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Reproductive biotechnologies in snake: a scienciometric analysis

Biotecnologias reprodutivas em serpentes: uma análise cieniométrica

Biotecnologias reproductivas en serpientes: una analisis cienciometrica

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Abstract
Several snake species are under some degree of extinction threat, generated mainly by human poaching, based on mystical-religious fear, and habitat loss. However, it is possible to avoid extinction processes by using reproductive biotechnologies with the purpose of producing offsprings in order to reinsert these species in their natural environment. In this regard, the objective of this study is to conduct a scientometric survey about assisted reproduction in snakes in the last 50 years, without a geographical cut-off. Web of Science, Scopus, Science Direct, Pub Med, Scielo and Google Academic databases were used with the term: snake sperm and snake artificial insemination. In total, 22 papers related to the theme were selected, 21 of them published in 16 different journals, and one book chapter. The Google Scholar database presented 100% of the selected materials, being the most complete platform among those evaluated. Regarding the authorship of these studies, 19 research institutions from five countries actively participated in their production. An average productivity of 0.53 studies per year was found, starting from the first publication in 1980. The species *Elaphe gutatta* was the most commonly used model, participating in 4 (19.04%) surveys. We conclude that reproduction biotechnologies applied to snakes has a good adherence in scientific journals, and has a high potential to be used as a conservationist tool in the taxon, however, new studies, with more solid methodologies, are necessary to validate this technological resource, especially in relation to artificial insemination, which is the major target of this field.
Keywords: Sperm; Cryopreservation; Artificial insemination; Germoplasm bank.

Resumo
Diversas espécies de serpentes encontram-se com algum grau de ameaça de extinção, gerados principalmente pela caça predatória humana, fundamentada no medo místico-religioso, e perda de habitat. Contudo, é possível evitar processos de extinção a partir da utilização de biotecnologias reprodutivas com a finalidade de produção de prole para reinserção no ambiente de ocorrência natural dessas espécies. Nesse sentido, o objetivo deste trabalho é realizar um levantamento cienciométrico sobre a reprodução assistida em serpentes, nos últimos 50 anos sem recorte geográfico. Foram realizadas buscas nas bases de dados: *Web of Science, Scopus, Science Direct, Pub Med, Scielo e Google Acadêmico*, utilizando os termos: *snake sperm* e *snake artificial insemination*. No total, foram selecionados 22 trabalhos relacionados à temática, sendo 21 deles publicados em 16 periódicos distintos, e um capítulo de livro. A base de dados Google Acadêmico apresentou 100% dos materiais selecionados, sendo a plataforma mais completa dentre as avaliadas. Em relação a autoria desses estudos, 19 instituições de pesquisa de cinco países participaram ativamente dessa produção. Calculamos uma média de produtividade de 0,53 estudos por ano, a partir da primeira publicação que data de 1980. A espécie *Elaphe gutatta* foi o modelo mais utilizado, participando de 04 (19,04%) pesquisas. Concluímos que as biotecnologias da reprodução aplicada no grupo das serpentes têm uma boa aderência em periódicos científicos, e possui elevado potencial para ser utilizado como ferramenta conservacionista no táxon, contudo, novos estudos, com metodologias mais sólidas, são necessários para validar esse recurso tecnológico, sobretudo em relação à inseminação artificial, que é o grande alvo dessa área.
Palavras-chave: Sêmen; Criopreservação; Inseminação artificial; Banco de germoplasma.

Resumen

Muchas especies de serpientes se encuentran en algún grado de amenaza de extinción, generada principalmente por la caza depredadora humana, basada en el miedo místico-religioso y la pérdida de hábitat. Sin embargo, es posible evitar los procesos de extinción mediante el uso de biotecnologías reproductivas con el fin de producir descendencia para la reinserción al medio natural de estas especies. En este sentido, el objetivo de este trabajo es realizar un relevamiento cienciométrico sobre la reproducción asistida en serpientes, en los últimos 50 años sin corte geográfico. Las búsquedas se realizaron en las siguientes bases de datos: Web of Science, Scopus, Science Direct, Pub Med, Scielo y Google Scholar, utilizando los términos: espermatozoide de serpiente y inseminación artificial de serpiente. En total se seleccionaron 22 artículos relacionados con el tema, de los cuales 21 fueron publicados en 16 revistas diferentes y un capítulo de libro. La base de datos de Google Scholar presentó el 100% de los materiales seleccionados, siendo la plataforma más completa entre las evaluadas. En cuanto a la autoría de estos estudios, 19 instituciones de investigación de cinco países participaron activamente en esta producción. Calculamos una productividad promedio de 0,53 estudios por año, desde la primera publicación de 1980. La especie *Elaphe gutatta* fue el modelo más utilizado, participando en 04 (19,04%) estudios. Concluimos que las biotecnologías reproductivas aplicadas al grupo de serpientes tienen una buena adhesión en revistas científicas, y tienen alto potencial para ser utilizadas como herramienta conservacionista en el taxón, sin embargo, se necesitan más estudios, con metodologías más sólidas, para validar este recurso tecnológico, principalmente en relación con la inseminación artificial, que es el principal objetivo en este ámbito.

Palabras clave: Semen; Criopreservación; Inseminación artificial; Banco de germoplasma.

1. Introduction

Snakes comprise a taxonomic group that is very vulnerable to extinction processes, so that of the 3128 existing species in the world, 73 are critically endangered, 152 endangered, and 136 vulnerable (IUCN, 2021). However, it is possible to mitigate and even reverse this situation through the use of reproductive biotechnologies with the purpose of producing offspring for the reinsertion into natural environments (Ballou, 1992).

In general, assisted reproduction presents itself as an excellent tool to be employed on behalf of conservation, especially to overcome reproductive difficulties faced due to population reduction of certain species (Clulow & Clulow, 2016). With the employment of biotechnological resources, the numerical reestablishment of some taxa can be initiated from just two specimens of different sexes (Oliveri et al., 2018). In this sense, even in more emblematic situations of extinction in the natural environment, such as the classic case of the blue macaw (*Cyanopsitta spixii*) (Wagler, 1832), which is an endemic bird of the Caatinga Biome in Brazil, could be reverted using specimens kept in captivity.

However, even with the inestimable relevance of reproductive biotechnologies in this context (Ballou, 1992; Clulow & Clulow, 2016), this resource is still little used among wild species, being more focused on domestic animals, given their economic importance (Young et al., 2021b). Nevertheless, the wealth of results obtained among domestic species can act as parameters for experimental models in wild species, providing materials and expertise to guide researchers in this area of study (Silva et al., 2021).

Thus, taking into consideration the importance of reproductive biotechnologies as a tool to mitigate deleterious processes, the objective of this work is to perform a scientometric analysis on assisted reproduction in snakes in the last 50 years, regardless of the geographic origin, the working group, and the area of occurrence of the species.

2. Methodology

Review Description

This is a scientometric analysis carried out in scientific databases that sought to include contextualized research on the subject of reproductive biotechnologies in snakes, according to the PRISMA methodology (main items to be reported in systematic review studies) (Moher et al., 2009).

Data Survey

A selection of materials was made on the following platforms: Web of Science, Scopus, Science Direct, SciELO, PubMed and Google Scholar, using the search commands: snake sperm and snake artificial insemination. For each command, the titles of the first 100 papers presented in each database were analyzed. Among these, the studies whose titles made some mention of the use of reproductive biotechnologies in snakes were fully read and included in the research, while the others were immediately disregarded. Papers that had titles that fit the scope of this research, but did not have free access to the full text, had their contents certified from citations in other studies and were counted.

After the database searches, the bibliographic references of each selected production served as a secondary indication of the search for new material, until no more unpublished references were found, according to the snowball technique (Bailey, 1994). In this modality, as well as in the primary form of search, the criteria for analysis of the titles and complete studies also were applied, following the same guidelines.

We selected only papers published in the last 50 years, in any country of the world, that were in line with an applied stage of assisted reproduction in snakes, involving at least one of the following stages: seminal evaluation, semen cryopreservation (cooling and freezing), and/or artificial insemination (AI). Studies related to sperm morphometry were not accounted, as well as duplicate publications due to corrections in the scope of the study.

Data processing

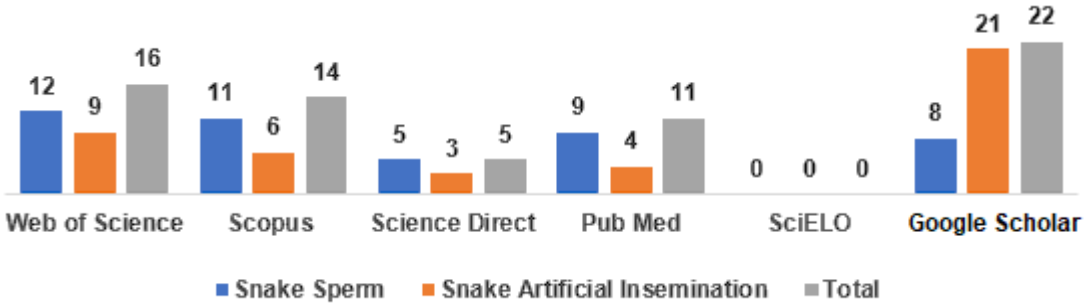
After the data survey, which took place until December 2021, the results obtained were evaluated in relation to the quantification of the literary production of each database; characterization of the journals with publications on the theme; indication of the countries and research institutions related to the studies; and categorization of the methodologies and main results obtained from these researches.

For comparison purposes, the total scientific production of the bovine species was quantified, which is one of the most studied domestic species in the context of assisted reproduction, using the same databases discriminated in this study. The search terms used were: bulls sperm and cattle artificial insemination, which correspond to those used in the searches related to snakes, distinguishing the taxa.

3. Results and Discussion

After searching the databases, 22 studies were selected, according to the scope of this work. Among them, 21 are published in journals, and one is a book chapter. The full text of the work by Mendgen et al., (1980) was the only inaccessible one, as it is a chapter of a physical book, which is the oldest research recorded here, however, its inclusion in the scope of this scientometric analysis was validated (Zacarioti & Guimarães, 2010; Oliveri et al., 2018). The Google Academic platform was the most representative in terms of publications of this nature, with 100% (n=22) of the selected material. The SciELO database was the least representative, with no publications related to the proposed theme (Figure 1).

Figure 1 - List of selected studies among the first 100 presented in Web of Science, Scopus, Science Direct, PubMed, SciELO and Google Scholar in the last 50 years worldwide.

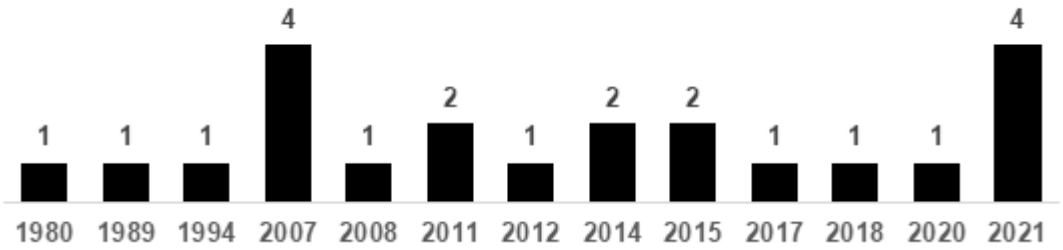


Source: Authors.

It is worth mentioning that the searches on the Web of Science, Scopus and Science Direct platforms using the term Snake Artificial Insemination, presented a file in addition to the one presented in Figure 1. However, this is a correction in the nomenclature of one of the authors of the research by Oliveri et al., (2018), being published in the year 2019, and it was disregarded in this study.

Using the secondary search methodology, no new studies were found. Thus, we calculated 41 years of scientific production related to the theme, counting from the first publication (Mendgen et al, 1980), and an annual average of productions until December 2021 of 0.53 (Figure 2). This low scientific productivity is worrying, given the great pressure of extinction exerted on the taxon (Alves-Filho et al., 2021) and the relevant ecological role of these biotechnological tools in the production of ex-situ offspring for reinsertion in areas of natural occurrence of these species, especially among animals at risk of extinction, thus avoiding deleterious processes (Ballou, 1992; Clulow & Clulow, 2016; Silva et al., 2021).

Figure 2 - Temporal relation of the scientific production on assisted reproduction in snakes from the last 50 years found in Web of Science, Scopus, Science Direct, PubMed, SciELO and Google Scholar.



Source: Authors.

Snakes have a high medical-pharmacological potential and an important ecological function, which may justify the motivation of researchers interested in the taxon. However, it is possible that mystical-religious aspects and fear, generated by the lethal potential of some animals, exert some negative influence on the recruitment of new scholars of the group, as is observed in part of the population, which may explain the low scientific production related to the taxon (Coelho et al., 2021; Onyishi et al., 2021; Fenandez-Badillo et al., 2021).

For comparison purposes, we adapted the search terms used in this scientometric survey to visualize an overview of the scientific production related to the bovine species, which is one of the main taxonomic groups targeted for research related to reproductive biotechnologies. We observed that all databases presented much higher results than the ophids group (Table 1).

Table 1 - Total list of studies presented in the databases Web of Science, Scopus, Science Direct, PubMed, SciELO and Google Scholar from searches using the terms: snake sperm, snake artificial insemination, bulls sperm and cattle artificial insemination.

Search words	Web of Science	Scopus	Science Direct	PubMed	SciELO	Google Scholar
SS	327	265	3,556	154	2	31.400
SAI	13	10	322	4	0	5.600
BS	5.103	5.485	22,314	7.748	83	556.000
CAI	3.256	5.522	10,889	4.385	73	80.900

SS = snake sperm, SAI = snake artificial insemination, BS= bulls sperm e CAI = cattle artificial insemination. Source: Authors.

Despite the low density of literary productions in the snake group, apparently there is a good acceptability of this material among scientific journals that publish in this field. The 21 scientific articles selected here were accepted and published in 16 different journals, only one of which was in Spanish and 20 in English. The journal Reproduction, Fertility and Development is the leader in this context, with 19.04% (n=4) of all accounted production (Table 2). The only study published as a book chapter was indexed by The Society for the Study of Amphibians and Reptiles.

Table 2 - List and classification of journals that published studies on assisted reproduction in snakes in the last 50 years worldwide. Classification according to the assessed parameters in 2021.

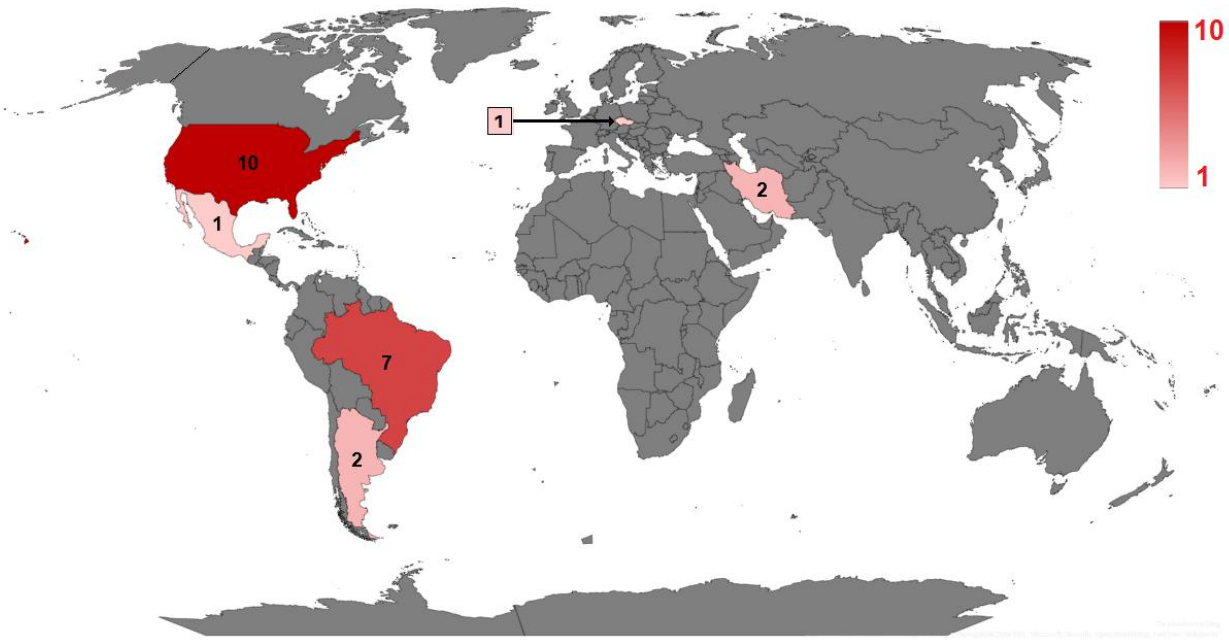
Journals	Productions	IF	hI
Animal Reproduction Science	1	2.145	104
Brazilian Journal of Biology	1	1.651	53
Brazilian Journal of Veterinary Research and Animal Science	1	0,300	17
Cell Journal	1	2.479	776
Ciencia y Mar*	1	-	-
Cryobiology	1	2,487	85
Herpetologica	1	1,676	50
Herpetological Conservation and Biology	1	1,134	23
Journal of Exotic Pet Medicine	1	0,453	32
Journal of Zoo and Wildlife Medicine	1	0,776	45
Journal of Zoology	1	2,322	96
Reproduction, Fertility and Development	4	2.311	76
International Zoo Yearbook	1	1,050	28
Research in Veterinary Science	1	2.534	72
Veterinary Research Forum	1	1.054	5
Zoo Biology	3	1,421	54

* = spanish language publication, IF = impact factor; hI = h index. Source: Authors.

Among the sample universe, it was identified that only six countries actively participated in these scientific productions (Figure 3). Of these, 21 were conducted by one or more research institutions of the same nationality, of which nine

were developed in the United States (USA), six in Brazil, two in Argentina, two in Iran, one in Mexico and one in the Czech Republic. Only one study was carried out in international cooperation, involving research entities from the USA and Brazil. Between authorship and co-authorship, 19 research centers participated in conducting these studies (Chart 1).

Figure 3 - Heatmap discriminating the 5 countries and their respective shares in the production of research related to assisted reproduction in snakes found in the databases: Web of Science, Scopus, Science Direct, PubMed, SciELO and Google Scholar in the last 50 years.



Source: Authors.

Chart 1 - List of countries and research institutions responsible for authoring studies related to assisted reproduction in snakes found in Web of Science, Scopus, Science Direct, PubMed, SciELO, and Google Scholar databases in the last 50 years worldwide.

Country	Instituition	Shares
Argentina	UNC	2
Brazil	BTT	5
	IPCT	1
	UFPel	1
	UFRG	1
	USP	5
United States	HDZ	2
	HZ	1
	LSU	1
	MZ	1
	OSU	1
	SDZ	3
	TE	1
	UF	1

Iran	IAU	2
	RVSRI	1
Mexico	UAM	1
	UABJO	1
Czech Republic	UVPSB	1

UNC = Universidad Nacional de Cordoba, BTT = Instituto Butantã, IPCT = Instituto de Pesquisa e Conservação de Tamanduás, UFPel = Universidade Federal de Pelotas, UFRG = Universidade Federal do Rio Grande do Sul, USP = Universidade de São Paulo, HDZ = Henry Doorly Zoo, HZ = Houston Zoo, KU = Kansas University, LSU = Louisiana State University, MZ = Memphis Zoo, OSU = Oregon State University, SDZ = San Diego Zoo, TE = Trancon Environmental, UF = University of Florida, IAU = Islamic Azad University, RVSRI = Razi Vaccine and Serum Research Institute, UAM = Universidad Autonoma Metropolitana, UABJO = Universidad Autonoma Benito Juárez de Oxaca, UVPSB = University of Veterinary and Pharmaceutical Sciences Brno. Source: Authors.

From the compilation of the methodologies and main results obtained in the 21 studies that had their complete texts analyzed, it was found that the species *Elaphe gutatta* (Baird & Girard, 1853) (corn snake) was the most representative in these experiments, with participation in four studies (19.04%) (Table 2). The corn snake is a species widely used as pets (Macedo & Protázio, 2022), moreover, they are non-poisonous and very docile animals, of adequate size for seminal collection management (Mattson et al., 2007; Oliveri et al., 2018), which may explain the higher interest in research involving the species.

The ventral massage collection method was the most used, being employed in 12 studies (57.14%) (Chart 2). One of the major problems with this method is the contamination of semen with urates and feces at the moment of ventral compression (Zacarioti & Guimarães, 2010). In this sense, some authors have applied local anesthetics in the pericloacal region in order to reduce muscle resistance and, consequently, the contamination of the ejaculates. Silva et al., (2015) and Silva et al., (2021) also performed a previous ventral massage to stimulate the emptying of feces and urine, then performed two intracloacal washes with 0.9% saline solution before the massage for seminal collection.

In a way, all the methodological efforts for good seminal handling and conservation come down to the potentiation of offspring production through AI. However, of the 21 studies reviewed here, only four (19.04%) performed AI procedures on the animals (Chart 2). Perhaps, this low adherence is justified by the difficulty of capturing and keeping in captivity some of these taxa (Silva et al., 2021). However, regardless of the low representativeness in the literature, the methodologies used in studies involving AI are not yet well established, especially in relation to the sample n used, which is too low to consolidate the data statistically (Zacariotti & Guimarães, 2010).

Chart 2 - Classification of methodologies and main results of studies related to assisted reproduction in snakes found in the Web of Science, Scopus, Science Direct, PubMed, SciELO and Google Scholar databases in the last 50 years worldwide.

Autors	Species	MN	CF	Extender	SC	TMA	PMA	Spz x10^6/mL	FN	SD	CFee	EN	BN
Quin et al., 1989	*Thamnophis marcianus*	7	EE	SS	F	> 50%	100%	-	1	Cl	0	-	0
	Thamnophis marcianus	7	EE	SS	F	> 50%	100%	-	2	O	50%	-	18
Langlada et al., 1994	*Crutalus durissus terrificus*	6	C	RS	F	-	-	-	3	RO	-	-	-
	Crutalus durissus terrificus	6	C	RS	F	-	-	-	3	LO	100%	9	9
Fahrig et al., 2007	*Elaphe guttata*	11	VM	TYB	R	> 50%	92,5%	852	-	-	-	-	-
	Elaphe guttata	11	VM	H F10	R	> 50%	92,5%	852	-	-	-	-	-
Mattson et al., 2007	*Elaphe guttata*	2	VM	THS	F	91,9%	89,3%	9,6	5	TO	-	-	5
	Elaphe guttata	2	VM	THS	R	85,6%	82%	6,14	5	TO	-	-	5
	Elaphe guttata	2	VM	THS	F	69,3%	75%	23,7	5	TO	-	47	22
	Elaphe guttata	2	VM	THS	R	48,1%	65%	11,2	5	TO	-	22	22
Tourmente et al., 2007	*Boa constrictor occidentalis*	7	VM	PBS	F	63%	95%	-	-	-	-	-	-
Zacariotti et al., 2007	*Crotalus durissus terrificus*	28	VM	-	F	63,88%	59%	138	-	-	-	-	-
Mattson et al., 2008	*Elaphe guttata*	-	VM	BG	F	72,5%	-	-	-	-	-	-	-
	Elaphe guttata	-	VM	BGG	C	27%	15%	-	-	-	-	-	-
Tourmente et al., 2011	*Waglerophis merremii*	13	C	BWW	F	> 50%	-	-	-	-	-	-	-
	Boa constrictor occidentalis	13	C	BWW	F	> 30%	-	-	-	-	-	-	-
Zacariotti et al., 2012	*Crotalus ruber*	4	VM	LAKE	C	0%	0%	-	-	-	-	-	-
	Crotalus ruber	4	VM	TY	C	50%	-	-	-	-	-	-	-
Mozafari et al., 2012	*Gloydius halys caucasicus*	26	C	-	F	> 60%	-	30	-	-	-	-	-
Moshiri et al., 2014	*Vipera albicornuta*	20	C	PBS	F	53%	-	182	-	-	-	-	-
Friesen et al., 2014	*Thamnophis sirtalis parietalis*	40	IC	-	F	> 50%	-	-	-	-	-	-	-
Silva et al., 2015	*Bothrops insularis*	18	VM	-	F	64,37%	63%	126	-	-	-	-	-
Meza-Manriques et al., 2015	*Boa imperator*	25	VM	Tyrode	F	91%	80%	122,4	-	-	-	-	-

Silva et al., 2017	*Erythrolamprus poecilogyrus sublineatus*	6	C	BTS	F	80%	-	330	-	-	-	-	-
Oliveri et al., 2018	*Elaphe guttata*	1	VM	SS	F	-	-	-	3	TO	66,6%	30	30
	Corallus hortulanus	1	VM	SS	F	-	-	-	1	TO	100%	-	7
	Sanzinia madgascariensis	1	VM	SS	F	-	-	-	1	TO	0%	0	0
	Hydrodynastes giga	1	VM	SS	F	-	-	-	1	TO	0%	0	0
Coeti et al., 2020	*Micrurus corallinus*	7	VM	H F10	F	85%	72%	1300	-	-	-	-	-
Sandfoss et al., 2021	*Pituophis ruthveni*	11	VM	H10	C	63%	52%	413	-	-	-	-	-
	Pituophis cantenifer	8	VM	H10	C	75%	65%	349	-	-	-	-	-
Silva et al., 2021	*Bothrops insularis*	6	VM	ACP 120®	R	45,5	10,6%	-	-	-	-	-	-
Young et al., 2021[a]	*Python bivittatus*	6	C	M199®	F	70%	47%	-	-	-	-	-	-
Young et al., 2021b	*Elaphe moellendorffi*	1	PM	M+D	C	0,058%							
				M+G	C	5,19%	-	-	-	-	-	-	-
	Dendroaspis viridis	2	PM	M+D	C	19.1%	-	-	-	-	-	-	-
				M+G	C	0,73%	-	-	-	-	-	-	-
	Phyton bivittatus	3	PM	M+D	C	2,98%	-	-	-	-	-	-	-
				M+G	C	0,02%	-	-	-	-	-	-	-
	Morelia viridis	1	PM	M+D	C	5,17%	-	-	-	-	-	-	-
				M+G	C	0,57%	-	-	-	-	-	-	-
	Bothrops erytrhomelas	1	PM	M+D	C	3,47%	-	-	-	-	-	-	-
				M+G	C	13,54%	-	-	-	-	-	-	-
	Crotalus scutulatus	2	PM	M+D	C	28,30%	-	-	-	-	-	-	-
				M+G	C	24,30%	-	-	-	-	-	-	-
	Crotalus viridis helleri	2	PM	M+D	C	15,20%	-	-	-	-	-	-	-
				M+G	C	14,50%	-	-	-	-	-	-	-

MN = male number; CF = collect forms, EE= eletroejaculation, VM = ventral massage, C = cirurgic, PM = *post mortem* IC = Intracloacal; SS = saline solution, RS = Ringer's Solution, TYB = Test-yolk buffer, H F10 = Ham's F10, THS = TH hepes solution, PBS = phosphate-buffered saline, BG = Biladyl + 20% of egg yolk, BGG = Biladyl + 20% of egg yolk + 17% of glycerol, BWW = Biggerst, Whitten and Whittinghan extender, BTS = Beltsville Thawing Solution, H10 = H10 = TL Hepes + 10% fetal bovine serum; M+D = M199® + 12% of dimetilsulfoxido, M+G = M199® + 12% of glycerol; SC = sperm condiction, F = fresh, R = cold, C = cryopreserved; TMA = total motility average; PMA = progresive motility average ; Spz = spermatozoa; FN = female number; SP = sperm deposition, Cl = cloaca, O = ovidute, TO = two ovidutes, RO = right ovidute, LO = left ovidute; CFee = conception fee; EN = eggs number; BM = births numbers (vivos e natimortos). Source: Authors.

From the data in chart 1, it can be seen that besides the fluctuation in the total motilities of these studies, justified by the authors mainly by semen contamination with urates and feces, it is also possible to observe a large variation in sperm concentrations, which in some cases may be associated with species, physiological age, or even body score. Yet, regardless of the variation, the results observed here for sperm concentration are much higher than desirable for commercialization in domestic mammals (CBRA, 2013). It is possible that this is due to the reproductive seasonality of these reptiles, which usually copulate only in a certain period of the year.

Another factor that may corroborate the understanding of this high sperm concentration is the reproductive potential of the females of these animals in maintaining the viability of male gametes in their organism for a high and still unknown time (Levine et al., 2021). Thus, fertilization can occur at different reproductive periods, as demonstrated in the study by Levine et al. (2021), in which females of *Crotalus atrox* (Baird; Girard, 1853) maintained viable sperm for six years after copulation, generating diploid pups, genetically corresponding to the male with whom they had the last sexual interaction.

4. Conclusion

Studies related to assisted reproduction in snakes have a good acceptance in scientific journals and are a little explored niche with enough literature demand. Therefore, it is extremely important to develop new researches, with solid methodologies, in order to validate assisted reproduction as a real resource to act in favor of the conservation of these reptiles, especially in relation to AI, which is the ultimate goal of this broad area of research.

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