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Research article

Mapping knowledge gaps about Assisted Reproductive Techniques related to EAZA Ex situ Programme managed populations

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Abstract
Many species face extinction due to anthropological impacts, leaving ex situ populations as valuable resources for conservation. Assisted reproductive techniques (ART) can broadly be defined as utilising oocytes and spermatozoa to generate offspring. ART holds potential as a conservation tool to support management of threatened populations. So far, what is known about ART use in species managed under the European Association of Zoos and Aquaria (EAZA) is unmapped. If ART is to be used as a conservation tool to improve population management, it is important to collate available knowledge and assess potential gaps to fill before the tool can be applied. Focusing on 13 selected ART topics, three scientific journal databases were searched for peer-reviewed scientific papers relevant to EAZA Ex situ Programmes (EEPs) through a systematic literature review. A total of 348 papers were found to address one or more of the 13 selected ART topics in an EEP taxon (family, genus, species or subspecies). The majority of papers focus on mammals (88%) followed by fish and birds (7% and 4% respectively). For these animal classes, the ART topics most frequently addressed are semen collection, evaluation and preservation. Only a single paper was found on amphibians, three on reptiles and none were found regarding invertebrates. With the information presented in this review, EEPs with little or no ART-related, peer-reviewed scientific knowledge and which may benefit from increased ART-related research can be identified thus helping to focus ART research and resources and help EEPs meet their conservation goals.

Introduction

Species face extinction worldwide due to anthropogenic impacts, with the current loss of species estimated to be between 1,000 and 10,000-fold higher than the natural extinction rate (Turvey and Crees 2019). Globally, population sizes of monitored mammals, fish, birds, reptiles and amphibians have declined by an average of 68% between 1979 and 2019 (Petersen and Almond 2020). Given the threats to species globally, there is a dire need for effective conservation actions. Ex situ species conservation management programmes such as those of regional zoo associations can improve conservation efforts and help counteract extinction for some species. Increasingly and in line with the One Plan Approach, conservation professionals are working to protect species in situ as well as ex situ (Byers et al. 2013). The goal of the One Plan framework is to foster interactive cooperation between ex situ and in situ efforts in order to identify strategies or actions necessary for in situ species conservation. Included in this work toward more comprehensive species conservation planning is the consideration of if and how ex situ species management may align with goals identified as important for species survival. Both in situ and ex situ populations can be small and at risk of losing genetic diversity, so it is crucial to maintain healthy and genetically diverse populations through conservation management programmes where possible.

Members of the European Association of Zoos and Aquaria (EAZA) cooperate on 449 management programmes (EAZA 2022) referred to as EAZA Ex situ Programmes (EEPs). The EEPs include vertebrate (mammals, birds, reptiles, amphibians, fish) and invertebrate species grouped under 39 EAZA Taxon Advisory Groups (TAGs; Supplementary Information). Each TAG oversees the taxa within their purview and advises on the need for intensive management (under an EEP), defining conservation role(s) and more. The taxa under a TAG are not necessarily all managed as EEPs, but for those which are, the breeding programme can target the subspecies (e.g. western lowland gorilla *Gorilla gorilla gorilla*), species (e.g. dama gazelle *Gazella dama*), genus (e.g. mouse lemurs *Microcebus* spp.) or family (e.g. mudminnows Umbridae) level (Supplementary Information).

The EEPs focus on minimising inbreeding and maximising genetic diversity through incorporating pedigree information into breeding decisions. For some EEPs, the low number of reproductively active animals contributing to the gene pool impacts the ability to achieve genetically healthy, self-sustaining populations and decreases their viability as insurance populations for their wild counterparts (EAZA 2019). While natural mating in these managed breeding programmes is preferred, reproductive challenges including mate incompatibility, lack of reproductive capacity in some individuals and loss of genetic diversity from underrepresented individuals may require intervention. These interventions often come with high costs and challenging logistics, such as those required for transporting animals large distances to form recommended breeding pairs. For some EEPs, assisted reproductive techniques (ART) are therefore recognised as an additional population management tool to reach the genetic aims of the programme. Unfortunately, the preservation of gametes and the use of ART in certain non-domestic species remains challenging, sometimes with little or no practical information available on how to implement these techniques (Campos et al. 2019; Presicce 2020; Wildt et al. 2010).

The term ART encompasses a wide array of different techniques, all with the general purpose of using gametes collected to create offspring with varying degrees of invasiveness and effort. ART may include both the collection and storage of spermatozoa and oocytes, and the subsequent application either in vivo (e.g. intrauterine insemination IUI), or in vitro (e.g. in vitro fertilisation IVF, or intracytoplasmic sperm injection ICSI). Despite ART having been utilised widely in domestic animals and acknowledged as a potential tool for wildlife conservation since the 1970s (Benirschke 1984; Ombelet and van Robays 2015), the reproductive physiology of only approximately 250 wildlife species has been studied so far (Bolton et al. 2022). Furthermore, only a few of these wildlife species have garnered enough attention or have been deemed in critical need to enable the significant collaborative efforts and investment required for the resource-intensive research needed to develop even preliminary species-specific ART protocols. The rapidly declining numbers of the northern white rhinoceros *Ceratotherium simum cottoni* generated increased interest in and extensive application of ART to combat this decline and save the subspecies. Similarly, ART has been used extensively for many years in the breeding programme for the giant panda *Ailuropoda melanoleuca.*

The use of ART is often hampered by a general lack of knowledge of basic reproductive or physiological knowledge (e.g. normal oestrus cycles or semen quality) required to develop species-specific reproductive techniques. The majority of ART protocols currently employed are based on those developed for a domestic counterpart or humans without factoring in the potential variation in anatomy and physiology; great differences may exist even between taxonomically close species (Pelican et al. 2006). The creation of species-specific protocols for each relevant species with practical guidance on how to optimally collect, store and use gametes would open up the possibility of using ART as a conservation tool for wildlife in both in situ and ex situ populations with a greater probability of success. With the EEPs, EAZA focuses population management efforts on 449 taxa. However, knowledge of ART in these taxa and whether it can be applied as a conservation tool is poorly described in published literature. This review focuses on 13 ART topics grouped into three main categories: semen, oocyte/embryo and in vitro/in vivo. The aim of this review was to systematically investigate what is currently known about use of ART for the different taxa managed as EEPs, and thereby identify the knowledge gaps that exist and what is needed to close them.

Materials and methods

To standardise the literature search, guidelines for Preferred Reporting for Systematic Reviews and Meta-Analyses (PRISMA) were followed to search for papers with relevant data (Figure 1). Searches were performed in three databases, selected on the basis of their comprehensiveness regarding wildlife peer-reviewed research and therefore being most likely to capture the relevant literature: PubMed (https://pubmed.ncbi.nlm.nih.gov/), BioOne (https://bioone.org/) and JZAR (https://www.jzar.org/jzar/search). For each of the databases, a search string was created (Supplementary Information) with the goal of identifying as many relevant papers as possible on the 13 defined ART topics (Figure 2) with a focus on EAZA EEP taxa and species in the same genus as an EAZA EEP taxon. Humans and domestic species were excluded from the search as these were outside the scope of this review. In PubMed, this was done using the filter 'Other animals'. The literature search in PubMed was performed from 1–15 September 2022, the search in BioOne 1–22 September 2022 and the search in JZAR 13–15 September 2022.

To exhaustively uncover any relevant papers, an individual search was conducted for each of the 449 EEP taxa (family, genus, species or subspecies) using the same parameters as the broad, ART-related search. For BioOne, the individual search was done on the specific EEP taxa (i.e. subspecies, species, genus or family) (EAZA 2022) including the search term from the ART search (Supplementary Information). In JZAR, an individual search for each of the 449 EEP taxa (family, genus, species or subspecies) was first conducted in conjunction with the ART search terms used previously and then using solely the taxonomic terms. All papers were subsequently individually screened for relevance (Supplementary Information). Both the common name and scientific name were used for all searches. If the search did not return any peer-reviewed scientific papers, the historical common names for the species of interest listed in the Integrated Taxonomic Information System (ITIS n.d.) were used. In PubMed, the same search strategy was employed as used for BioOne and JZAR databases, however as this resulted in few papers retrieved the search was also conducted on the family level for all EEP taxa. The species and subspecies were not included as search terms but were manually retrieved afterwards. One exception to the family level search was the family Bovidae, where the search was done on the subfamily level as the amount of ART research done in beef and dairy cattle occluded the work done in non-domesticated Bovidae species. Using the subfamily in the search provided the most efficient way of filtering out domestic animals. For some of the family-based searches on the list, there existed a controlled vocabulary thesaurus used for indexing articles in the PubMed database (i.e. Medical Subject Headings (MeSH) terms) and these were included where relevant (Supplementary Information).

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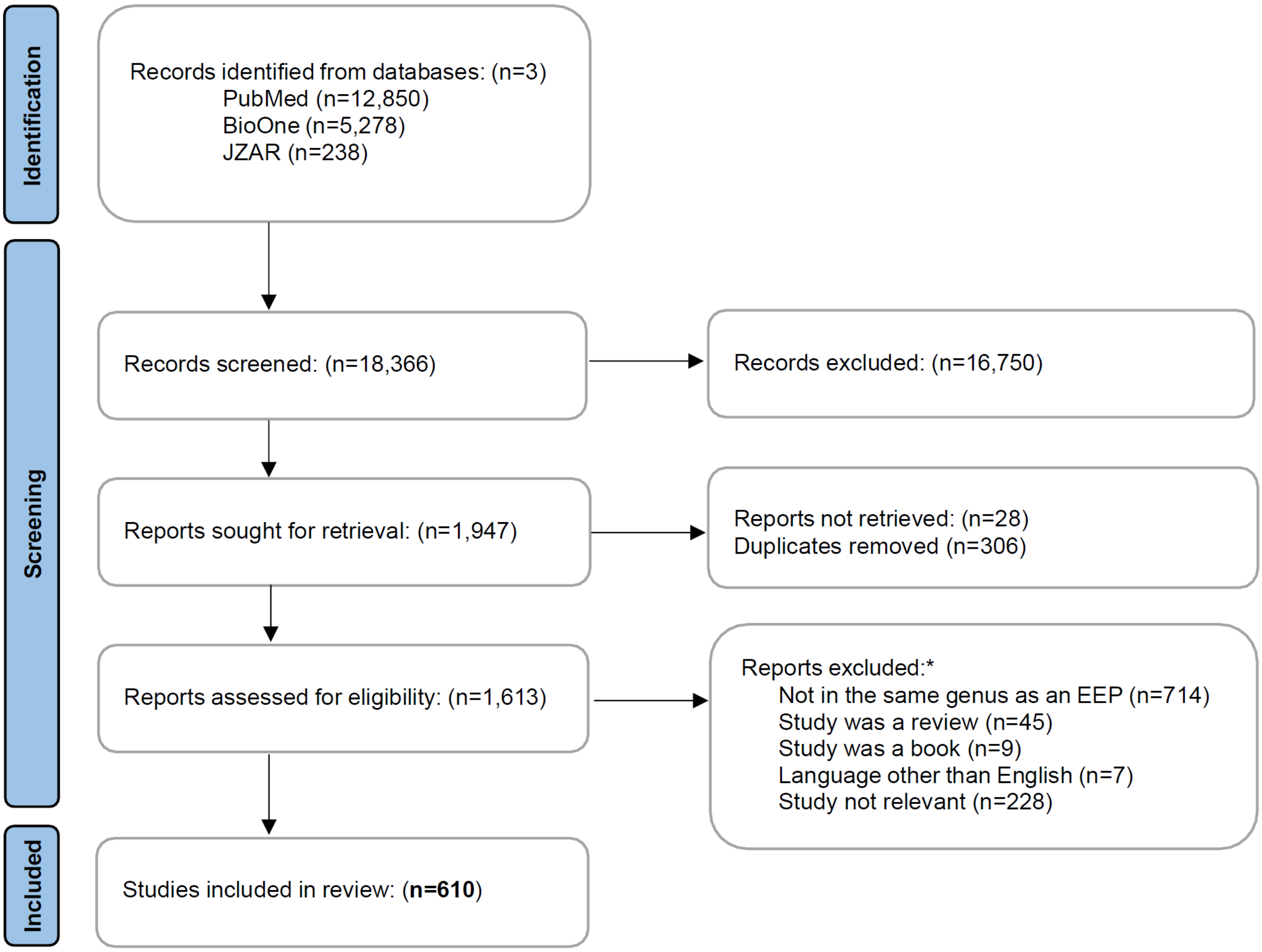

Figure 1. PRISMA flow diagram illustrating the inclusion process of the relevant literature (Page et al. 2010). * All exclusions were done manually.

After the literature search was completed, all titles and abstracts from the resultant papers were evaluated for relevance. Papers were deemed relevant if they included any information on an EEP taxon or a related species regarding the three main topical groupings: semen (semen collection, semen preservation, semen evaluation and sex-sorting of semen), oocyte/embryo (reproductive assessment, hormone stimulation, oocyte collection, work related to embryos (harvesting, transfer, preservation), hormone evaluation and preservation of oocytes and ovaries) and in vitro/in vivo (artificial insemination, in vitro techniques and biotechnologies such as xenografting, stem cell procedures and nuclear cell transfer). All relevant information was extracted and compiled into an Excel spreadsheet and further refined by excluding reviews and books as the search string had included the papers referenced in these.

A comprehensive cross check was conducted to ensure that all papers were captured in the search string. No date limits were implemented in the search. All the papers identified in the JZAR searches were open access. The papers identified through the PubMed search were accessed through the University of Copenhagen, Royal Danish Library's website (rex.kb.dk). Papers from BioOne were accessed through a membership of the European Association of Zoo and Wildlife Veterinarians (EAZWV). If a paper was not open access, or not available through the library or the membership, the paper was excluded (28 papers in total; Figure 1). Papers written in languages other than English were excluded apart from one Japanese paper where the results were reported in English (seven papers were excluded by this criterion).

Results

After removing duplicate papers, a total of 610 papers were found to cover one or more of the 13 selected ART topics in reference to an EEP taxon (family, genus, species or subspecies) specifically or a species within the same genus as an EEP taxon. Of the 610 papers, 84% (514) were retrieved from PubMed, 15% (88) from BioOne and 1% (8) from JZAR. The papers included in this review span the years 1967–2022 with the majority (82%) being published after 2000.

Out of the 610 publications, 348 focused on a specific EEP taxon covering 30% of EEPs (133 of 449; Supplementary Information). Grouping the papers by animal class, 88% (306) were on mammals (Figure 2) covering 40% (98/240) of the mammal EEPs. This was followed by 7% (26) related to fish covering 24% (4/17) of the fish EEPs and 4% (14) related to birds covering 21% (29/137)

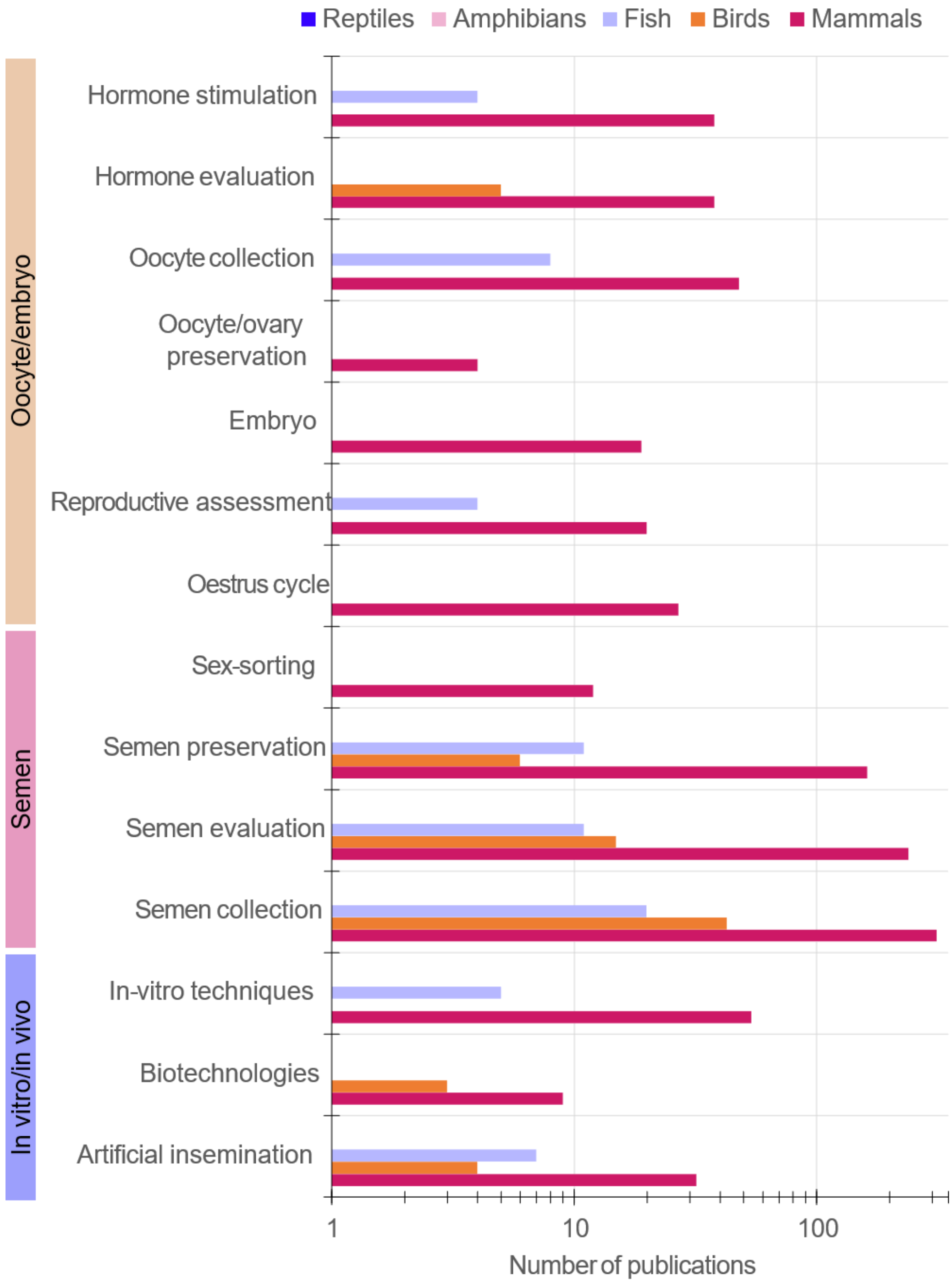

Figure 2. Distribution of ART topic publications per EEP taxa animal class.

of the bird EEPs. The remaining taxonomic classes are poorly represented in scientific publications related to ART topics with the reptile and amphibian classes each represented in 1% (1) of ART topic papers published. The invertebrate class had no ART-related representation in the literature. Of the 30% of EEP taxa (133/449) specifically mentioned in the ART topic-related literature (Supplementary Information), only the elephant, rhinoceros and penguin TAGs had all the TAG-listed taxa (subspecies and species) represented in scientific publications. Within these publications, the focus falls predominantly within the semen category of ART topics, specifically, semen collection, assessment and preservation. Among all of the selected ART topics pertaining to EEP taxa described in the literature, semen collection is by far the most represented procedure described, with a total of 415 results. For EEP-managed families, species and/or subspecies, 24% of the EEPs (109/449) had at least one publication discussing a semen collection method of which 74% (81/109) were on mammal EEPs. Within the topic of semen collection, 57% of the protocols discussed electroejaculation/electrostimulation, making this the most common collection technique reported (Figures 2 and 3).

Within the three ART topic categories outlined above (semen, oocyte/embryo and in vitro/in vivo), the 348 EEP taxa-related publications were further categorised by 826 separate descriptions, as one paper could report on multiple techniques and/or taxa. Semen-related topics, particularly in relation to mammals, were the most referenced of the three categories.

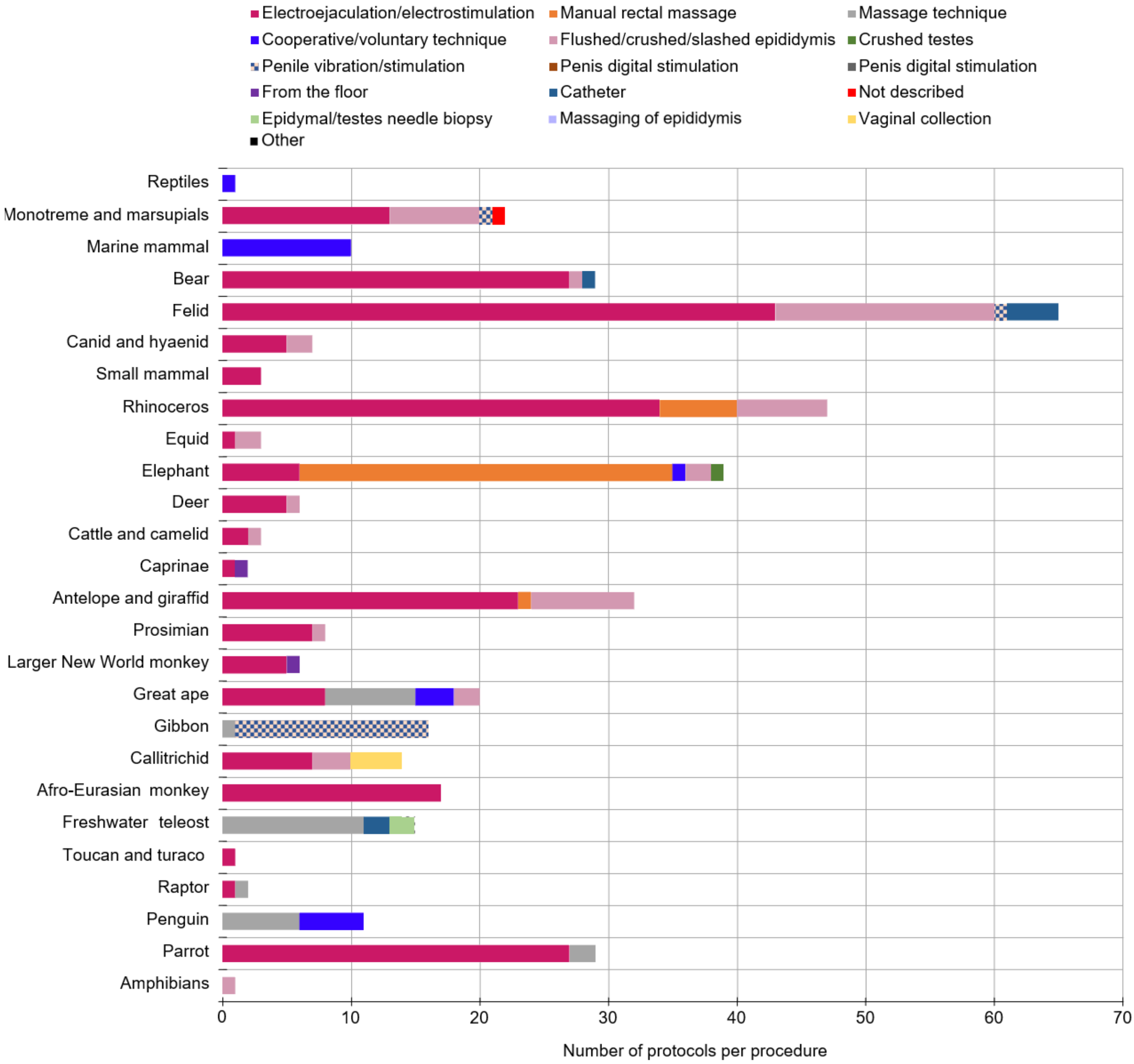

Figure 3: Published semen collection methods by TAG. 26/42 TAGs represented

For mammals 50% (416) of descriptions from all publications related to this topic, 20% (166) of described topics related to the oocyte/embryo category and 11% (95) related to the in vitro/in vivo category. After mammals, the next most abundant group represented in the literature, birds, had 8% (64) of all publications related to the semen category, 0.5% (4) publications in the oocyte/embryo category and 0.8% (7) related to the in vitro/in vivo category. Fish were represented in just 5% (42) of the publications for semen-related topics, 2% (17) on oocyte/ovaries topics and 1.5% (12) related to the in vitro/in vivo category. Lastly, reptiles and amphibians were represented in only 0.3% (2) and 0.1% (1) respectively of all publications and these were related to the semen category (Figure 2).

Even for the three TAGs with all EEP taxa mentioned in the ART-related publications (elephant, rhinoceros and penguin TAGs), gaps in knowledge still exist. For the elephant TAG, there were no publications found on oocyte collection/preservation, in vitro techniques or work related to embryos. For the rhinoceros TAG, no publications on oocyte preservation were found. For the penguin TAG, publications were missing on hormone stimulation, in vitro techniques/biotechnologies, oocyte collection/preservation, reproductive assessment, embryos and sex-sorting of semen. Broken down by class, mammals had information covering all selected ART-related topics for at least one taxon. For fish, information was missing on hormone evaluation, biotechnologies, embryos and sex-sorting of semen and within the bird class there

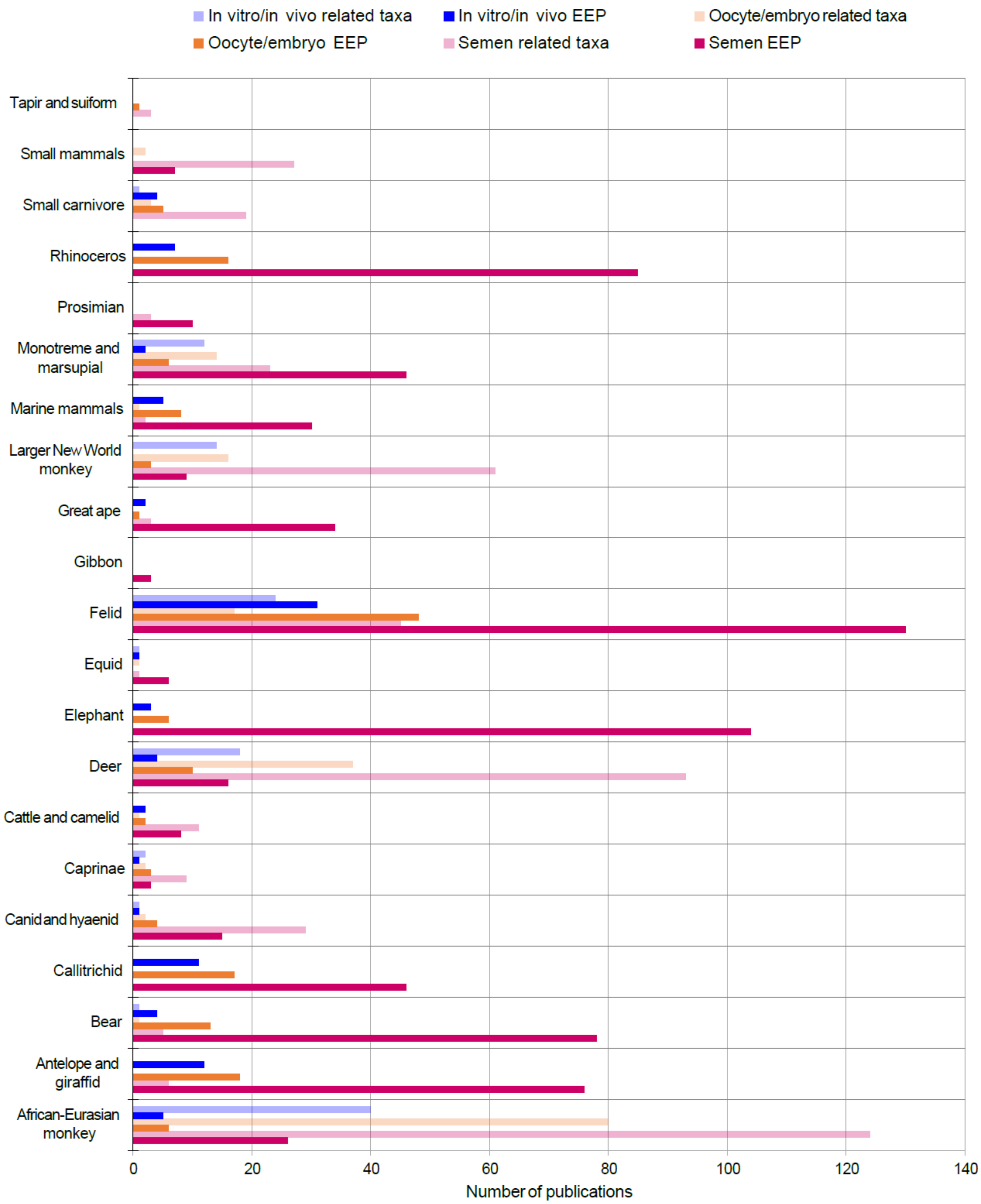

Figure 4. ART topic publications by TAG belonging to the mammal class, for EEP taxa and EEP-related taxa. ART topic category colour coding includes purple for in vitro/in vivo, pink for semen and orange for oocyte/embryo. As the publications can report on multiple species/taxa and/or procedures, they may count more than once in this figure. 21/42 TAGs represented

were no publications on hormone stimulation, oocyte collection or reproductive assessment or in vitro techniques (Supplementary Information). Out of the EAZA TAGs, the highest number of ART topics in peer-reviewed scientific publications were returned for the felid, elephant, bear, rhinoceros and antelope and giraffid TAGs (Figure 4), with 45, 36, 35, 28 and 28 publications respectively. These publications reported on 209, 113, 108, 106 and 94 ART topics on EEP taxa for felid, elephant, rhinoceros, antelope and giraffid and bear respectively. The same trend that was observed in the overall EEP taxa results (i.e. that the semen category was present in the majority of ART topic papers returned) was observed for these five TAGs (Figure 4).

Furthermore, within the 262 papers pertaining to a species within the same genus as an EEP species (i.e. not a specific EEP family, genus, species or subspecies), semen is also the category most reported on (Figure 4). Widening the search returned publications for related mammal species in the Afro-Eurasian monkey, deer and larger New World monkey TAGs, with 246, 148 and 92 additional ART-related publications, respectively. For birds, only the parrot TAG had a large number of additional publications returned when widening the search to TAG-related species, resulting in an additional 96 relevant publications on an ART topic. These findings show that searching for literature outside of specific EEP taxa may still be useful for gaining supplemental ART-related knowledge as it pertains to the EEP.

Discussion

Knowing how to collect, store and use gametes from a particular species in a way that maximises viability requires specific protocols encompassing all the steps of ART for both males and females. Of the 13 selected ART topics, only 30% of the 449 EEP families, species and subspecies were represented in the literature in at least one of the ART topics. As such, there is still a large knowledge gap to fill before species-specific protocols can be developed for use of ART for all EEPs if deemed relevant and needed. Future research should focus on identifying the EEP-managed species for which no ART knowledge is available and which might benefit from ART now or in the future, and work to fill those gaps. As with many clinical aspects and as evidenced by the body of literature under review, the first step towards developing new techniques in any species is to look at what is available for their closest domestic counterpart or humans in the case of primates. ART procedures in domestic animals and humans were outside the scope of this review but basic knowledge and techniques used in wildlife ART stem from those previous developments and future research should commence by reviewing the information available for these criteria. However, caution is advised for direct application of knowledge or techniques developed for one species on a closely-related species, as physiological variations often result in potential failures as exemplified by different use of essential ion-channels between mice and human sperm (José et al. 2015) and in different cryopreservation success between wombat and koala sperm (Johnston et al. 2006).

The animal class appearing most often among the ART topics in peer-reviewed scientific literature was mammals with 98 out of 240 EEP-managed taxa being reported in the publications. The fish class was the second most represented class with four out of 17 EEP taxa mentioned and the bird class came third with 29 out of 137 EEP-managed taxa described (Supplementary Information). A potential explanation for this finding lies in the taxonomic level at which the different EEPs are managed. The nine freshwater teleosts are managed at the family level, whereas none of the bird EEP taxa are managed at the family level. Historically, ART science has focused on mammals both in regard to its use and advancement in humans and in optimisation or increase of livestock and poultry productivity. Similarly, the bias towards more charismatic species such as large herbivores and felids in the literature may also reflect societal and cultural views on wildlife and conservation. In that regard, the attention afforded to birds is interesting as they are often not perceived to be as highly valued as mammals (Bradley et al. 2020) and ART methodologies have only been developed for some bird species due to their importance in agriculture or the pet trade (Blanco et al. 2009). Specifically, the value of more exotic bird species in the pet trade may also explain why protocols exist for some of the more prominent species (Pires 2012) and why birds are generally relatively well represented in the ART literature. The species most heavily represented among the birds are also the most charismatic and popular such as species of penguins and parrots, which tend to receive more attention and funding for research (Gunnthorsdottir 2001).

Somewhat surprisingly, the great apes are not well represented among the ART topics in peer-reviewed scientific literature, with only 18 publications on the topics of semen collection, evaluation and preservation, reproductive assessment and in vitro techniques. This, despite these species often being highly valued culturally and for their similarity to humans, is surprising (Gunnthorsdottir 2001). As this review only focused on peer-reviewed scientific papers and did not include grey literature, there is a possibility that ART protocols and documents on these as well as other species exist internally in institutions and were omitted here. Any relevant protocols developed internally within an institution would be a great resource on which to build future ART research, especially for underrepresented EEP taxa. Therefore, publication and/or wider dissemination of such protocols is of great importance to advance the global wildlife ART community and is strongly encouraged.

With the PRISMA guidelines, a standardised literature search was performed to comprehensively extract literature on the use of ART in EEP-managed taxa. The broadness of the search needed the addition of several filters to the search string which may have led to the unintentional exclusion of relevant papers and therefore potential underestimation of the representation of specific taxa. The search was conducted for English language publications and as such relevant publications in other languages were excluded. In addition, some families, species and subspecies might have had changes in taxonomy over time, leading to papers referring to outdated or obsolete nomenclature to possibly be excluded from the search. To counteract this, an individual, family or genus search, depending on the database, was used as a way of incorporating as many papers on the relevant EEP taxa as possible. As only EEP taxa and species in the same genus as these were selected as the scope for this review, significant ART-related research in other, more distantly related, non-domestic species might have been omitted. For example, research in non-EEP species (e.g. reptiles) may prove useful as foundational knowledge for development of species-specific protocols that could be applicable to EEP species and as such the authors advise that future research take a wider breadth of species into consideration, with the appropriate caution as previously mentioned.

Of the 13 ART topics focused on in this paper, semen collection was by far the most described topic with 109 taxa having some information reported (Figure 3). Mammals is the main class reported on, closely followed by birds. The most common method of collecting semen across all taxa was electroejaculation/ electrostimulation which made up nearly 60% of the reported techniques in both mammals and birds. The success rate of the technique varied between taxa but overall it produced consistent and reliable results in both in situ and ex situ situations (Wildt et al. 1987). The largest disadvantage of this method is the need for the animal to be anaesthetised, which in addition to the increased risk of animal mortality under anaesthesia (Arnemo et al. 2006) can affect the ease of collection or the quality of the sample due to effects of the sedatives (Zambelli et al. 2007). For some of the EEP species, semen collection protocols and data may exist for a related species in the same genus (Figure 4) and although species-specific variations exist it would be beneficial to attempt to use these protocols as foundations for the EEP taxa of interest. It could therefore be beneficial in the future to implement the collection, evaluation and preservation of semen during routine veterinary procedures as much as possible in order to facilitate the development of species-specific protocols. Experimenting with techniques may allow for optimisation and/or creation of protocols for semen collection in species where these do not yet exist, as well as serve to gather basic semen parameter reference data for a wider array of species. Ideally, these developments would be made before there is a more urgent need to implement ART methods in an EEP species.

For a few species, animals have been trained for voluntary semen collection, mostly with great success. This has primarily been done in primates but has also been successful in the bottlenose dolphin (Yuen et al. 2009) and the Philippine crocodile (Sandmaier et al. 2022). If animals in ex situ situations can be conditioned to voluntarily submit to semen collection, it might hold advantages in terms of animal welfare and avoiding risks of anaesthesia. In addition, samples collected from awake animals are often more viable than samples obtained from forced ejaculates (Schneiders et al. 2004). However, the increased resources needed to successfully train and maintain behaviours of the animals for this purpose and the added risk to personnel safety must be taken into account. Alternatively, if training for voluntary sample collection is not feasible, penile vibrostimulation could be an option to avoid anaesthesia and still get a semen sample, since it is performed on awake animals. However, it does require a skilled handler and an animal that is small enough to be manually restrained during the stimulation (Schneiders et al. 2004; Yeoman et al. 1998). Several techniques exist that may be relevant for population management and could be options for EEP taxa that still lack specific collection protocols.

In the literature, most of the research has focused on the collection and preservation of male rather than female gametes, which holds especially true for mammal-focused ART research. Only 41 out of 240 EEP mammal species have data related to female hormone assessment, which is crucial to understanding the female cycle in the different species. Of the publications on hormone assessment, 47% (34) used faecal samples to test hormone levels and 43% (31) used blood samples. Non-invasive samples such as faeces could potentially be implemented in the routine management of a species to get a better understanding of the female cycle in the different EEP species but it would require a large sample set to define normal reference ranges. To date, only 27 EEP species have information on how to collect ovaries postmortem, which allows for collection of mature or immature oocytes which can then either be used for IVF or ICSI after appropriate in vitro maturation. A total of 29 out of 240 mammal EEPs have published data on oocyte collection and only seven species have data on embryo collection. This discrepancy may be due to the increased invasiveness and difficulty of female gamete or embryo collection.

More overall knowledge of species-specific female anatomy and physiology is needed to increase the probability of successful and viable sample collection. Due to the size difference, and thus cryoprotectant permeability, between male and female gametes, the storage of collected female gametes is more challenging (Clulow et al. 2019). The increased volume and lower permeability of oocytes increase the likelihood of intracellular ice crystal formation within the sample, destroying the cells and rendering it nonviable for IVF or ICSI (Clulow et al. 2019). While cryopreservation of oocytes and embryos has been performed successfully in some mammal species, size becomes more of a challenge in other taxa such as amphibians due to the increased size of oocytes as compared to those of mammals (Clulow et al. 2019). Successful preservation of semen faces similar challenges in that there is broad interspecies variation in resistance to cold stress and toleration of different cryoprotectants. For example, the commonly used cryoprotectant glycerol is cytotoxic in macropods at physiological temperatures (McClean et al. 2007). This underlines the necessity of establishing functional species-specific cryopreservation protocols to ensure that the samples are being preserved successfully and will be viable after thawing.

Currently, the most common preservation technique used for semen, oocytes and embryos is slow freezing cryopreservation but newer techniques such as vitrification and directional freezing are emerging and producing encouraging results (O'Brien and Robeck 2014; O'Brien et al. 2013; Cerdeira et al. 2021). The limitations of these newer techniques include the expenses involved with acquiring the equipment as well as the lack of field application. The latter could be mediated by utilising temporary cooled storage during transport to a dedicated cryo-facility, albeit there is minimal knowledge of how well semen from most animals tolerates prolonged cooling. Lyophilisation or freeze drying of semen has also been utilised in some species (Kaneko et al. 2014) but for the sperm to result in viable offspring afterwards, in vitro techniques such as ICSI are needed due to the lack of sperm motility that results from the freeze-drying process. As ICSI allows the opportunity to work with semen samples of lower quality and mobility, research on 13 EEP species—all mammals—has already been undertaken to try to implement this technique. However, the oocytes must still be matured and ready for insemination. Thus, with any preservation technique employed, better basic knowledge of how to collect gametes of different species is required and the development and publication of these species-specific protocols is essential to expand the use of new techniques into ART in EEP and population management plans (Howell et al. 2022).

Secondary to understanding the gaps in EEP species-specific ART topic protocols in the current literature, this review has uncovered the current lack of standardisation in reporting and describing collection, evaluation and preservation of gametes. This is particularly evident for the evaluation of collected semen, where there is no uniform way of reporting semen parameters. For example, reports of sperm motility varied highly, either reported as a score of 1–5, a percentage of motile sperm or a calculated number based on different formulas. It is often not specified whether the sperm motility reported is the total motility, progressive motility or some other metric. These ambiguities create difficulties when trying to establish a normal reference interval for an individual species, let alone make interspecies comparisons. It would therefore be beneficial to establish guidelines on how to prospectively report not only semen parameters, but also other parameters and metrics of cryopreservation and gamete storage. This would facilitate reproducibility of research and allow for more comparable and accessible results. However, certain limitations would persist, especially with suboptimal samples such as those from an animal outside its prime reproductive period or those otherwise compromised (e.g. during collection or transport). Protocols cannot take all the variables into consideration but with more research published, a better understanding of these variations can inform how to adapt and implement ART as a conservation tool for EEP taxa if needed.

In summary, there is still much to learn about ART and its associated disciplines used within EEP-managed taxa and beyond. Besides a few (sub)species, it is more common that wildlife species do not have specific ART protocols developed, despite facing dire conservation threats. ART holds great promise for ex situ management of wildlife, yet significant gaps exist in its application especially within reptiles, amphibians and fish. Knowledge gaps exist for many threatened mammal and bird species, making the use of ART less feasible as a conservation tool in these species. One major challenge lies in the diversity of reproductive strategies and physiological characteristics among species within these groups. Additionally, there is a lack of species-specific knowledge regarding gamete biology, reproductive anatomy and hormonal regulation which are essential for the development of ART techniques. Furthermore, limited research and funding directed towards reptiles, amphibians and fish compared to mammals and

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birds have contributed to the scarcity of validated ART methods tailored to these taxa.

Many EEP species across all taxa have complex reproductive cycles influenced by factors such as temperature, photoperiod and social interactions, making it difficult to develop standardised ART protocols applicable across even closely related species. As such, it is important to start identifying which species and subspecies may need ART in order to ensure healthy and genetically diverse populations, both in situ and ex situ, and provide the best foundation for their future preservation. Addressing these gaps requires collaborative efforts among researchers, conservation organisations and reproductive and biotechnology experts to conduct species-specific studies, develop innovative techniques and prioritise funding for ART research aimed at improving ex situ management of reptiles, amphibians, fish and even underrepresented bird and mammal species at risk of extinction. To initiate a broader implementation of ART in the conservation work already being done, it is critical to allocate more resources and efforts to establish best practices for the collection, evaluation and preservation of gametes. Groundwork laid now, preferably on a species level but at minimum at a genus level, can work to assure that in situ and ex situ wildlife populations will have this conservation tool available if and when needed. By bridging these knowledge and technology gaps, ART has the potential to become a valuable tool for conservation breeding programmes and the preservation of biodiversity in the face of global environmental challenges.

Conclusion

To date, only 30% of EEP-managed genera, families, species and subspecies have ART topics published in peer-reviewed scientific literature, leaving information on more than 300 EEP taxa unaccounted for, some of which might benefit from ART. Most of the publications include the mammal class with 98 out of 240 of the EEP mammal taxa being published on. Surprisingly 29 out of 137 EEP bird taxa had a publication related to ART. The most described ART topic among the relevant publications across all animal classes was semen collection where 109 EEP taxa had at least one protocol describing a semen collection method. The collection method most described in the literature was electroejaculation/-stimulation, which overall produced reliable results.

There are still knowledge gaps regarding gamete preservation and currently the most common technique reported is slow freezing cryopreservation, but newer, emerging techniques are showing encouraging results to support their usage. The general lack of standardisation in reporting and describing ART limits the broader use and comparability of data, especially semen evaluation, where the lack of consistency makes a comparison of semen parameters in the same species almost impossible. Identifying EEPs in need of ART and developing species-specific protocols for collecting, evaluating, preserving and using gametes requires prioritisation of resources and dedicated efforts, but once established, can prove ART to be a powerful tool to help EEPs meet their conservation goals.

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