work for ecological, genetic, and conservation researchers. The background, previous studies, and the importance of their review were explained in enough detail. They also mentioned the knowledge gap present in this field and how interdisciplinary it is which will be appreciated in different fields such as evolutionary biology, population genetics, ecology, and conservation. This implies the impact of this manuscript in those fields involved in the outcome of the project.

The search strings and methods employed by the authors are elaborated and justified in a clear manner, however, they did not mention if the deduplication was done on Rayyan or another online platform (Zotero). While the material, figures, and tables are presented in a satisfactory manner, I would suggest that Figure 2 be slightly enlarged to enhance visibility. The statistical analysis of the results is also explained thoroughly. In the result and discussion, the authors explained their reason and justified the exclusion of some articles that did not qualify for this review even after the first and second screening. They managed to summarise the main methods used in those articles and they provided statistical information on studies done in different continents and countries and over a century. The discussion and conclusion sections effectively support and corroborate the findings. The overall presentation of the article is commendable, with clear and accurate English language usage. The authors have avoided unnecessary wordiness, and extra tables and figures. The literature and references are appropriately used and cited within the text. As this is a systematic review that reports the work of others, there is no need for concern regarding animal care and ethical issues. Overall, this article contributes significantly to current research in the fields of behavior, ecology, and evolution due to its originality and the high quality of science it offers to scientists in these fields.

With the limited time that the authors had and the large number of papers they had to screen to prepare this manuscript, they managed to prepare a good-quality text. Considering that the presented manuscript

adheres to both the journal's guidelines and Biology Letters format and because of its potential impact on multiple fields of study it is recommended that this article be published in this journal.

6. Zuzanna Rauk

The present paper provides a systematic review of the research on hybridization events in wild canids and their impact on species fitness, dispersion, and genetic variation—factors crucial for the conservation of the species.

The influence of hybridization on species fitness, dispersion, and genetic variation is not described in detail (positive/negative, observed phenomena, and dependencies). However, as far as I understand, it was not the aim of the article to provide such detailed information. Nevertheless, the title of the paper suggests that it contains this detailed information; therefore, I suggest paraphrasing it.

The abstract presents the authors' aims, summarizes the most important results, and highlights the discovered knowledge gap.

In the introduction, the authors provide a background of the study, describe the phenomenon of interspecies hybridization, and highlight the need for a better understanding of this process in wild canids as a group with a particular vulnerability to hybridization due to the popularity of domestic dogs. The authors clearly state their aims while conducting this systematic review and formulate the research question on the presence of data about fitness, dispersion, and genetic variation in the research papers describing intraspecies hybridization.

The methodology is presented in detail, with a straightforward description of inclusion and exclusion criteria in the table. I would suggest presenting the search string in a copyable form to enable its use and testing instead of/in addition to showing it as a part of a figure. The string seems suitable for the review's aims, as it includes the names of species of interest and phenomena to be analyzed. The PRISMA diagram is clear and informative. The categories used for papers classification are named; however, the 'country' category requires clarification, as it is not clearly stated whether the particular country is a place of samples collection or the affiliation of the paper authors. The clarification occurs in the Figure 2A description, but I suggest clarifying it in the text (Line 75).

The results section is well organized. Specifying and describing the categories one by one makes the text easy to follow and provides the reader with a clear image of the obtained results. I appreciate the map of countries where hybridization was studied, as it presents a nice overview of the countries engaged in this type of research and supplies the interested researchers with ideas about potential international cooperation. Figure 2B, presenting the species whose hybridization was studied, is informative and indicates the pairs of the most frequently analyzed hybridizing species, thereby showing the current stage of knowledge. I would suggest magnifying the font in the plot, as the names of species are unreadable. Similarly for Figure 2C, with measured fitness parameters and recommendations provided. Figure 3 provides a nice summary of the article, as it shows

that the review objectives (analyzing the presence of measured fitness parameters and recommendations in papers about hybridization) were met.

Overall, the manuscript was easy to read, had a satisfactory level of English language, and fulfilled all the criteria required by the Biology Letters journal. The study may attract the attention of other researchers as it provides a valuable contribution to the field, highlighting current trends in intraspecies hybridization research and identifying a research gap regarding the impact of hybridization on canid reproduction and survivability.

Final version

The scientific evidence on hybridisation events in wild canids and its effect on species fitness, dispersion and genetic variation

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Summary

Interspecific hybridisation, i.e. interbreeding between distinct species, has become an increasingly important factor carrying major implications for population genetics, species ecology and conservation. This systematic review focuses on evaluating existing research on hybridisation in wild canids, particularly its impact on species fitness, genetic variation, and dispersion. By analysing 133 relevant publications from an initial pool of 3.466 records, we gained valuable information on the prevalence and direction of the consequences of hybridisation events studied by scientists. The studies used various methods to detect hybridisation and its effects, with an emphasis on genetic analysis (61% of studies) or its combination with morphology (23%). The majority of publications (89%, n=118) detected hybridisation between canid species. Researchers put an emphasis on measuring genetic variation (n=81) and animal dispersion (n=49), providing methodological (n=78) and conservation (n=75) recommendations. This review revealed a scientific gap in the assessment of recent hybridisation events from the perspective of canid reproduction and survivability, which are the key factors in the conservation of numerous species. In addition, despite the increasing number of human-wildlife conflicts, only 12 papers mentioned any societal recommendation regarding canid hybridisation.

Introduction

Interspecific hybridisation is a complex phenomenon and a subject of study in many areas of biology, such as evolutionary biology, population genetics, ecology, and conservation. It occurs when two distinct, but not entirely reproductively isolated species come into contact and interbreed (Schwenk, Brede & Streit, 2008). This may result in the creation of offspring that can also be partially or completely fertile and interbreed with one or both of its parent species. In such cases, through repeated backcrossing, introgression – or the flow of genes between different species – may occur (Aguillon *et al.*, 2022).

Hybridisation and introgression can have varying consequences for the hybridising populations (Adavoudi & Pilot, 2021). They may have negative impacts, such as the creation of infertile offspring and a consequent drastic reduction in fitness (Phadnis & Orr, 2009), introduction of harmful alleles through introgression (Rinker *et al.*, 2020), or a reduction in the genetic diversity of the population (Fraïsse *et al.*, 2014). On the other hand, introgression sometimes introduces favourable gene variants and can improve the fitness of the individuals in the population, in which case it is referred to as adaptive introgression (Hedrick, 2013).

Hybridisation is of particular interest in wild canids. Dogs (*Canis lupus familiaris*), one of the most common pets and an invasive species (Young *et al.*, 2011), share a common ancestry with the grey wolf (*C. lupus*), and can interbreed with many canid species, including wolves, coyotes (*C. latrans*), dingoes (*C. dingo*), and golden jackals (*C. aureus*), producing fertile offspring (Leonard *et al.*, 2014). This can lead to the introgression of undesirable traits, such as a lowered apprehension towards humans, which can increase human-wildlife conflict and threaten species conservation (Pilot *et al.*, 2021; Lescureux & Linnell, 2014).

Foxes, including the red fox (*Vulpes vulpes*) and the arctic fox (*V. lagopus*) are other species bred by humans. Both are known to escape from fur farms and hybridise with several wild fox species, potentially threatening their conservation (Noren *et al.*, 2005; Wierzbicki *et al.*, 2021). Furthermore, wild canids can interbreed with each other – for example, coyotes and wolves (Bohling & Waits, 2015), or red wolves (*C. rufus*) and grey wolves (Jenks & Wayne, 1992). Such hybridisation can have positive or negative impacts on genetic diversity, fitness, and conservation status of species. However, the effects of hybridisation on wild populations appear to be understudied and there is a need to identify the knowledge gap in this area.

In this review, we investigated the scope of research on wild canid hybridisation, whether with human-bred species or with each other, examining hybridisation events occurring since the year 1900. We aimed to create an overview of the field and examine whether studies assessing hybridisation in wild canids report its effects on fitness, dispersion, and genetic variation of the examined species. We also examined whether the studies provide recommendations on methodology of research or wildlife

conservation policy and societal action. This study can improve our understanding of the research gap on canid hybrids and provide the context for ecological, genetic, and conservation researchers.

Materials and Methods

The systematic review started with the formulation of a research question: “Do studies assess species fitness, dispersion and genetic variation when interspecific hybridisation occurs?”. To capture the comprehensive range of relevant studies, a search string was created, incorporating key terms and synonyms related to the topic of interest (Fig. 1). On 04.06.2024, titles and abstracts were collected from two databases, Web of Science and Scopus. Those abstracts were deduplicated using Zotero (www.zotero.org), then systematically screened using Rayyan software (Ouzzani *et al.*, 2016), with every abstract screened by at least two people. There was no limit on the time scope of publication and only articles fully written in English were considered. All the articles fulfilling the criteria outlined in Table 1 passed into full-text screening. Studies were reviewed to confirm their relevance and adherence to the research question and subsequent coding of these studies was conducted using Google Forms. We decided to only include papers that were readily available in the open access or by Jagiellonian University subscriptions.

Table 1. Criteria used to determine inclusion/exclusion of articles for the review.

INCLUSION CRITERIA	
Hybridisation study	The article must be a hybridisation study with the usage of any genetic, morphological, both genetic and morphological or other methods used to detect hybridisation events
Species	Any wild-living canid species as one of the parents of a specimen that is a suspect of hybridisation
Time of event	The study includes hybridisation or introgression event that occurred since the last century (1900)
Language	The study must be published in English
Publication	It must be a research article (may be a preprint) with analysis of data for a new study
EXCLUSION CRITERIA	
Irrelevant	Articles that do not study hybridisation, or the method used is not applicable for hybridisation detection (for e.g., only mtDNA genetic analysis)
Phylogenetic	Article studies species on time scale not including hybridisation events happened after year 1900
Species	Articles in which both parents come from non-wild (captive) population
Misclassified	Articles that studied species other than canid in terms of hybridisation or hybridisation is inter-, not intraspecific (for e.g., between different populations or subspecies)
Accessibility	The full text was unavailable and we could not check if the article met the criteria
Publication	Any review, conference paper, book chapter was excluded since they did not report primary data or the data may be duplicated

Upon inclusion, we extracted the following information: data on species studied (genus and species name); country where samples were collected; analytical method of assessing hybridisation (genetic, morphological, both genetic and morphological, and others); parameters accessed

(reproduction, survivability, genetic variation, dispersion); and if methodological, conservation or societal recommendations provided. This information is available as metadata attachment (SM1). The construction of graphs and visual representations of the data was performed using R environment, v 4.2.2 (R Core Team, 2023) and assorted packages (“network3D”, “webshot2”, “rworldmap”) to illustrate key findings and trends within the literature.

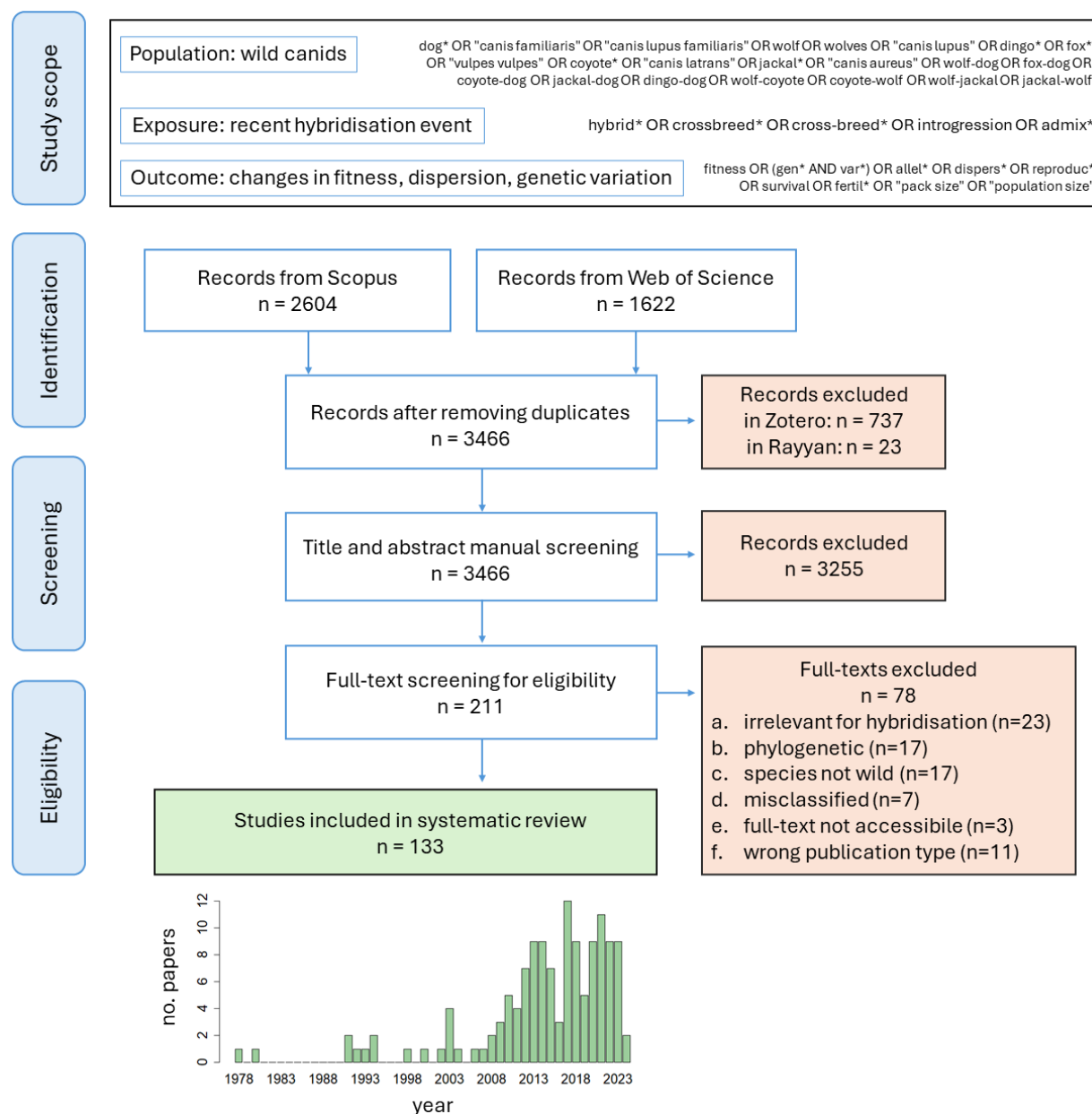


Fig. 1. PRISMA flow diagram (Page *et al*, 2021) showing the procedure of literature search and selection according to inclusion/exclusion criteria summarised in Table 1. The three main parts of the search string (relevant for Population, Exposure and Outcome) were connected with Boolean operator “AND”. The full search strings can be found in metadata of SM1.

Results and discussion

Our review revealed 133 articles, out of which in 89% of studies (n=118) hybridisation was detected. Out of 15 studies where hybridisation was not detected, 11 studies measured genetic variation and 8 studies provided methodological recommendations.

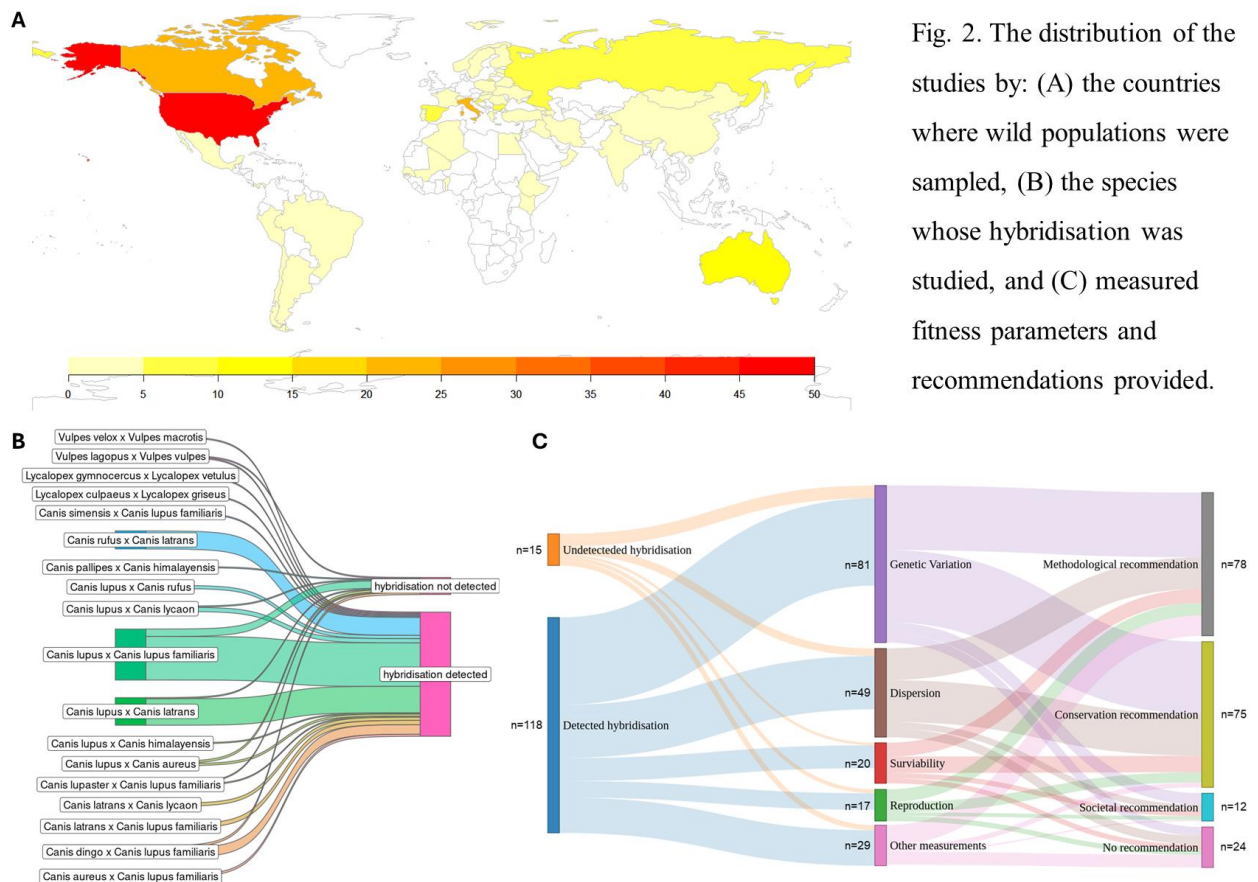
The main methods used in the studies were: genetic analysis (n=81, 61% of the studies), followed by both genetic and morphological analyses (n=31, 23%) and morphological (n=13, 10%). Other methods included: acoustic (n=1); semen analysis (n=1) and mathematical model (n=1); both genetic and radio-tracking (n=2); both genetic and mathematical model (n=2); genetic, morphological and histological (n=1). The predominance of genetic analysis as a method reflects the importance of molecular tools in identifying hybridisation events.

Research on hybridisation in wild canids is conducted globally, indicating widespread interest in this topic on every continent; with research conducted in study sites set in 53 different countries. The percentage breakdown shows the highest interest in hybridisation research in wild canids, namely wolves, coyotes and domestic dogs in North America, which accounts for 49.6% (n=66) of all the articles reviewed. Europe is the second most active region with 42.9 % of the research. Asia, South America, and Australia have smaller but still significant shares in the global study of hybridisation in wild canids (Fig. 2A). These intensive studies highlight the importance of hybridisation for conservation and population genetics. These studies frequently focus on genetic diversity and the ecological and evolutionary impacts of these interactions. While North American countries – USA (n=44) and Canada (n=22) – dominate in the number of papers, in Europe Italy is leading in the hybridisation research (n=21). This may be connected to strong wolf research by one of the pioneers of wolf conservation in Europe, Luigi Boitani and his team (for e.g., Boggiano *et al.*, 2013; Ciucci *et al.*, 2003). Other notable research states are Australia (n=10), Croatia (n=8), Russia (n=8), Spain (n=7), Bulgaria (n=7) (Fig. 2A). The detection of hybridisation in many countries representing all continents suggests that hybridisation is a worldwide phenomenon with potential implications for genetic diversity and species conservation.

Out of 13 Canidae genera, only the following three were represented in hybridisation studies: wolves *Canis* (n=128); followed by *Vulpes* genus (n=3). The domestic dog appeared as a source of introgression in almost half of the studies (n=67). Moreover, *C. lupus* x *C. l. familiaris* were the focus in 48 studies, while 10 other Canidae genera were not detected within our research scope.

This review revealed the problem of distinct species recognition on the span of continuing evolution. As an example, a *C. lycaon* species used to be a subspecies *C. lupus lycaon* (Sears *et al.*, 2003), but has since then been included into the “*Canis* hybrid swarm” in North America (Rutledge *et al.*, 2010). In addition, there was a recent distinction of two more separate species: *C. pallipes* (formerly *C. lupus*

pallipes) and *C. himalayensis* (formerly *C. lupus chanco*) (Srinivas & Jhala, 2023). Accordingly, we tried to unify how we address the species in Fig. 2B.



Out of all studies, 61% (n=81) measured genetic variation in context of hybridisation in wild populations. The next by popularity was dispersion, measured in 49 studies (37%), followed by survivability and reproduction parameters accessed in 20 and 17 studies respectively. Nowadays, genetic variation is one of the main outcomes of hybridisation events that is being studied, whereas there is a knowledge gap in dispersion, survivability and reproduction studies. The relatively high number of studies focusing on dispersion indicates a growing interest in understanding how hybrid individuals spread and integrate into wild populations (Fig. 2C).

Majority of studies were methodological- and/or conservation-oriented, with 59% (n=78) and 56% (n=75) of studies providing methodological and conservation recommendations respectively. Only 12 studies provided societal recommendations in the context of hybridisation.

The findings from this review underline the complexity and prevalence of hybridisation events among wild canid populations. Our study suggests that hybridisation is a worldwide phenomenon with major implications for species fitness, genetic variation, and distribution. Most studies have focused on genetic analysis, highlighting the importance of molecular tools for understanding hybridisation. Although many studies have provided valuable methodological and conservation recommendations, major gaps remain

in assessing the impacts of recent hybridisation events on reproduction and viability, key factors for effective species conservation. Closing these gaps and incorporating societal recommendations into future studies will be critical for mitigating human-wildlife conflict and protecting wild canid populations.

Author contributions

All authors contributed to data identification, screening of abstracts and full-texts and preparation of this paper. J.P. and M.C. organised the work division, E.R. and R.M. organised data processing and compilation, R.M. and J.P. provided statistics visualisation, M.C., P.D., E.R. and M.S. worked the most on the text.

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Topic 2 - The effects of maternal exposure to anaesthesia during pregnancy on the offspring neurodevelopment – a systematic review of studies in small mammal models

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**The effects of maternal exposure to anesthesia during pregnancy on the offspring
neurodevelopment – a systematic review of studies
in small mammal models**

**Martyna Gorkowska, Szymon Kantor, Martyna Marzec,
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Summary

The prevalence of neurodevelopmental disorders (NDDs) such as autism spectrum disorder (ASD) and attention-deficit hyperactivity disorder (ADHD) among children under 18 has risen significantly in recent decades, varying globally between 4.70% and 88.50%, influenced by diagnostic practices and socioeconomic factors. This increase underscores the need for deeper insights into NDDs etiology and the development of preventive strategies. Emerging research highlights prenatal exposure to maternal stress, immune activation, undernutrition, drugs, and anaesthesia as potential risk factors for abnormal neurodevelopment. Anesthetic exposure is particularly critical as non-obstetric surgery is performed on 0.5%-2% of pregnant women. The fetal nervous system, highly sensitive throughout gestation, undergoes crucial developmental processes such as neural tube formation, neuronal migration, synaptogenesis, and gliogenesis. While prior reviews have examined molecular, cellular, and cognitive impacts of fetal anaesthesia exposure, a comprehensive synthesis of these findings and their link to neurodevelopmental outcomes is needed. This systematic review aims to consolidate current knowledge on the association between maternal anaesthesia and offspring cognitive and behavioral abnormalities, alongside macroscopic structural nervous system changes. The findings serve as a critical resource for medical professionals managing pregnant patients and researchers using animal models, guiding them in mitigating anaesthesia-related risks to ensure both clinical and experimental integrity.

1. Introduction:

According to the recently published systematic review, the prevalence of neurodevelopmental disorders (NDDs) such as autism spectrum disorder (ASD) and attention-deficit hyperactivity disorder (ADHD) among children below the age of 18 has increased in recent decades. It fluctuates globally between 4.70% and 88.50%, depending on the country and applied estimation procedures (Francés et al., 2022). This upward tendency results from more effective diagnostic strategies and an intensified activity of the countries in the diagnosis and training of professionals, but on the other hand, it indicates a need for a better understanding of NDDs' etiology and developing preventive strategies. Recently, studies describing the impact of prenatal exposure to maternal stress, immune activation, undernutrition, drugs, or anaesthesia as risk factors for abnormal nervous system development have attracted attention. Among these factors, the anesthetics are of particular note, as their use in pregnant women is sometimes

inevitable due to emergency situations. Approximately 0.5% - 2% of all pregnant women undergo nonobstetric surgery during their pregnancy (Perna, Loughan, Le, & Hertza, 2015). The developing nervous system is sensitive to external factors from the beginning of gestation, as neural tube formation takes place in the first weeks, followed by neuronal migration, synaptogenesis, and gliogenesis in the 2nd and 3rd trimester (Doi, Usui, & Shimada, 2022). To date, several reviews describing the molecular and cellular changes or cognitive abnormalities after fetal anaesthesia exposure have been published (Perna, Loughan, Le, & Hertza, 2015; Li, Jiang, & Zhao, 2021; Olutoye, Style, & Menchaca, 2021). However, there is a need to systematize the current knowledge on the relationship between maternal anaesthesia and cognitive and behavioral abnormalities in the offspring, constituting the potential components of neurodevelopmental disorders. Moreover, the current state of research on macroscopic structural aberrations of the nervous system after prenatal anaesthesia should be reviewed. The present review may provide a sort of a guide for medical professionals (e.g., doctors, obstetricians, and anesthesiologists, etc.) who work with pregnant women or those planning to become pregnant. It can help them consider the potential risks for embryos associated with using general anaesthesia during pregnancy when medical interventions (planned or emergency) are required. Furthermore, it may be helpful for professional breeders and scientists (e.g., physiologists, neuroscientists, toxicologists, etc.) conducting research on animal models, as it may guide them in adjusting the use of general anaesthesia, especially during emergency surgeries, to prevent any adverse effects on the offspring, thereby ensuring the accuracy and reliability of their experimental results and avoiding potential biases in their research.

2. Methods:

2.1 Literature Search

We searched for relevant peer reviewed papers in two main databases (Scopus and Web of Science) without restrictions on the year of publication. Initial search terms using anesthesia, pregnancy and neurodevelopment were used. Based on our search string, a total of 5244 papers were found with 2526 from Web of Science and 2718 from Scopus. The details regarding applied search strings are provided in Table 1.

Table 1. The search strings used in Scopus and Web of Science search for the articles.

SCOPUS search string	
Search field	Keywords
TITLE-ABS-KEY	(rodent* OR rat OR rats OR rattus OR mice OR mouse OR mus OR hamster* OR mesocricetus OR vole* OR clethrionomys OR myodes OR rabbit* OR oryctolagus OR "guinea pig*" OR cavia OR ferret* OR mustela)

	AND
	(maternal* OR mother* OR prenatal* OR pregn* OR "in uter*")
	AND
	(surger* OR anaesthe* OR anesthe* OR isoflurane OR sevoflurane OR desflurane OR enflurane OR halothane OR propofol OR thiopental OR benzodiazepine* OR barbiturate* OR etomidate OR methohexital OR methohexitone OR "chloral hydrate" OR "nitrous oxide" OR ketamine)
	AND
	(fetus* OR feta* OR embryo* OR pup* OR offspring* OR litter*)
	AND
	(patholog* OR brain* OR develop* OR behavio* OR neuro* OR cogni*)
	Web of Science search string
Search field	Keywords
All Fields	(rodent* OR rat OR rats OR rattus OR mice OR mouse OR mus OR hamster* OR mesocricetus OR vole* OR clethrionomys OR myodes OR rabbit* OR oryctolagus OR "guinea pig*" OR cavia OR ferret* OR mustela)
	AND
	(maternal* OR mother* OR prenatal* OR pregn* OR "in uter*")
	AND
	(surger* OR anaesthe* OR anesthe* OR isoflurane OR sevoflurane OR desflurane OR enflurane OR halothane OR propofol OR thiopental OR benzodiazepine* OR barbiturate* OR etomidate OR methohexital OR methohexitone OR "chloral hydrate" OR "nitrous oxide" OR ketamine)
	AND
	(fetus* OR feta* OR embryo* OR pup* OR offspring* OR litter*)
	AND
	(patholog* OR brain* OR develop* OR behavio* OR neuro* OR cogni*)

All articles were imported into the Zotero reference manager (<https://www.zotero.org/>) and went through a deduplication process, with the use of Zotero Duplicates Merger v1.1.5 plugin, which resulted in 3969 unique records being extracted for further processing. Articles were uploaded to Rayyan (<https://www.rayyan.ai/>) and screening based on titles, abstracts and the selected keywords was carried out by three pairs of scientists in our group with each independently scanning selected abstracts. Conflicts in results were discussed and resolved between each pair. In total, 123 articles were included for full text screening. The articles that did not have the PECO outlines were excluded. The considerable number of exclusions was a result of strict exclusion criteria, as presented in Table 2.

Table 2. An overview of the PECO statement applied in the review.

PECO	EVIDENCE	TERMS
POPULATION	Offspring of small mammal mothers exposed to general anesthesia during pregnancy	(rodent* OR rat OR rats OR rattus OR mice OR mouse OR mus OR hamster* OR mesocricetus OR vole* OR clethrionomys OR myodes OR rabbit* OR oryctolagus OR “guinea pig*” OR cavia OR ferret* OR mustela)
EXPOSURE	Most commonly used general anesthetics, administered to the mother during the pregnancy	(maternal* OR mother* OR prenatal* OR pregn* OR “in uter*”) AND (surger* OR anaesthe* OR anesthe* OR isoflurane OR sevoflurane OR desflurane OR enflurane OR halothane OR propofol OR thiopental OR benzodiazepine* OR barbiturate* OR etomidate OR methohexital OR methohexitone OR “chloral hydrate” OR “nitrous oxide” OR ketamine)
COMPARATOR	<i>Not applicable</i>	<i>Not applicable</i>
OUTCOMES	Disturbances in neurodevelopment (macroscopic structural changes in nervous tissue and behavioral/cognitive impairments)	(fetus* OR feta* OR embryo* OR pup* OR offspring* OR litter*) AND (patholog* OR brain* OR develop* OR behavio* OR neuro* OR cogni*)

Only texts written in English and studies conducted on small mammals which reported neurodevelopmental, cognitive, and behavioral changes caused by prenatal exposure to anaesthesia were included. Reviews, book chapters and conference abstracts, studies on in vitro models and humans were rejected. Papers were also excluded if they reported on the administration of anesthesia during labor or a caesarean section, rather than during pregnancy itself. Full text screening of 123 articles was done by 6 researchers - approximately 20 papers per person and additionally 20% of texts were double screened. Articles were sorted based on (1) species (2) types of anesthesia (3) types of dosing (4) time of exposure during pregnancy (5) sex and sample size of offsprings (6) sample size of mothers (7) route of administration (8) age of the offspring when the changes were studied and (9) whether changes studied were behavioral, cognitive, macroscopic or both. The literature search and selection procedures are summarized in PRISMA diagram (Fig 1.).

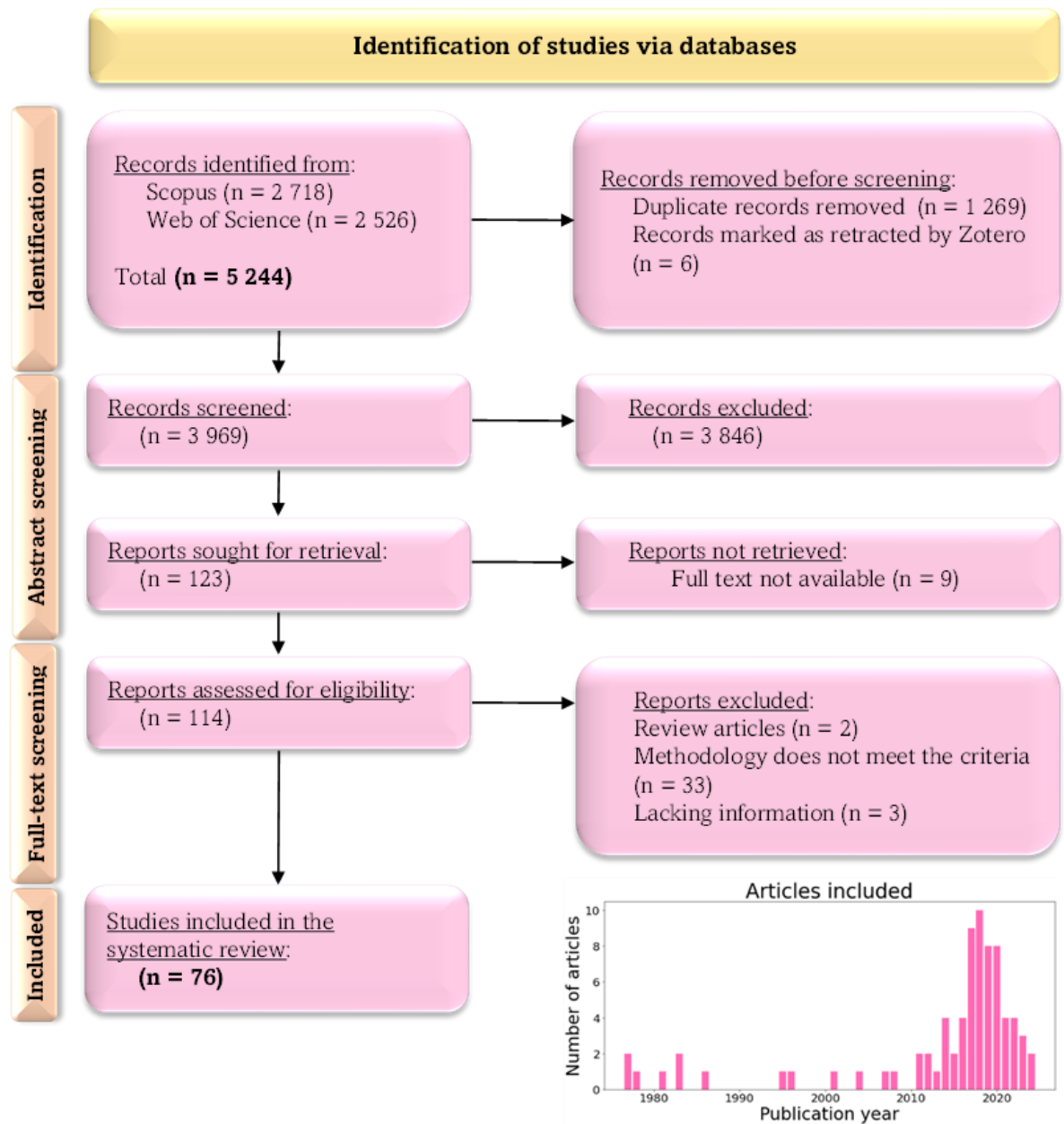


Figure 1. The PRISMA diagram illustrates the selection process of studies for this systematic review with an insert bar plot (bottom right corner) showing the annual publication frequency. (From: Page et al., 2020)

2.2 Data visualization

The data extracted from the selected articles were analyzed using Microsoft Excel for initial sorting and categorization. Custom-made Python scripts and Flourish Studio platform (<https://flourish.studio>) were employed for further analysis and data visualisation. Finally, CorelDRAW software was used for the assembly and refinement of the figures.

3. Results and discussion:

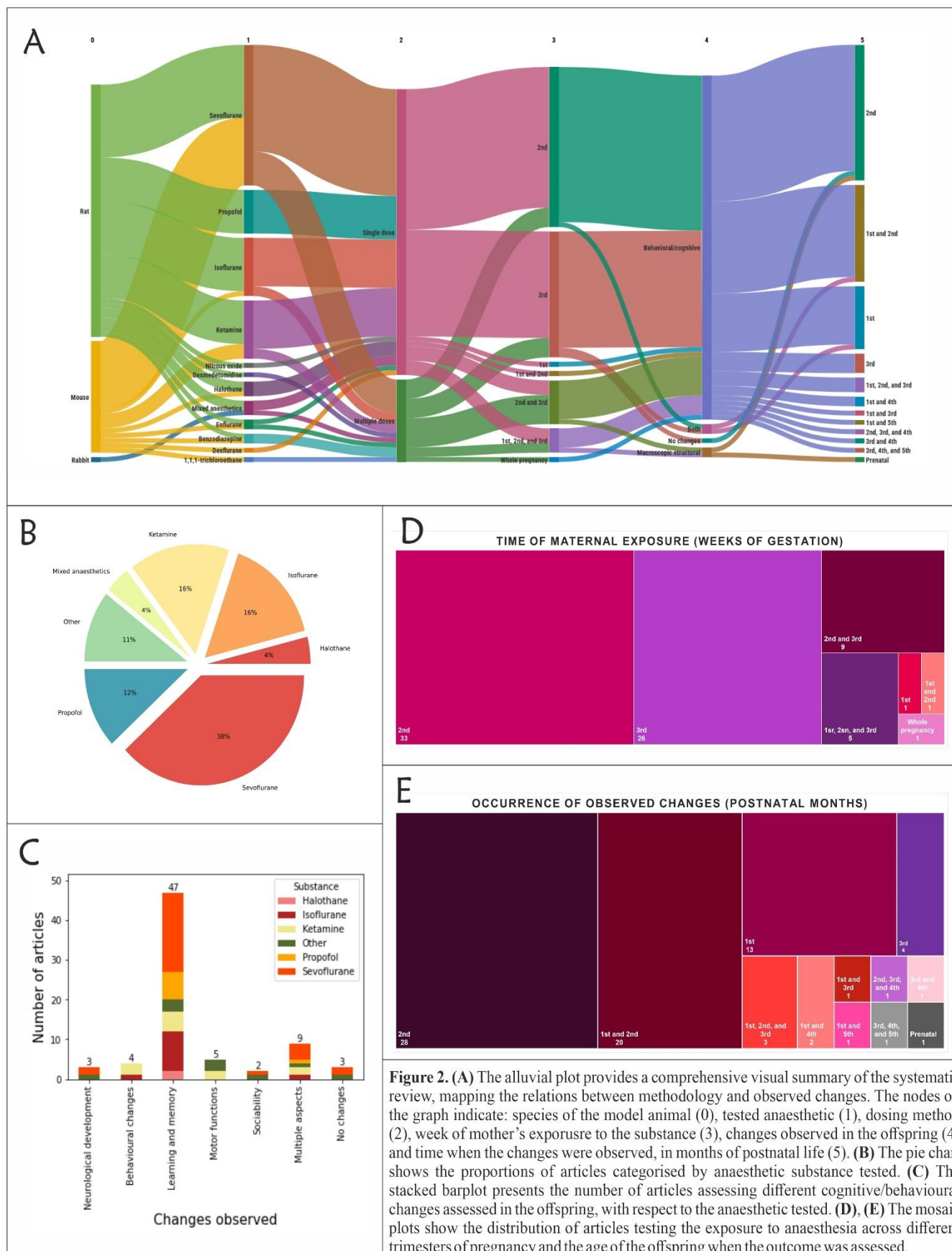
A total of 76 studies were included in this systematic review, spanning publication years from 1977 to 2024. These studies primarily focused on the effects of various frequently used anesthetics (such as sevoflurane, isoflurane, ketamine, and propofol, etc.) administered during pregnancy on the neurodevelopment and cognitive and behavioral functions of offspring in animal models. In a comprehensive analysis of 76 research papers, the inclusion of sex information among offspring subjects was found to be lacking in a substantial 56.6% (n=43) of studies. Only 21.1% (n=16) of papers included both male and female subjects, while 22.4% (n=17) focused exclusively on male subjects. Moving to species analysis, rats were the most frequently utilized, featuring prominently in 68.4% (n=52) of the papers, followed by mice at 30.3% (n=23). In contrast, rabbits were notably underrepresented, appearing in a mere 1.3% (n=1) of the studies. Anaesthesia practices varied widely, with sevoflurane emerging as the predominant choice in 38.2% (n=29) of papers. Other commonly employed anesthetics included ketamine (17.1%; n=13), isoflurane (15.9%; n=12), and propofol (14.5%, n=11). Less frequently used agents comprised halothane (3.9%; n=3), enflurane (2.6%; n=2), and benzodiazepine (2.6%; n=2). Nitrous oxide, desflurane, dexmedetomidine, and 1,1,1-trichloroethane were the least utilized anesthetics, each featuring in 1.3% (n=1) of the reviewed papers (Fig. 2B). In terms of timepoints of anaesthesia, exposure day E14 (embryonal day 14) was the most frequently reported (Fig. 2D), which corresponds to mid-gestation in humans, when most non-obstetric surgeries with the usage of anaesthesia are performed. Additionally, this period is characterized by high sensitivity and vulnerability of the nervous system to external factors. The E14 is crucial for neural tube critical stages of formation and differentiation, neurogenesis and synaptogenesis-processes that can be influenced and interrupted by anaesthesia administration (Tau & Peterson, 2010). Such disruptions in neurodevelopmental mechanisms may result in behavioral and cognitive changes observed in adulthood, as well as major structural changes in the brain. Only 4 out of 76 analysed papers mentioned macroscopic structural changes indicating small brain weight, worse brain-to-body weight ratio, and enlarged size of ventricles after exposure to anaesthesia (enflurane, combination of propofol and sevoflurane, and halothane, respectively). The majority of publications described behavioral and cognitive implications (96%; n=73) of prenatal anaesthesia exposure in juvenile and adult rodents.

Among all analysed papers, the following categories of behavioral and cognitive changes were distinguished: learning and memory deficits (including spatial learning), behavioral impairments (anxiety-, ADHD- and depressive-like behaviors), sociability, motor functions, neurological development (Fig. 2C). Behavioral tests such as open field activity test (locomotor activity and anxiety-like behavior), forced swimming test (depressive behavior), and Morris water maze (spatial learning and memory) were used to assess cognitive and behavioral changes in animals. The effects

were assessed through behavioral tests at various postnatal timepoints, with postnatal day 28 (P28) being the most commonly studied (30%, n=23), followed by postnatal day 30 (P30) (20%, n=15; Fig. 2E). The majority of the papers described learning and memory deficits (62%, n=47), which indicates this is the main cognitive domain affected by prenatal anaesthesia exposure. Five of the papers mentioned the retardation in neurological development, including delayed occurrence of reflexes, delayed eye opening, and hearing impairment. Locomotor deficiencies, such as impaired motor coordination, alternations in the spontaneous motor activities and delayed motor development were reported in 5 issues. Only 4 of the screened papers reproduced human clinical circumstances and tested the exposure to anaesthesia combined with surgery, reporting no effect of anesthetic without surgery on the offspring performance (n=2) or exacerbation of anaesthesia induced learning impairment when combined with surgery (n=1). The visual summary of the abovementioned results is provided in Figure 2A.

Based on our data, learning and memory tests are among the most commonly used protocols in rodent behavioral studies, which makes the results subjective and limits the possibility of detecting other behavioral abnormalities. On the other hand, sevoflurane is indeed one of the most commonly used anesthetics, also in pregnant women and its huge prevalence over other anesthetics is predictable. However, this points out a possible knowledge gap and indicates that presumably more studies over influence of different anesthetics on offsprings' neurodevelopment should be conducted. One of the limitations of these studies was that researchers commonly and intentionally focus on a single sex, as the methodological complexities associated with the neuroprotective effects of female hormones may unnecessarily complicate the experimental design, at least at the initial stage of basic research. This reveals the concerning fact about methodological sections of the included papers. The subjects of studies should always be reliably described to enable the study replication. Nonetheless, due to possible sex differences in vulnerability to medications and behavioral changes caused by various factors, studies in both sexes would be of a high value. The other limitation of rodent studies is that they do not reproduce all the aspects of human use of anesthesia in pregnancy, as in the majority of studies anesthesia itself is tested in animals, without accompanying surgery. This finding indicates the need for carefulness while planning the rodent model experiments and interpreting results of such research.

In conclusion, this review underscores the critical need for more comprehensive studies on the effects of prenatal anaesthesia exposure, particularly concerning the importance of including both sexes in future research to address potential differences in response to anaesthesia, as well as the necessity for more realistic rodent models to better mimic human clinical scenarios.



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Author contributions:

M.M. conceived the original idea for the research topic.

Conceptualisation: M.G., S.K., M.M., Z.R., J.R. and A.S.; deduplication of the articles: Z.R., A.S., J.R.; abstract and full-text screening: M.G., S.K., M.M., Z.R., J.R. and A.S.; MS Forms survey design, collection and exporting the data: Z.R.; data analysis and visualisation: S.K., M.M., J.R.; preparation of article draft: M.G., M.M., Z.R., A.S.; Each author contributed to the critical revision of the manuscript and gave their approval for the final version.

Conflicts of interest:

The authors declare no conflicts.

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<https://doi.org/10.1038/NPP.2009.115>

Reviews

1. Dr Magdalena Zaniewska

The systematic review by *Gorkowska et al.* summarizes the influence of maternal anesthesia on various aspects in pups, including behavioral functions and neurodevelopment. This review clearly indicates the article screening and exclusion criteria. A major strength of this study is the wide variety of animal species and factors considered in the analysis. However, before the review can be considered for publication, some issues need to be addressed:

1. Instead of the phrase “neuronal migration”, it would be more appropriate to mention “neurogenesis”, the process that occurs during fetal brain development and consists of various stages, including proliferation, neuronal migration, differentiation, and maturation.
2. It would be essential to provide examples of the types of surgeries performed on pregnant women.
3. "medical professionals (e.g., doctors, obstetricians, and anesthesiologists)." – Authors should clarify this statement, as anesthesiologists are types of doctors as well, so the differentiation might be unnecessary and could lead to confusion.
4. The last sentence in the **Introduction** needs clarification. It is not clear which emergency surgeries could be conducted by scientists on pregnant animals. I agree that not only professional breeders but also veterinary doctors could benefit from this review. However, I would be very careful when mentioning scientists, as it is unlikely that scientists would opt for emergency surgery on a pregnant animal. Neuroscientists who work with animal models are aware that any disruption during pregnancy may increase the risk of psychiatric disorders in offspring, so they try to avoid such situations. The review, of course, may serve neuroscientists by summarizing research findings on the effect of anesthesia on neurodevelopment.
5. The Table 1 caption should provide a comprehensive description of its content. It should mention what was searched in the Scopus and Web of Science databases.
6. Fig. 2 caption: Instead of “dosing method”, I would call it “dosing regimen”.
7. I would not refer to the analyzed animals as “models”. An example of an animal model could be a model of depression, in which animals are exposed to e.g., chronic unpredictable stress, or a model of addiction, in which animals are exposed to addictive substances.

8. Results and discussion:

- The sample size of offspring and mothers, as well as the route of administration, have not been discussed.

- The analysis should indicate which month of 2024 was the last month included in the analysis.
- There are missing references that need to be cited: “In terms of timepoints of anaesthesia, exposure day E14 (embryonal day 14) was the most frequently reported (Fig. 2D), which corresponds to mid-gestation in humans, when most non-obstetric surgeries with the usage of anesthesia are performed.” and “Such disruptions in neurodevelopmental mechanisms may result in behavioral and cognitive changes observed in adulthood, as well as major structural changes in the brain.”
- Instead of the term “implications”, I suggest that the Authors use the term “effects”.
- “Behavioral and cognitive changes” (lines 132, 136, 137, 142) - Cognitive effects are also behavioral effects; therefore, it would be more appropriate if the Authors differentiate these effects as e.g., cognitive (learning and memory), affective (depression and anxiety), and motor (locomotor activity) effects.

Minor issues:

- Authors should provide their affiliations, indicate the corresponding author, and provide contact details.
- The list of keywords should be provided.
- Please use the term “nonobstetric” or “non-obstetric consistently.
- Please change “should be reviewed” to “need to be examined”.
- Please change “The present review may provide a sort of a guide...” to “The present review may serve as a guide...”.
- There is inconsistency between Fig. 1 and the Methods section regarding the number (114 vs. 123) of articles considered in the full-text screening stage - this should be clarified.
- Please insert a period when citing the Fig. 1 in the text.
- Fig. 1 caption is missing a short title before describing what it contains.
- Please change “species of the model animal to “animal species”.
- Fig. 2 caption: please correct spelling: “mother’s exporusre”.
- Instead of the name “open field activity test” for the behavioral test, please use “open field test”.
- In lines 140 to 141, to clarify the specific effects each behavioral test is designed to evaluate, it would be essential to add in the parenthesis “useful for studying...”
- Please remove the unnecessary “The m” before the sentence: “The majority of the papers described learning and memory deficits (62%, n=47), which indicates this is the...”.
- Use British or American spelling consistently; e.g., British “categorised”, “anaesthesia”, “analysed” vs. American “behavioral”, “anesthesia”.

2. Mateusz Chechetkin

Overall, this systematic review is of high quality and the remarks I have are relatively minor. The review adheres to the standards of biology letters, including appropriate formatting and word limit. The title and abstract accurately describe the contents of the article. Based on the summary and introduction, the review is of high interest to the field of neurobiology. The purpose and the need of conducting the systematic review is well-argued with several arguments provided. The layout is clear, though readability of the article is slightly reduced because of large fragments of text that are presented with no paragraph breaks. The list of citations is very short, with only one article cited in the results and discussion section. The text has a typo on line 144 with no other typos that I can see.

Introduction very briefly overviews the topic and effectively argues for why there is a need for systematic reviews in the field. However, almost half of the introduction is justification of the need to conduct this particular systematic review and description of who it is written for and who can use the results. In my opinion, it would be beneficial to include more information on e.g. how anesthesia can influence the developing brains of the embryos, what anesthetics are commonly used and why, as well as mention what limitations animal studies have compared to studies on humans. In general, the introduction is well-written and has good flow of points, but I would prefer it to go more into detail on specific points I outlined.

The methods section is thorough and describes every step of the process that would be required to replicate the findings of the study. Methodology itself is sound and executed correctly to achieve the goals stated in the introduction. This part of the review also includes some information that is more well-suited to the results section, such as the number of articles that were identified by the search string and the numbers of articles that passed into full text screening and into further analysis. Methodology clearly outlines inclusion and exclusion criteria and justifies low number of records that passed from abstract screening to full text screening. There is a table included showing the full search string for both Web of Science and Scopus which, in my opinion, could be moved to supplementary materials.

The results comprehensibly describe the outcome of the analysis, pointing when needed to relevant graphs. This section includes important and interesting information, such as the observation that less than 50% of the articles examined included data on the sex of the offspring and information on what tests were used to assess the possible impairments in the offspring. For me, what is lacking is the discussion part of the results and discussion – the whole section includes only one citation and there is not a lot of additional information as to e.g. why it is important to include both sexes of offspring in research or how the use of only male animals can bias or skew the results. The graphs effectively represent the results and are accurately described. The conclusion follows from the text and concisely summarizes the presented research.

Overall, the information is effectively presented without unnecessary or superfluous fragments. The quality of the written English is high and only requires very minor revisions. The study is very well made, though some information might be re-arranged and in my opinion it would benefit from a more extensive introduction and discussion with more research cited.

3. Michał Strączyński

The paper “The effects of maternal exposure to anaesthesia during pregnancy on the offspring neurodevelopment – a systematic review of studies in small mammal models” discusses the use of anaesthesia in mothers and is aimed at medical professionals looking for information and data on its influence on the health of the offspring. The title and abstract of the paper both accurately describe the contents of the article. The abstract provides a good and to-the-point summary of the background, methodology used and the results of the study as well as the conclusions and its relevance to the field of neurobiology.

The introduction outlines the existing knowledge surrounding the development of neurological functions of animals and the influence of the use of anaesthesia on pregnant mothers on post-natal fitness of the offspring. Additionally, the authors highlight the existing knowledge gap formed by the unconnected, dispersed nature of various studies measuring behavioural abnormalities and research measuring macroscopic structural nervous system changes. The introduction also shows the potential, broader consequences of this knowledge gap, for example in human pregnancy care and safety. The research question outlined in the paper appears novel and important in the context of the presented knowledge gaps.

The methods used for data collection are proper for the aims of the study and in accordance with the PECO rules adapted by the authors. The authors describe the steps taken to search for articles in two separate databases: Scopus and Web of Science, using specialised search strings for each. The search strings as well as the abstract and full-text screening criteria were clearly communicated using tables. The data was analysed well and in a detailed way, with several categories used to compartmentalise the information about the contents of the papers selected for analysis. In general, the methodology was communicated clearly, and it is possible to repeat these steps and the study for cross-referencing purposes.

The description of the results and conclusions in the discussion flows clearly from the methodology, thoroughly outlining the aftermath of each step. The presentation of these results is notably clear and concise, allowing for easy comprehension. The article effectively utilizes generated graphics, frequently referring to them and the authors ensured that the statistics presented in tables and charts are easily understandable at a glance. The results are discussed in satisfactory detail, particularly regarding the representation of categories adopted during the data analysis phase. The discussion describes numerous aspects of the papers chosen, providing an understanding of the findings. One point of criticism is that while the language used in this part is mostly clear and easily understandable, there are a few places in which substituting some words would make the text clearer. For instance, in line 133 it is not exactly clear what is meant by the term “worse”. It can be intuited that the exposure causes

the ratio in question to be lower, however as somebody not knowledgeable on the subject I cannot be sure, so a more specific word or phrase could be used here. Later in the paragraph, it could be described what the authors mean by “worse”. In line 134, I believe it would be useful to specify that the subject being exposed to anaesthesia is the mother as this sentence might read as the offspring being subjected which adds unnecessary confusion. The passage from 135 to 139 could use some frequencies (n=) assigned to the categories mentioned to make this information more available, even if they are also separately shown on a graph, even if the graph itself is being referenced here. The literature used in this study was cited correctly and is sufficient to support the points made in the introduction. However, the discussion/conclusion part of the paper should have just a few more citations, to better prove the interpretation of the results and give more credence to the future research recommendations. Maybe the authors should try to look for research into similar methodology in humans and draw parallels, while further emphasising the need to use small mammal/rodent models in new ways. Apart from the already mentioned small readability issues, there are some spelling errors, such as in line 144.

I believe that-to the best of my knowledge and understanding of the field- this paper is of good methodological quality and has the potential to be a valuable addition to the existing body of knowledge available to neuroscientists looking for information on maternal exposure to anaesthesia and presents interesting research questions and gaps to be solved in the future. I recommend that it should be accepted for publication after language corrections.

4. Ekaterina Rostovskaya

This is the systematic review on the state of art in understanding of the association between maternal anaesthesia and offspring cognitive and behavioural abnormalities. With a 200-word summary and 1879 words of the main body of the manuscript, two tables and two graphs, it fits into formal requirements provided by the journal. Among required sections, this manuscript is missing the addresses and affiliation of the authors. In addition to this, the section of references does not contain the links mentioned in the section of materials and methods (for e.g., to Zotero and Rayyan). No metadata with publications used for this review paper is provided.

This is the work of general interest to neurobiologists, providing an overview towards the studies of foetal neurodevelopment in the context of anaesthetic exposure in small mammal models. The title accurately describes the content of this manuscript and is sufficient in understanding the scope of the research. Nevertheless, the summary does not seem fully sufficient, since it does not contain any information on results accessed by the authors.

The authors inform about three existing reviews on the effect of prenatal exposure to anaesthesia on molecular, cellular and neurological factors, although underline that these reviews did not fully cover the microscopic structural aberrations occurring in the nervous system. There are no hypotheses stated, nevertheless, there are provided possible implications of this review, such as guidance on the anaesthesia risks for medical professionals, as well as guidance for professional breeders and scientists on how to adjust the use of general anaesthesia to prevent any postponed effects on foetus development. It is not clear if the adjustment will involve the use of any other type of anaesthetic or will include any other possible solutions.

The authors used search strings and considered only peer-reviewed papers from Scopus and Web of Science databased. They clearly explained which application tools (Zotero, Rayyan) were used and provided a clear protocol of the work. Nevertheless, I have a comment regarding the content of the tables that seems interchangeable. In Table 1, two identical search strings are provided, with the exception of search fields that differed between the databases. In Table 2, the information on the terms used for the PECO statement are redundant, as they include the parts of the search string from the Table 1. My recommendation would be to reload the superfluous Fig. 2 into one table and one graph.

Out of 3969 articles, 123 studies were included for full-text screening (3.1% success rate), where 20% of manuscripts were double-screened. The authors collected information on the studied species, type and dosage of anaesthesia, time of intervention, sample sizes, offspring sex and age when studied, which changes were studied and which administration route was involved. The total of 76 articles got included in the systematic review.

The Fig. 1 in the section of materials and methods is the PRISMA diagram illustrating the process of studies selection. It is a good method for presentation of the methodology of systematic review work, although I would recommend citing the methodology source “(From: Page et al., 2020)” in the main text, otherwise the reader might conclude that the figure got directly taken from the before-mentioned publication. Further data processing and visualisation are missing the information on full-text screening (from the acknowledgement part I presume that authors used the Google Forms).

In the results and discussion section, the authors revealed a substantial information gap: offspring sex was recorded only in 43.4% of studies, a half of which studied male offspring and a half included both female and male pups. This scientific review provides the list of the most frequently studied species (rats and mice) and anaesthetics used (sevoflurane, ketamine, isoflurane and propofol), as well as time of anaesthesia and the consequences analysed. The maximal number of 38.2% studies tested learning and memory changes, and the authors conclude that it makes the result of such behavioural change subjective.

The Fig. 2 consists of five parts (A-E) and is quite complicated. An alternative can be placement of the figures 2D and 2E into a table, that will allow to give more explanation on the most frequent time when maternal exposure occurred and when the changes were detected, especially in case if there have been any periods not studied at all.

To conclude, this manuscript requires only a few language corrections before being published. For example, authors should keep with one spelling of “anesthesia” (American English) vs “anaesthesia” (British English). This article does not require an additional statistical review, since the scientific review paper do not provide statistics. After application of the provided comments, I give a positive review to this manuscript.

5. Patrycja Dziurawicz

The review focuses on the crucial issue of how anesthesia exposure during pregnancy affects neurodevelopment, with far-reaching implications for understanding behavior and cognitive functions in mammals. The review draws on various studies to identify gaps in current knowledge and offers a synthesis that advances understanding in this vital area. The title and abstract explicitly mention maternal anesthesia exposure and its influence on the neurodevelopment of offspring.

The review is very thorough and well done. The authors searched thoroughly through two major databases, Scopus and Web of Science two major databases, without any time constraints. They used specific keywords to guarantee complete coverage of relevant studies. The selection process was rigorous, based on PECO guidelines, which align with best practices for systematic review. However, the review should give a more detailed account of the inclusion and exclusion criteria to help the selection process. Prisma diagram is a good one but it could be expanded on to give more detail on how articles should be arranged.

One suggestion for improving the clarity of the review. Figure 2 should be in the body of the text, right after the description. This would help readers understand the text and the figure simultaneously, enhancing overall comprehension.

Another area for improvement is the consistency in language use. The review inconsistently uses British and American English. For example, it alternates between "anaesthesia" (British) and "anesthesia"(American), and "behavioural" (British) and "behavioral" (American). Also, they used American forms such as "labor" and "pediatric". The inconsistencies serve as evidence that the review should incorporate consistent language. Only one version of English would make the review more consistent and clearer.

Despite these minor issues, the review is a valuable resource that significantly contributes to understanding the effects of anesthesia on neurodevelopment in offspring. Its strengths include a detailed methodology, a thorough selection process, and a thorough analysis of results. To further enhance the review's clarity and coherence, it would be beneficial to provide a more detailed PRISMA diagram and unify the language terminology used.

This review provides a comprehensive analysis of how maternal anesthesia exposure affects the neurodevelopmental status of small mammal models. It is a significant advancement in the field, aiding in identifying gaps in existing knowledge and providing a comprehensive overview of current research. I think it can be made more effective and accessible to readers by addressing the areas for improvement above, like describing the prisma diagram, and ensuring language is consistent.

6. Rebeca Miszczak

The scientific review entitled “The effects of maternal exposure to anesthesia during pregnancy on the offspring neurodevelopment – a systematic review of studies in small mammal models” focuses on a very interesting and important topic. As the authors stated, approximately 0.5%-2% of pregnant women undergo non-obstetric surgery requiring anesthesia, which may impact the developing fetal nervous system. Prenatal exposure to anesthesia during surgery can affect fetal neurodevelopment. Previous research focused on molecular and cellular changes, while this review centers on cognitive and behavioral outcomes. The authors focused on research involving small mammal models.

The methods of this systematic review are well designed and adhere to current standards for conducting scientific reviews. The authors conducted their research in two databases: Scopus and Web of Science, yielding 5244 articles. Deduplication and screening of abstracts based on clearly stated strict PECO criteria (Population, Exposure, Comparator, Outcomes) resulted in 123 articles for full-text screening. For the final review, 76 articles were included. The final exclusion of articles was mostly based on the methodology of the article not meeting the criteria. It would be beneficial to state in more detail why these articles were excluded to avoid missing crucial information due to this part of the methodology.

Finally, 76 papers were sorted based on (1) species used, (2) types of anesthesia administered, (3) types of dosing regimens, (4) timing of exposure during pregnancy, (5) sex and sample size of offspring, (6) sample size of mothers, (7) route of administration, (8) age of the offspring when changes were studied, and (9) whether the changes studied were behavioral, cognitive, macroscopic, or both. The complexity of this sorting is a strong point of this scientific review. Learning and memory deficits were the most reported outcomes (62% of studies). Impairments in motor functions, delays in neurological development, and behavioral impairments (such as anxiety-like behaviors) were also observed. Most studies (56.6%) did not specify the sex of the offspring, with male individuals being more commonly studied. Most studies reported anesthesia exposure around the 14th day of embryonic development, which corresponds to mid-gestation in humans, a critical period for neurodevelopment. The authors identified many limitations of such studies. Many did not replicate the full clinical scenario of human anesthesia use, often excluding the accompanying surgery. The lack of sex-specific analysis limits the generalizability of findings. Behavioral tests predominantly focused on learning and memory, potentially overlooking other relevant behavioral abnormalities.

The manuscript follows the structure of Biology Letters, with a limit of 2500 words (excluding the text on the title page and references). The manuscript includes 2 tables and 2 figures, which is the allowed maximum of displays (4). Most of the displays are presented clearly. Figure 5 has two parts that could be improved. Figure 5a represents an alluvial plot – some nodes are coded as 1st, 2nd, 1st AND 2nd, etc. The authors should code data so that there are only separate parts, with nodes connected to them

(there should not be parts of the node such as 1st AND 2nd). This would result in fewer factors in nodes, making the Sankey diagram much clearer. In Figure 5c, the y-axis should be broken to show results with lesser counts in a more detailed way. The conclusions are adequately supported by the data shown. The paper appropriately cites published work. The quality of written English is suitable for publication, although the authors should choose between British or American English for consistency.

7. Jakub Plachta

Dear Authors,

I find this review well thought out. The text itself reads well, it is put together in an orderly and logical manner. It is immediately obvious that the research team put time and effort into formulating the research problem. The question of the effects of anaesthesia and its effects on postnatal offspring is an interesting topic. General anaesthesia is sometimes unavoidable, especially in cases not even involving the foetus. That is of relevance to both medical practitioners as well as any researcher using these procedures in their animal studies. The authors also mentioned breeders, which is not an immediately obvious choice, but accurate nonetheless. An obvious choice, however, would have been to mention veterinarians. Medical professionals (line 57) refers to those “who work with pregnant women or those planning to become pregnant”. Veterinarians probably got lost somewhere between medical practitioners and breeders, since anaesthesiologists work with both humans and non-human animals.

While the title mentions that this review concerns “small mammal models”, I think it would be helpful to include a quick mention of that in Summary and Introduction, the latter in particular which heavily emphasising human applications.

The methodology is well-described, detailed and fully reproducible. The query looks extensive, with multiple synonyms considered and returns a healthy amount of papers. However, an alternative spelling of fetus (foetus) was omitted. While not being particularly influential in terms of the number of results (an increase of around a dozen papers in both Scopus and WoS), it is important to include both British and American spellings of the search terms. In Table 2, I think that the first part of Outcomes (fetus etc.) should be moved to Population, since that part is more suitable and even mentions the “offspring”. Lastly here, my most pressing questions concern the date range, stated as “without restrictions” (line 73/74) and resulting in a reported timespan of 47 years (1977-2024). I will get into details in the following section.

The authors clearly and accurately point out the gaps and areas for improvement in the contemporary literature. There is, however, a possible confounding factor stemming from the afore-mentioned date unrestricted date range. Anaesthetics, as any part of science and medicine in particular, is in a constant process of change and development. This results in new knowledge on the chemicals used, sometimes resulting in their fading out of use or outright bans. This is the case for three of the substances: halothane, enflurane and 1,1,1-trichloroethane (TCE). Halothane as an anaesthetic has lost popularity in the 90’s and is no longer available in the U.S. and rarely used in Europe. As a result, the production of this agent has been discontinued. This is due to its hepatotoxicity. Enflurane is not commonly used, either, and also no longer available on the U.S. market. The reason is more connected to the scope of this review. Enflurane has been shown to cause seizures in patients. The last one, TCE, has been banned by the FDC and the European Union as an inhaled anaesthetic. This was due to a link to Parkinson’s disease.

The FDC can happen, coincidentally, in 1977. While these substances are still in use in some developing countries, their neurotoxicity is well-understood. In my opinion this should be mentioned in the article. If more time was available, a map of the usage of anaesthetics mentioned in this review would've been useful to understand if the inclusion of those toxic substances had an impact on the results. I would also group these three substances and analyse them separately as to not bias the neurodegenerative effects shown in Figure 2. Having said that, I understand the time and graph space limitations of the review, so a paragraph in the Results and Conclusions section would suffice. Also, to link these results to human applications, I think more in-text literature support is needed here. I would also like to see a bit more citations in this section. As for the graphs, they are ordered and described well, however the amount of graphs squeezed due to journal limitations hurts readability. A larger font size on the graphs would definitely help.

A couple language/formatting issues:

Please add affiliations to your names.

Line 144 – “The mThe” Please correct

Line 151 – add hyphen to “anaesthesia induced”

Also, please remove double spaces.

Overall, I am pleased with both the text and graphical representation of the results. I think these findings are important for anaesthesia usage and widely applicable. The authors have treated this important issue with due diligence. The problem, knowledge gap and usefulness were presented clearly; the methodology was extensively described; analysis was performed correctly; and the conclusions followed the results. With all that considered, I would suggest the Editor to accept this review with minor corrections.

With regards,

Jakub Płachta

**The effects of maternal exposure to anaesthesia during pregnancy on the offspring
neurodevelopment – a systematic review of studies
in small mammal models**

**Martyna Gorkowska, Szymon Kantor, Martyna Marzec,
Zuzanna Rauk, Joanna Roszkowska, Anbarieh Saadat**

Affiliation: Institute of Zoology and Biomedical Research, Jagiellonian University

Summary

The prevalence of neurodevelopmental disorders (NDDs) has risen significantly in recent decades, which underscores the need for deeper insights into NDDs aetiology and the development of preventive strategies. Emerging research highlights prenatal exposure to maternal stress, immune activation, undernutrition, drugs, and anaesthesia as potential risk factors for abnormal neurodevelopment. Anaesthetic exposure is particularly critical as non-obstetric surgery is performed on 0.5%-2% of pregnant women. This systematic review aims to provide a comprehensive synthesis of the current studies on the association between maternal anaesthesia and offspring cognitive and behavioural abnormalities, alongside macroscopic structural nervous system changes. Scopus and Web of Science were searched from inception until 4th of June 2024. Papers describing the studies on small mammal models were chosen during abstract screening, and 76 studies undergone full text screening. In the selected papers, sevoflurane was the most frequently used anaesthetic, followed by ketamine, isoflurane and propofol. Rats were the most frequently used model and embryonal day 14 the most frequently chosen timepoint of anaesthesia exposure. Only 4 out of 76 analysed papers mentioned macroscopic structural changes, whereas the majority of publications described behavioural and cognitive implications (n=73) of prenatal anaesthesia exposure in juvenile and adult rodents, including learning and memory deficits, behavioural impairments (anxiety-, ADHD- and depressive-like behaviours), sociability, motor functions and neurological development. Learning and memory deficits were described by the majority of papers (n=47). Lack of information about the offspring sex, or focusing on the only one sex, and not combining the anaesthesia with surgery were the main limitations of analysed studies. In conclusion, the review presents a significant impact of prenatal anaesthesia exposure on nervous system, and underscores the critical need for more comprehensive studies in this field, including experiments on both sexes and developing more realistic rodent models.

1. Introduction:

Recently, studies describing the impact of prenatal exposure to maternal stress, immune activation, undernutrition or drugs as risk factors for abnormal nervous system development have attracted

attention. The prevalence of neurodevelopmental disorders (NDDs), such as autism spectrum disorder (ASD) and attention-deficit hyperactivity disorder (ADHD) among children below the age of 18 has increased in recent decades, which indicates a need for a better understanding of NDDs' aetiology and developing preventive strategies. Among the risk factors, the anaesthetics are of particular note, as their use in pregnant women is sometimes inevitable due to emergency situations. Approximately 0.5% - 2% of all pregnant women undergo non-obstetric surgery during their pregnancy (Perna, Loughan, Le, & Hertza, 2015).

The developing nervous system is sensitive to external factors from the beginning of gestation, as neural tube formation takes place in the first weeks, followed by neuronal migration, synaptogenesis, and gliogenesis in the 2nd and 3rd trimester (Doi, Usui, & Shimada, 2022). To date, several reviews describing the molecular and cellular changes or cognitive abnormalities after foetal anaesthesia exposure have been published (Perna, Loughan, Le, & Hertza, 2015; Li, Jiang, & Zhao, 2021; Olutoye, Style, & Menchaca, 2021). However, there is a need to systematize the current knowledge on the relationship between maternal anaesthesia and cognitive and behavioural abnormalities in the offspring, constituting the potential components of neurodevelopmental disorders. Moreover, the current state of research on macroscopic structural aberrations of the nervous system after prenatal anaesthesia needs to be examined. The present systematic review will provide a robust and unbiased view of this research field.

The paper may serve as a guide for medical professionals (e.g., doctors, obstetricians, and anaesthesiologists, etc.) who work with pregnant women or those planning to become pregnant. It can help them consider the potential risks for embryos associated with using general anaesthesia during pregnancy when medical interventions (planned or emergency) are required. Furthermore, it may be helpful for professional breeders and scientists (e.g., physiologists, neuroscientists, toxicologists, etc.) conducting research on animal models, as it may guide them in adjusting the use of general anaesthesia, especially during emergency surgeries, to prevent any adverse effects on the offspring, thereby ensuring the accuracy and reliability of their experimental results and avoiding potential biases in their research.

2. Methods:

2.1 Literature Search

We searched for relevant peer reviewed papers in two main databases (Scopus and Web of Science) on 4 June 2024 without restrictions on the year of publication. The details regarding applied search strings are provided in Table 1. Based on our search string, a total of 5244 papers were found with 2526 from Web of Science and 2718 from Scopus.

All articles were imported into the Zotero reference manager (<https://www.zotero.org/>) and went through a deduplication process, with the use of Zotero Duplicates Merger v1.1.5 plugin, which resulted in 3969 unique records being extracted for further processing. Articles were uploaded to Rayyan (<https://www.rayyan.ai/>) and screening based on titles, abstracts and keywords was carried out by three pairs of scientists with each person in pair independently scanning selected abstracts. Conflicts in results were discussed and resolved within each pair. The PECO statement applied for abstract screening is presented in Table 1. The articles that did not meet the criteria outlined in PECO were excluded. Only texts written in English and studies conducted on small mammals which reported neurodevelopmental, cognitive, and behavioural changes caused by prenatal exposure to anaesthesia were included. Reviews, book chapters and conference abstracts, studies on *in vitro* models and humans were rejected. Papers were also excluded if they reported on the administration of anaesthesia during labour or a caesarean section, rather than during pregnancy itself. In total, 123 articles were included for full text screening. Full text screening of those articles was done by 6 researchers - approximately 20 papers per person and additionally 20% of texts were double screened.

Table 1. An overview of the PECO statement and search string applied in the review. The three main parts of the search string (relevant for Population Exposure and Outcome) were connected with Boolean operator “AND”.

PECO	EVIDENCE	SEARCH STRING
POPULATION	Offspring of small mammal mothers exposed to general anaesthesia during pregnancy	(rodent* OR rat OR rats OR rattus OR mice OR mouse OR mus OR hamster* OR mesocricetus OR vole* OR clethrionomys OR myodes OR rabbit* OR oryctolagus OR “guinea pig*” OR cavia OR ferret* OR mustela)
EXPOSURE	Most commonly used general anaesthetics, administered to the mother during the pregnancy	(maternal* OR mother* OR prenatal* OR pregn* OR “in uter*”) AND (surger* OR anaesthe* OR anaesthe* OR isoflurane OR sevoflurane OR desflurane OR enflurane OR halothane OR propofol OR thiopental OR benzodiazepine* OR barbiturate* OR etomidate OR methohexital OR methohexitone OR “chloral hydrate” OR “nitrous oxide” OR ketamine)
COMPARATOR	<i>Not applicable</i>	<i>Not applicable</i>
OUTCOMES	Disturbances in neurodevelopment	(fetus* OR feta* OR embryo* OR pup* OR offspring* OR litter*) AND (patholog* OR

(macroscopic structural changes in nervous tissue and behavioural/cognitive impairments)	brain* OR develop* OR behavio* OR neuro* OR cogni*)
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Articles were sorted based on (1) species (2) types of anaesthesia (3) types of dosing (4) time of exposure during pregnancy (5) sex and sample size of offsprings (6) sample size of mothers (7) route of administration (8) age of the offspring when the changes were studied and (9) whether changes studied were behavioural, cognitive, macroscopic or both. The literature search and selection procedures are summarised in PRISMA (Page et al., 2020) diagram (Fig. 1).

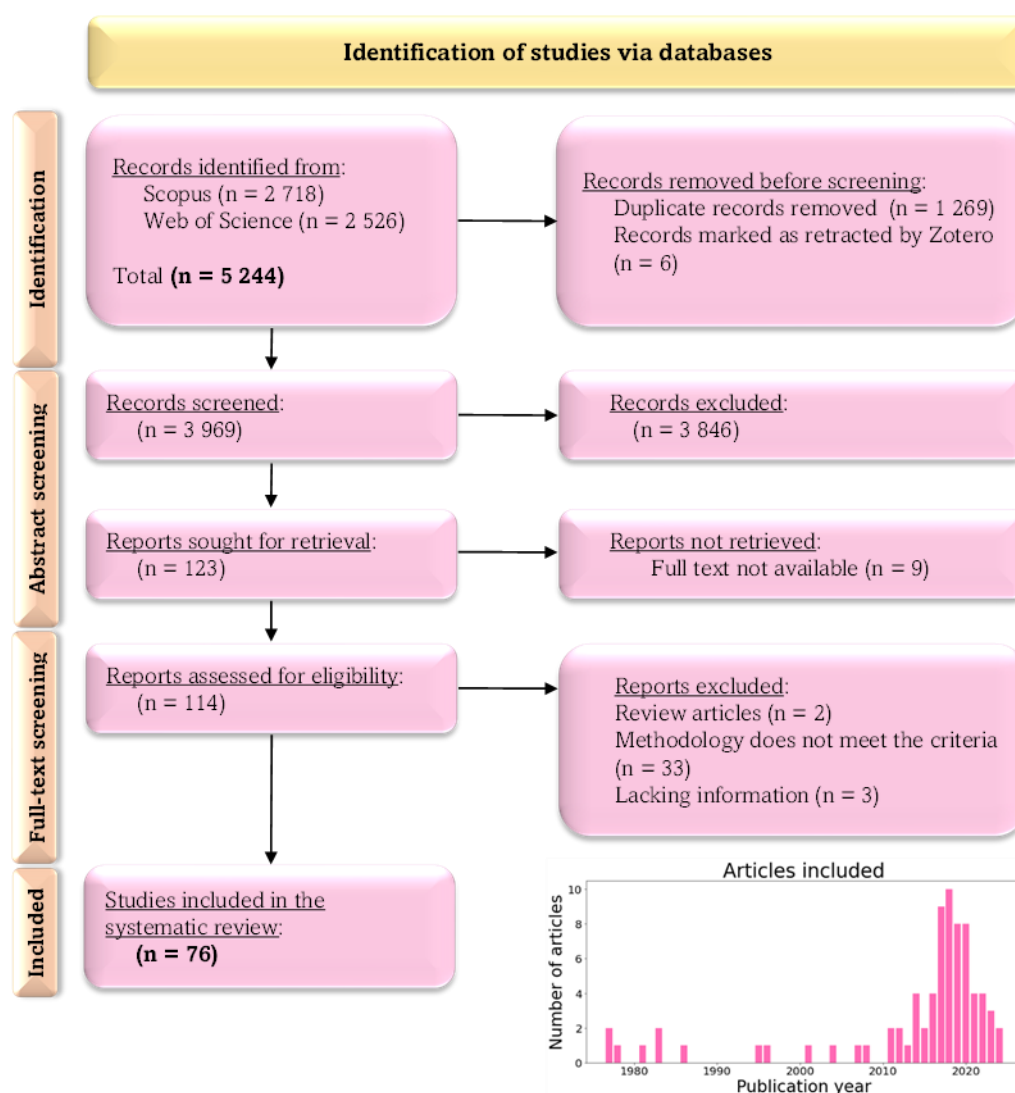


Figure 1. The PRISMA diagram illustrates the selection process of studies for this systematic review with an insert bar plot (bottom right corner) showing the annual publication frequency. *2.2 Data visualisation*

The data extracted from the selected articles were analysed using Microsoft Excel for initial sorting and categorisation. Custom-made Python scripts and Flourish Studio platform (<https://flourish.studio>) were employed for further analysis and data visualisation. Finally, CorelDRAW software was used for the assembly and refinement of the figures.

3. Results and discussion:

A total of 76 studies were included in this systematic review, spanning publication years from 1977 to 2024 (Fig. 1). These studies primarily focused on the effects of various frequently used anaesthetics (such as sevoflurane, isoflurane, ketamine, and propofol, etc.) administered during pregnancy on the neurodevelopment, cognitive, and behavioural functions of offspring in animal models.

In a comprehensive analysis of 76 research papers, a substantial 56.6% (n=43) of studies failed to include information on the sex offspring subjects. Only 21.1% (n=16) of papers included both male and female subjects, while 22.4% (n=17) focused exclusively on male subjects. This reveals the concerning fact about methodological sections of the included papers. The subjects of studies should always be reliably described to enable the study replication. One of the limitations of these studies was that researchers commonly and intentionally focus on a single sex, as the methodological complexities associated with the neuroprotective effects of female hormones may unnecessarily complicate the experimental design, at least at the initial stage of basic research. Nonetheless, due to possible sex differences in vulnerability to medications and behavioural changes caused by various factors, studies in both sexes would be of a high value.

In terms of species, rats were the most frequently used, featuring prominently in 68.4% (n=52) of the papers, followed by mice with 30.3% (n=23). Anaesthesia practices varied widely, with sevoflurane emerging as the predominant choice in 38.2% (n=29) of papers. Other commonly employed anaesthetics included ketamine (17.1%; n=13), isoflurane (15.9%; n=12), and propofol (14.5%, n=11). Less frequently used agents comprised halothane (3.9%; n=3), enflurane (2.6%; n=2), and benzodiazepine (2.6%; n=2). Nitrous oxide, desflurane, dexmedetomidine, and 1,1,1-trichloroethane were the least utilised anaesthetics, each featuring in one of the reviewed papers (Fig. 2B). As sevoflurane is indeed one of the most commonly used anaesthetics, also in pregnant women and its huge prevalence over other anaesthetics is predictable. However, this points out a possible knowledge gap and indicates that presumably more studies over influence of different anaesthetics on offsprings' neurodevelopment should be conducted.

With regard to sample sizes, the number of mothers included in the studies was rarely reported, while the sample size of the offspring varied for different tests mentioned in the texts. Usually, 10-20 animals per group were used in each behavioural test, while many studies did not specify the exact number or only reported the total sample size.

In terms of timepoints of anaesthesia, exposure day E14 (embryonal day 14) was the most frequently reported (Fig. 2D), which corresponds to mid-gestation in humans, when most non-obstetric surgeries with the usage of anaesthesia are performed. Additionally, this period is characterised by high sensitivity and vulnerability of the nervous system to external factors. E14 is crucial for neural tube critical stages of formation and differentiation, neurogenesis and synaptogenesis-processes that can be influenced and interrupted by anaesthesia administration (Tau & Peterson, 2010). Such disruptions in neurodevelopmental mechanisms may result in behavioural and cognitive changes observed in adulthood, as well as major structural changes in the brain.

Only 4 out of 76 analysed papers mentioned macroscopic structural changes indicating small brain weight, worse brain-to-body weight ratio, and enlarged size of ventricles after exposure to anaesthesia (enflurane, combination of propofol and sevoflurane, and halothane, respectively). The majority of publications described behavioural and cognitive implications (96%; n=73) of prenatal anaesthesia exposure in juvenile and adult rodents. Among all analysed papers, the following categories of behavioural and cognitive changes were distinguished: learning and memory deficits (including spatial learning), behavioural impairments (anxiety-, ADHD- and depressive-like behaviours), sociability, motor functions, neurological development (Fig. 2C). Behavioural tests such as open field test (locomotor activity and anxiety-like behaviour), forced swimming test (depressive behaviour), and Morris water maze (spatial learning and memory) were used to assess cognitive and behavioural changes in animals. The effects were assessed through behavioural tests at various postnatal timepoints, with postnatal day 28 (P28) being the most commonly studied (30%, n=23), followed by postnatal day 30 (P30) (20%, n=15; Fig. 2E).

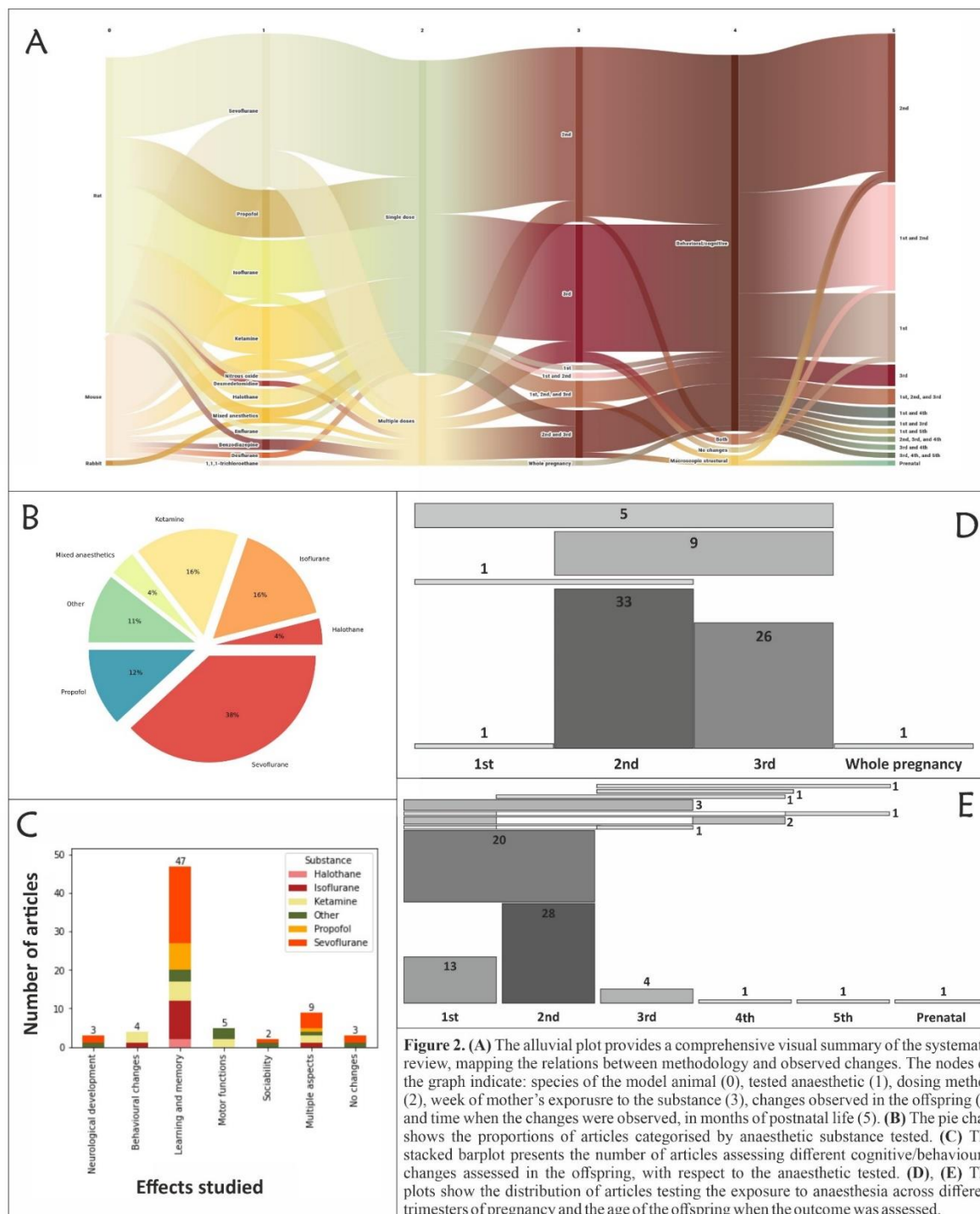
Five of the papers mentioned the retardation in neurological development, including delayed occurrence of reflexes, delayed eye opening, and hearing impairment. Locomotor deficiencies, such as impaired motor coordination, alternations in the spontaneous motor activities and delayed motor development were reported in 5 articles.

The majority of the papers described learning and memory deficits (62%, n=47), which indicates that cognition is the most researched and studied domain for the prenatal anaesthesia exposure topic. Learning and memory tests are among the most commonly used protocols in rodent behavioural studies following use of anaesthetics, which makes the results subjective and limits the possibility of detecting other behavioural abnormalities. It should also be kept in mind that the current review revolves around neurodevelopmental effects only, while the effects of prenatal anaesthesia exposure are much broader

when it comes to foetal development. Lastly, the research articles that tested the substances at subanaesthetic doses, e.g. as painkillers, were excluded, but the effects assessed in these protocols could also be relevant.

One crucial limitation of rodent studies is that they do not reproduce all the aspects of human use of anaesthesia in pregnancy, as in the majority of studies anaesthesia itself is tested in animals, without accompanying surgery. Only 4 of the screened papers reproduced human clinical circumstances and tested the exposure to anaesthesia combined with surgery, reporting no effect of anaesthetic without surgery on the offspring performance ($n=2$) or exacerbation of anaesthesia induced learning impairment when combined with surgery ($n=1$). This finding indicates the need for carefulness while planning the rodent model experiments and interpreting results of such research. The visual summary of the abovementioned results is provided in Figure 2A.

In conclusion, this review underscores the critical need for more comprehensive studies on the effects of prenatal anaesthesia exposure, particularly concerning the importance of including both sexes in future research to address potential differences in response to anaesthesia, as well as the necessity for more realistic rodent models to better mimic human clinical scenarios. It also provides a list of studies where cognition and behavioural changes were assessed – a possible basis for future meta-analyses.



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Author contributions:

M.M. conceived the original idea for the research topic.

Conceptualisation: M.G., S.K., M.M., Z.R., J.R. and A.S.; deduplication of the articles: Z.R., A.S., J.R.; abstract and full-text screening: M.G., S.K., M.M., Z.R., J.R. and A.S.; MS Forms survey design, collection and exporting the data: Z.R.; data analysis and visualisation: S.K., M.M., J.R.; preparation of article draft: M.G., M.M., Z.R., A.S.; Each author contributed to the critical revision of the manuscript and gave their approval for the final version.

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