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A systematic review and meta-analysis of ovarian lavage sperm artificial insemination and its impact on reproductive performance in external fertilization of fish

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Abstract

Ovarian lavage sperm artificial insemination (OLSAI) is a human-assisted fish breeding technique that involves delivering sperm into the ovary via a catheter tube, which allows internal gamete association. This systematic review and meta-analysis compared OLSAI to dry external fertilization procedures, with the parameters focused on fertilization and hatching rates. We conducted a literature search on only external fertilization fish species to collect publications. After screening and satisfying the inclusion criteria, the final systematic review and meta-analysis included eleven peer-reviewed articles. We conducted random-effects meta-analyses to incorporate data on fertilization and hatching rates, given the anticipated heterogeneity. The trim and fill analysis revealed significant variability between studies. The fertilization and hatching rate Tau-squared (τ^2) values were 910.57 and 1313.98, respectively. Moreover, heterogeneity statistics indicated a higher I^2, representing a 97.05% fertilization rate and a 99.12% hatching rate. The pooled rates of fertilization for OLSAI and dry external fertilization were 52.78% and 67.92%, respectively. The pooled hatching rate was also 52.91% for OLSAI and 73.89% for dry external fertilization. Despite these numerical differences, a Welch two-sample t-test revealed no significant difference between OLSAI and dry external fertilization in either fertilization or hatching rates, likely due to several sources of heterogeneity. This study revealed that OLSAI could be an alternative method of freshwater fish species propagation to the conventional dry external fertilization technique without compromising the success rate. To capitalize on the benefits of OLSAI and contribute to advancing our knowledge, further research across species is needed.

Keywords: Ovarian lavage, Artificial insemination, Meta-analysis, Fertilization rate, Hatching rate

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Introduction

The increasing population and interest in healthier diets are driving the demand for fish (Hoga et al., 2018). A sustainable aquaculture practice is required to meet the rising demand with an estimated amount of 80 million tons by 2050 (Zamri et al., 2022). Nonetheless, an important barrier to increasing intensive fish farming is the inconsistent, insufficient, time-consuming, and commercially unviable supply of natural water fish seeds (Adebayo & Popoola, 2008).

Sustainability can be ensured in captivity through obtaining high-quality seed (Mylonas et al., 2010). The generation of massive seeds aids in domestication and safeguards endangered species. Scientists use methods like collecting gametes and fertilizing them outside the fish, which are important for breeding programs and can involve mixing different fish species. This method is essential and often the only practical way to make changes like genetic modification or to use frozen sperm.

Stripping followed by *in vitro* fertilization faces several problems, like the challenge of knowing exactly when ovulation happens (Ittzés et al., 2020; Müller et al., 2018a; Mylonas et al., 2010), the unreliable production of hybrid seeds that requires a lot of effort (Perera et al., 2017), the need to sacrifice male fish in some species like the *Ictalurus furcatus* (Bart & Dunham, 1996; Dunham & Argue, 2000; Dunham et al., 2000; Hu et al., 2011; Myers et al., 2020a, 2020b), and the risk of contamination that can lead to early gamete activation (Dunham & Masser, 2012).

In dealing with these problems, ovarian lavage sperm artificial insemination (OLSAI) is a way to help fish reproduce by taking sperm and putting it into the female's reproductive system using a catheter tube, which is a hopeful choice for increasing fish populations and protecting them (Ittzés et al., 2020; Müller et al., 2018a, 2018b, 2019, 2020a). This procedure requires sperm and/or hormone insemination into the ovary to encourage ovulation and/or gamete association. Indicative optimistic results have been reported in various species, including *Danio rerio*, *Sander lucioperca*, *Clarias gariepinus*, and *Rhamdia quelen*, demonstrating effective spawning and genetic diversity preservation (Gazsi et al., 2021a, 2021b; Müller et al., 2018a). Additionally, injecting hormones directly into the ovaries avoids the need for uncomfortable needle injections in fish such as *Dichotomyctere nigroviridis*.

Using ovarian sperm insemination and hormone injections in common carp (*Cyprinus carpio*) helps the sperm stay alive for up to 12 hours while still being able to fertilize eggs, as the sperm becomes inactive and saves energy (Müller et al., 2018a). This method also allows for short-term sperm storage for up to 24 hours (Okomoda et al., 2023). Interestingly, sperm from 5 to 10 different males is combined to fertilize the eggs, which helps increase genetic diversity (Müller et al., 2020a). Furthermore, it helps to hybridize two different catfish species (Quyến et al., 2022). The OLSAI method may be appropriate for applying manipulated, cryopreserved, or genetically altered sperm (Müller et al., 2018a, 2018b).

Lastly, after hormone and/or sperm application via OLSAI, the brood returns to their rearing unit that allows spontaneous spawning (Quyến et al., 2022); this helps the fish to practice their natural breeding behaviors, leading to coordinated gamete release, high fertilization success, and seasonal fecundity (Mylonas et al., 2010). Therefore, this systematic review and meta-analysis compared fertilization and hatching rates in external fertilizing fish, focusing on OLSAI and dry methods.

Methodology

Literature search strategy

Based on studies conducted between 1990 and 2024, a comprehensive bibliographic research project was initiated to create an investigational meta-database. Various research engines (Google Scholar, ResearchGate, PubMed, Scopus, Semantic Scholar, Science Direct, Web of Science, Wiley, Taylor & Francis, and Springer Nature) were used to collect information for meta-analysis and systematic review by searching keywords like ovarian lavage, artificial insemination, ovarian sperm insemination, and fish. Finally, the collected literature was screened to identify research on fish species that engage in external fertilization.

Eligibility criteria and data extraction procedure

After gathering all the literature results, the search engine systematized them based on issues like the subject matter and document type. This allows for an effective inspection of the information, indicating the required while leaving out the unnecessary. The screening procedure involved eliminating conference proceedings, review papers, and press publications while keeping scientific articles. Additionally, we eliminated research articles that focused on OLSAI in livebearer fish species (internal fertilization).

Further article cleansing involved classifying and maintaining research articles about fish species reproducing in external fertilization based on their titles and abstracts. The last sorting criteria were identifying research articles that concurrently ad-

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dressed either fertilization rates, hatching rates, or both exclusively in English. Fig. 1 indicates a Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow chart for literature search, screening, and the number of included and excluded studies for the last systematic reviews and meta-analyses. Furthermore, through personal communication from the corresponding author, raw data for a single publication was obtained directly, from which the mean and standard deviation were extrapolated.

According to the rules set by Higgins & Green (2011) in the research investigation framework, the treatments consist of different groups, each with its details like mean and standard deviation. The main goal of this process is to calculate and combine studies with various means and standard deviations, leading to a conclusion that fits perfectly for the next meta-analysis. Utilizing a precisely constructed dataset, the necessary important information was carefully included in an R script.

Statistical analysis method

Using R programming language software version 4.3.1, the "metafor" package, specifically the "rma.uni" function, was employed to fit the models to the data, estimate the effects, and generate forest and funnel plots (Viechtbauer, 2010). The effect size was the fertilization and hatching rate reported as a percentage in each study. This rate with the standard deviations was used to estimate the standard errors. Due to the inherited treatment differences across studies, a random effects model was used to account for both within-study and between-study heterogeneity.

Statistical analysis entails developing meta-analysis models for both fertilization and hatching rates. Since the assumption that the variances are the same is not met, Welch's Two Sample *t*-tests were used (Delacre et al., 2017; Derrick et al., 2016) to compare the fertilization and hatching rates of different sperm application methods, which are dry external fertilization and OLSAI. Furthermore, meta-regression models were fitted for

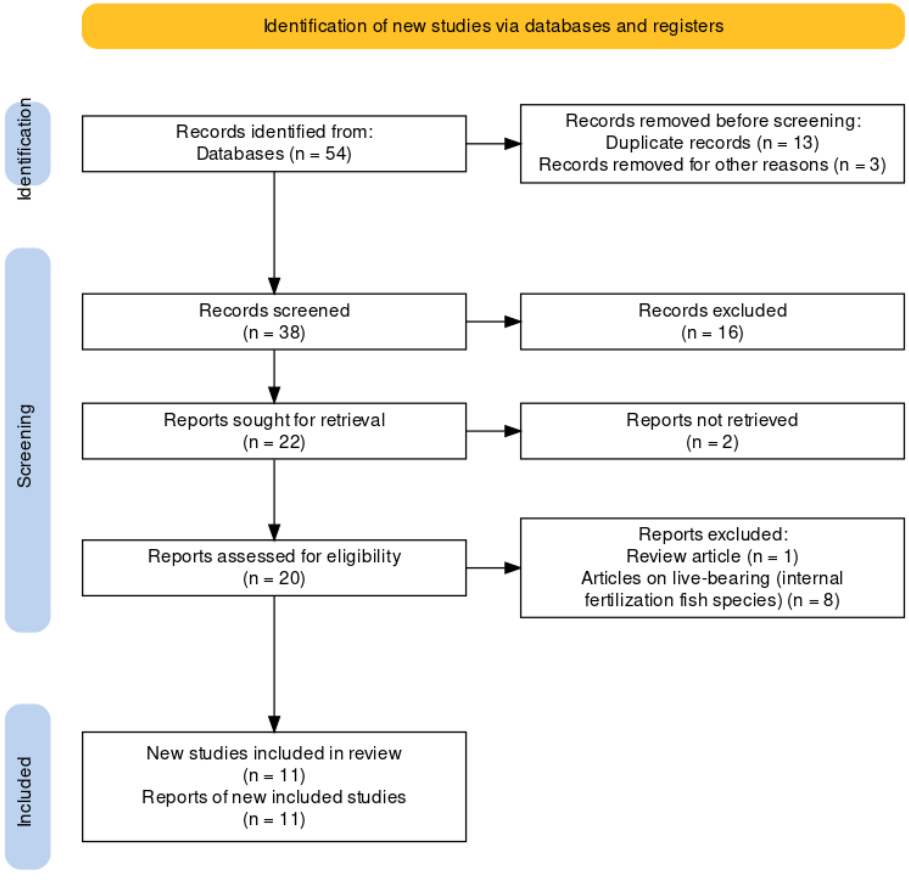

Fig. 1. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart shows the literature identification, screening, and inclusion/exclusion process made with these of the online tool developed by Haddaway et al. (2022). Data from PRISMA (2025).

countries/continents, sperm treatment types, and species acting as moderators.

Higgins' I^2 was used to examine heterogeneity between studies, calculating the percentage of total inter-study variation that could not be explained merely by chance but rather by the number of studies examined. The Higgins' I^2 values, greater than 50%, indicated significant heterogeneity among studies, with higher values indicating bigger differences (Higgins & Thompson, 2002).

The funnel plot demonstrated effect size estimates from each study, enabling the identification of potential publication bias associated with the meta-analysis studies. Without publication bias, the effects obtained were uniformly dispersed around the genuine effect size. When publication bias was evident, however, the distribution was not homogeneous. The statistical test linked with this heterogeneity was the Egger Test (Egger et al., 1997), which modeled the relationship between effect sizes and precision to see if the linear regression line's intercept was null. In the situation of asymmetry, the intercept had not crossed zero (Lin & Chu, 2018). Furthermore, further analysis of publication bias was performed with the Trim and Fill Method (Shi et al., 2019), and Begg's Test (Begg & Mazumdar, 1994). Forest plots were also generated to visualize individual study effects and the overall pooled effect sizes. The *p*-value threshold for statistical significance was set to 0.05.

Results

Descriptive results of eligible studies

Following the application of various selection filters to the literature search, the meta-analysis retained 11 studies. Out of them, 9 were carried out in Europe/Hungary (81.8%), while the remaining two studies were conducted in Africa/Nigeria and Asia/Iran. The majority of the studies (36.4%) were performed on the fish species *C. gariepinus*, and five other distinct species and hybrids (*Clarias gariepinus* × *Heterobranchus longifilis*) were included. Since artificial insemination in external fertilizing fish is still in its infancy, we cannot incorporate extensive data and enforce the inclusion of studies conducted between 2018 and 2024 (Table 1). Ten of the eleven studies contain the fertilization rate, five include the hatching rate, and only four include both fertilization and hatching rates (Fig. 2).

Additionally, the studies included in this systematic review and meta-analysis came from various sources of variability, such as three continents or countries, two different sperm application methods, multiple fish species including one hybrid, and surprisingly, more than 10 types of sperm treatments (Table 2).

Table 1. Description of studies included in this systematic review and meta-analysis

Parameters	Frequency	Proportion (%)
Continent/country		
Europe/Hungary	9	81.8
Africa/Nigeria	1	9.1
Asia/Iran	1	9.1
Species		
Clarias gariepinus	4	36.4
Danio rerio	2	18.2
Cyprinus carpio	1	9.1
Rhamdia quelen	1	9.1
Hybrid (*Clarias gariepinus* × *Heterobranchus longifilis*)	1	9.1
Sander lucioperca	1	9.1
Esox lucius	1	9.1
Year of publication		
2018	2	18.2
2019	1	9.1
2020	1	9.1
2021	2	18.2
2022	4	36.4
2024	1	9.1

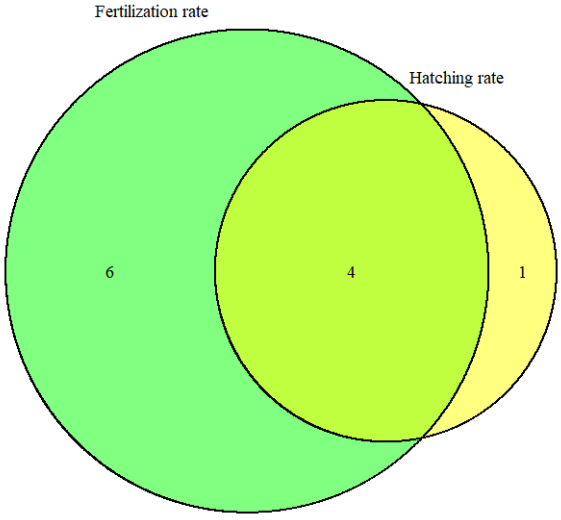

Fig. 2. The Venn diagram shows the distribution of studies reporting fertilization rates (green), hatching rates (yellow), and their overlap.

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Meta-analysis

In various studies, fertilization rates ranged from 17.6% to 93% and 21.7% to 91.13% for OLSAI and dry external fertilization, respectively. Meanwhile, for hatching rates, the OLSAI ranged from 17.6% to 93%, and for dry external fertilization, it was found between 21.7% and 91.13%. Table 3 shows the results of the Welch two-sample *t*-test, which looked at the fertilization and hatching rates for the two different methods, OLSAI and dry external fertilization. There is no significant difference in fertilization rates between the two procedures ($p = 0.11$), as indicated by the *t*-test result of –1.65 with a confidence interval of [–34.09, 3.81]. Similarly, in hatching rates, there was no significant difference ($p = 0.085$) between procedures with the *t*-test statistic of –1.88 [–45.29, 3.34]. These findings suggest that OLSAI achieves comparable reproductive parameters.

The degree of variation in fertilization and hatching rates across conditions can be interpreted as the effect size. The forest plot from the meta-analysis on fertilization rates shown in Fig. 3 includes studies like Okomoda et al. (2023), which show a bigger effect size with tighter confidence intervals, meaning their estimates are more accurate, while Gazsi et al. (2021b) show a smaller effect with wider confidence intervals, indicating more uncertainty. Correspondingly, Fig. 4 shows a forest plot for hatching rates, where the study by Nargesi & Gorouhi (2024) has a larger effect size and narrower confidence intervals, indicating their estimates are more precise.

Table 2. Summary statistics for research included in the final systematic review and meta-analysis

Authors	Country	Sperm type	Sperm app	Species	FR	SD	HR	SD
Gazsi et al. (2021b)	Hungary	Fresh sperm spawning III	OLSAI	*Danio rerio*	42.9	27.5	na	na
Gazsi et al. (2021b)	Hungary	Fresh sperm spawning IV	OLSAI	*Danio rerio*	41.5	23.7	na	na
Gazsi et al. (2021b)	Hungary	Fresh sperm spawning I	EF	*Danio rerio*	21.7	30.7	na	na
Gazsi et al. (2021b)	Hungary	Fresh sperm spawning II and III	EF	*Danio rerio*	43.3	29.4	na	na
Müller et al. (2019)	Hungary	Cryopreserved sperm	OLSAI	*Clarias gariepinus*	23.5	16.1	17.7	13.2
Müller et al. (2019)	Hungary	Cryopreserved + fresh sperm	OLSAI	*Clarias gariepinus*	17.6	13.7	12.5	9.3
Müller et al. (2019)	Hungary	Fresh sperm	EF	*Clarias gariepinus*	71.0	14.4	61.0	11.5
Müller et al. (2018a)	Hungary	Fresh sperm 2 hrs. before stripping	OLSAI	*Cyprinus carpio*	46.7	15.0	na	na
Müller et al. (2018a)	Hungary	Fresh sperm 12 hrs. before stripping	OLSAI	*Cyprinus carpio*	41.0	15.7	na	na
Müller et al. (2018a)	Hungary	Fresh sperm	EF	*Cyprinus carpio*	85.8	4.2	na	na
Ittzés et al. (2020)	Hungary	Fresh sperm	OLSAI	*Rhamdia quelen*	82.1	9.4	na	na
Ittzés et al. (2020)	Hungary	Fresh sperm hormone mix	OLSAI	*Rhamdia quelen*	76.5	4.4	na	na
Ittzés et al. (2020)	Hungary	Fresh sperm	EF	*Rhamdia quelen*	75.6	9.3	na	na
Müller et al. (2018b)	Hungary	Fresh sperm hormone mix	OLSAI	*Clarias gariepinus*	74.7	18.4	na	na
Müller et al. (2018b)	Hungary	Fresh sperm hormone mix + external fresh sperm	OLSAI	*Clarias gariepinus*	75.1	11.5	na	na
Müller et al. (2018b)	Hungary	Fresh sperm	OLSAI	*Clarias gariepinus*	69.7	5.2	na	na
Müller et al. (2018b)	Hungary	Fresh sperm to the left ovary and NaCl-dissolved hormone to the right ovary	OLSAI	*Clarias gariepinus*	87.7	8.7	na	na
Quyến et al. (2022)	Hungary	Fresh sperm	EF	*Clarias gariepinus*	81.4	1.62	77.2	0.9
Quyến et al. (2022)	Hungary	Fresh sperm	OLSAI	Hybrid (*Clarias gariepinus* × *Heterobranchus longifilis*)	74.9	6.6	71.1	11.3
Okomoda et al. (2023)	Nigeria	Fresh sperm	EF	*Clarias gariepinus*	91.1	4.33	82.3	2.2
Okomoda et al. (2023)	Nigeria	Fresh sperm	OLSAI	*Clarias gariepinus*	93	3.57	81.8	4.2
Gazsi et al. (2021a)	Hungary	Fresh sperm	EF	*Danio rerio*	81.3	10.4	na	na
Gazsi et al. (2021a)	Hungary	Fresh sperm from transgenic males but without males	OLSAI	*Danio rerio*	12.6	9.2	na	na
Gazsi et al. (2021a)	Hungary	Fresh sperm from transgenic males and males separated by a transparent plastic screen	OLSAI	*Danio rerio*	11.8	16.3	na	na

Table 2. Continued

Authors	Country	Sperm type	Sperm app	Species	FR	SD	HR	SD
Varga et al. (2022)	Hungary	Fresh sperm	OLSAI	*Sander lucioperca*	16.1	26.9	na	na
Nargesi & Gorouhi (2024)	Iran	Fresh sperm	EF	*Esox lucius*	62.4	1.9	88.5	1.7
Nargesi & Gorouhi (2024)	Iran	Fresh sperm and hormone insemination	EF	*Esox lucius*	65.6	3.6	91.2	1.7
Nargesi & Gorouhi (2024)	Iran	Fresh sperm after 72 hrs. of hormone injection	OLSAI	*Esox lucius*	60.5	2.4	86.1	3.4
Nargesi & Gorouhi (2024)	Iran	Fresh sperm after 72 hrs. of hormone insemination	OLSAI	*Esox lucius*	54.8	2.5	86.8	2.9
Kucska et al. (2022)	Hungary	Fresh sperm	EF	*Clarias gariepinus*	na	na	55.1	16.1
Kucska et al. (2022)	Hungary	Fresh sperm hormone mix	OLSAI	*Clarias gariepinus*	na	na	39.1	18.3
Kucska et al. (2022)	Hungary	Fresh sperm	EF	*Clarias gariepinus*	na	na	61.9	12.0
Kucska et al. (2022)	Hungary	Fresh sperm	OLSAI	*Clarias gariepinus*	na	na	46.0	9.2
Kucska et al. (2022)	Hungary	Fresh sperm hormone mix	OLSAI	*Clarias gariepinus*	na	na	35.1	21.2

FR, mean fertilization rate; HR, mean hatching rate; app, application; OLSAI, ovarian lavage sperm artificial insemination; Spawning I, II, III, and IV, eggs spawning from 1 to 4, respectively; EF, dry external fertilization.

Table 3. The Welch two-sample *t*-test results for the parameters fertilization and hatching rate

Parameters	Fertilization rate	Hatching rate
Ovarian lavage (mean in %)	52.78	52.91
Dry external fertilization (mean in %)	67.92	73.89
t-test value	–1.65	–1.88
95% confidence interval	–34.09, 3.81	–45.29, 3.34
Degrees of freedom	22.68	12.22
p-value	0.11	0.085

Publication bias and small study effect assessment

Table 4 represents funnel asymmetry tests, or Egger's test for publication bias, to assess the presence of bias in the published research on both fertilization and hatching rates. For the fertilization rate, a Z-score of –3.35 ($p = 0.0008$) indicates significant asymmetry, strongly suggesting the presence of publication bias. The intercept (b) value was 80.28 with a confidence interval [67.83, 92.73], significantly deviating from zero, affirming further evidence for the presence of publication bias.

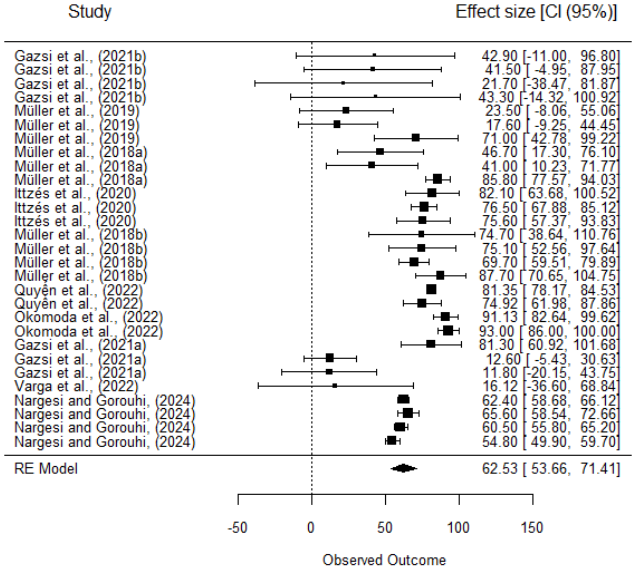

Fig. 3. Forest plot on fertilization rates. CI, confidence interval; RE Model, random effects model.

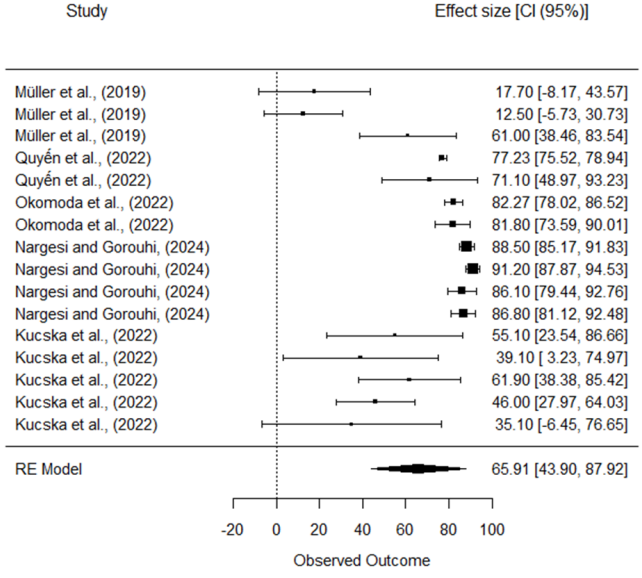

Fig. 4. Forest plot on hatching rates. CI, confidence interval; RE Model, random effects model.

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Table 4. Funnel asymmetry tests or Egger's test for publication bias

Parameters	Fertilization rate	Hatching rate
Z-score	–3.35	–4.4
p-values	0.0008	0.0001
Intercept (b)	80.28	89.47
95% confidence interval	67.83, 92.73	77.1, 101.83
Publication bias	Present	Present

Similarly, in the hatching rate, a Z-score of –4.4 (0.0001) shows a significant asymmetry indicating the possibility of publication bias. The intercept (b) was 89.47 with a confidence interval of [77.1, 101.83], significantly different from zero, providing further evidence of publication bias. Figs 5 and 6 show asymmetries in the funnel plots for fertilization and hatching rates. Remarkably, some studies with extreme impact sizes depart from the funnel plot's anticipated range. This asymmetry may be due to methodological variations in different studies, mainly in continent, species, and the type of sperm used.

Table 5 demonstrates the analysis results of the trim and fill output, which was used to correct for publication bias in meta-analyses. The estimated total heterogeneity (τ^2) values, which show real differences not caused by random sampling error, were 910.57 for fertilization rates and 1313.98 for hatching rates, indicating significant differences in both rates. Furthermore, the percentage of total variability due to true differences between

Table 5. Trim and fill analysis for publication bias

Parameters	Fertilization rate	Hatching rate
Random-effects model (τ^2- estimator: REML)	K = 29	K = 16
- estimated amount of total heterogeneity (SE)	910.57 (257.96)	1,313.98 (445.39)
τ (square root of estimated τ^2 value)	30.18	36.25
I^2 (total heterogeneity/total variability) (%)	97.05	99.12
H^2 (total variability/sampling variability)	33.93	113.12
Q test for heterogeneity (*p*-value)	407.97 (< 0.0001)	310.6 (< 0.0001)
Meta-regression		
Estimate (SE)	79.22 (6.01)	81.23 (8.1)
z-value	13.19	10.01
95% confidence interval	67.45, 90.99	65.33, 97.13
p-value	< 0.0001	< 0.0001

REML, restricted maximum likelihood; K, number of observations.

studies was 97.05% for fertilization rates and 99.12% for hatching rates, as indicated by the total heterogeneity/total variability values (I^2). Furthermore, the differences in results were found to be significant ($p < 0.0001$) for both fertilization and hatching rates, with Q test values of 407.97 and 310.6, respectively.

The meta-regression analysis gave estimates of 79.22 for the fertilization rate and 81.23 for the hatching rate, with *z*-values of 13.19 and 10.01 and confidence ranges of [67.45, 90.99] for fertilization and [65.33, 97.13] for hatching. Additionally, for both fer-

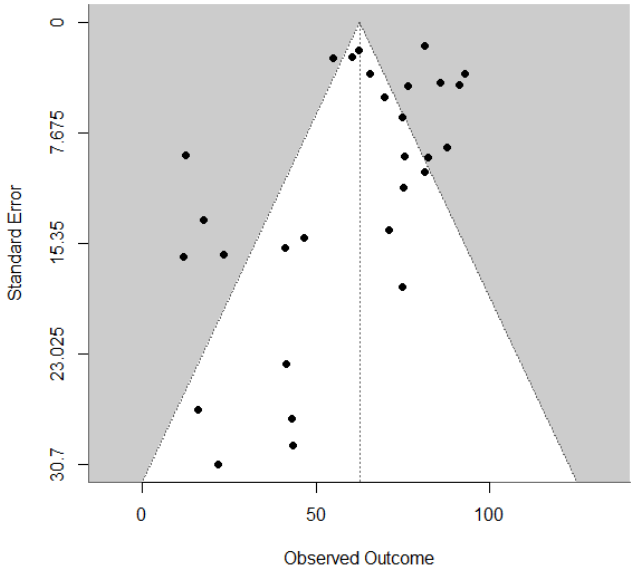

Fig. 5. Funnel plot that assesses publication bias for fertilization rate.

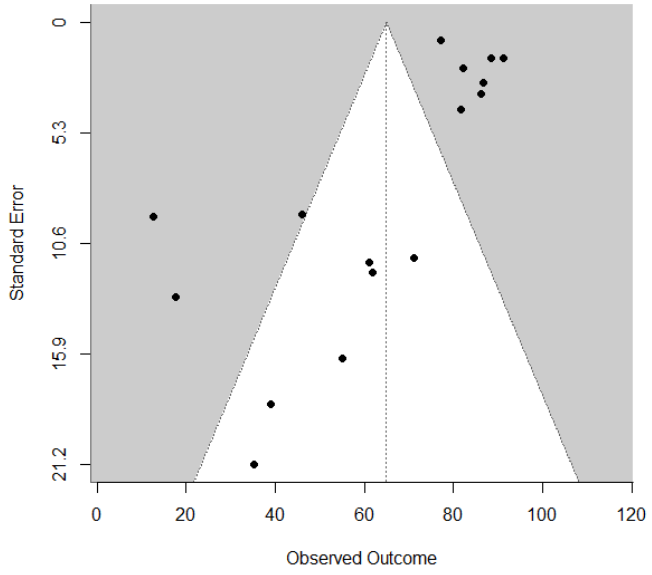

Fig. 6. Funnel plot that assesses publication bias for hatching rate.

tilization and hatching rates, a significant link was found between multiple parameters and observed heterogeneity, with *p*-values < 0.0001. These findings showed that many different factors affecting fertilization and hatching rates were quite complicated, highlighting the need to carefully look at and understand the data because of the differences observed and possible publication bias.

Moreover, Begg's test for publication bias was carried out using the rank correlation test to determine the presence of publication bias. For fertilization and hatching rates, Kendall's Tau values were obtained: –0.21 (p = 0.011) and –0.41 (p = 0.027), respectively. These significant *p*-values for both fertilization and hatching rates are further revealed by the asymmetry in the funnel plots in Figs 5 and 6, respectively, which supports the existence of publication bias.

Subgroup meta-analysis: sources of heterogeneity

Table 6 summarizes a multivariable meta-regression model result examining the impact of different parameters on fertilization and hatching rate. Those parameters considered include continent/country of study, sperm application method, fish species, and sperm treatment types used. A study performed in Africa (Nigeria) showed a significantly higher fertilization rate as compared to those conducted in Europe (Hungary) and Asia (Iran). Research investigations conducted in Africa (Nigeria) and Asia (Iran) had significantly greater hatching rates than those conducted in Europe (Hungary). Furthermore, different species had different effects on the model compared to the reference species, *C. gariepinus*. Hence, *D. rerio* produced a significantly lower fertilization rate. *Esox lucius*, however, had a noticeably higher hatching rate.

In the case of the sperm type used, when cryopreserved sperm was combined with fresh sperm for dry external fertilization, when using sperm from transgenic males, or when insemination occurred after 72 hours of hormone treatment, fertilization rates were found to be significantly lower compared to other types. Similarly, hatching rates decreased significantly under different sperm treatments, including cryopreserved sperm, cryopreserved sperm combined with fresh sperm for dry external fertilization, and fresh sperm with the hormone mix. Furthermore, in the fertilization rate, the I^2 value for sperm treatment types was smaller than others, which suggests less residual variation among groups.

The amount of heterogeneity accounted for R^2 values expressed in % presented in the data sheds light on the extent to which the observed variability was explained by distinct factors. To begin with, when continent or country impacts were considered a factor, the R^2 values for fertilization rate and hatching rate were both 9.92% and 57.55%, respectively. In addition, the R^2 values for fertilization and hatching rates were 10.22% and 9.15%, respectively, when sperm application methods were taken into account.

Furthermore, the species effect showed modest explanatory power, with R^2 values ranging from 20.3% to 30.75% for fertilization and hatching rates, respectively. This indicates that the differences in species support the explanation of inconsistencies in these results. Finally, the sperm treatment types with R^2 values of 78.32% and 76.18% for both fertilization and hatching, respectively, indicated the strongest explanatory power. This result suggests that the type of sperm applied was responsible for a substantial amount of the variation found in fertilization and hatching rates.

Discussion

To our knowledge, this study is the first systematic review and meta-analysis to investigate the efficacy of OLSAI on reproductive results in external fertilization of fish. The scope of this review is limited to species that use dry external fertilization, noting the differences in parameter measurements between external and internal fertilization fish species. The study period runs from 2018 to 2024, reflects the young nature of research in this subject, and aims to include all relevant papers. As a result, the final meta-analysis includes just eleven studies that cover various treatment modalities, containing 29 observations for fertilization rate and 16 for hatching rate. Fertilization rates in included studies range from 11.8% to 93%, while hatching rates range from 12.5% to 91.2%; this variability is mostly due to the superiority of fresh sperm over manipulated types.

The fertilization rates with OLSAI versus *in vitro* dry external fertilization were 52.78% and 67.92%, respectively. Furthermore, ovarian lavage resulted in a hatching rate of 52.91%, whereas dry external fertilization produced a hatching rate of 73.89%. The lower average rates seen for OLSAI compared to dry external fertilization might be because there was less success in fertilization and hatching in the initial data, especially in studies that used cryopreserved sperm (Müller et al., 2019) and sperm from genetically modified males (Gazsi et al., 2021a). Additionally, only five studies reported hatching rates following OLSAI, including Kucska et al. (2022), Müller et al. (2019), Nargesi & Gorouhi (2024), Okomoda et al. (2023), and Quyến et al. (2022).

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Table 6. Sources of heterogeneity/multivariable meta-regression model estimates (SE) of the effects of study characteristics

Effects	Fertilization rate	Hatching rate
Continent/country		
Europe/Hungary (*Reference*)	0	0
Africa/Nigeria (SE)	32.35 (15.4)*	32.18 (12.68)*
Asia/Iran (SE)	1.08 (11.37)	38.31 (9.9)***
I^2 (%)	93.77	93.63
R^2 (amount of heterogeneity accounted for) (%)	9.92	57.55
Sperm application		
Dry external fertilization (*Reference*)	0	0
Ovarian lavage (SE)	–16.45 (9.03)	–19.54 (12.23)
I^2 (%)	94.23	98.24
R^2 (amount of heterogeneity accounted for) (%)	10.22	9.15
Species		
Clarias gariepinus (*Reference*)		0
Danio rerio (SE)	–34.38 (12.28)**	-
Cyprinus carpio (SE)	–9.3 (14.58)	-
Rhamdia quelen (SE)	6.27 (13.67)	-
Hybrid (*Clarias gariepinus* × *Heterobranchus longifilis*) (SE)	3.22 (21.2)	16.22 (23.58)
Sander lucioperca (SE)	–55.58 (33.6)	-
Esox lucius (SE)	–10.9 (11.74)	33.29 (11.97)**
I^2 (%)	93.5	97.15
R^2 (amount of heterogeneity accounted for) (%)	20.3	30.75
Sperm treatment types		
Fresh sperm (*Reference*)	0	0
Cryopreserved sperm (SE)	–53.4 (19.23)***	–55.52 (18.1)**
Cryopreserved sperm + fresh sperm for dry external fertilization (SE)	–59.3 (17.27)***	–60.72 (15.46)***
Fresh sperm 2 hrs. before stripping (SE)	–30.2 (18.32)	-
Fresh sperm 12 hrs. before stripping (SE)	–35.9 (18.9)	-
Fresh sperm hormone mix (SE)	–3.50 (8.12)	–35.90 (16.73)*
Fresh sperm hormone mixes + fresh sperm for dry external fertilization (SE)	–1.8 (15.6)	-
Fresh sperm to the left ovary and NaCl-dissolved hormone to the right ovary (SE)	10.8 (13.65)	-
Fresh sperm from transgenic males but without males in aquaria (SE)	–64.3 (13.97)	-
Fresh sperm from transgenic males and males separated by a transparent plastic screen in aquaria (SE)	–65.1 (19.4)***	-
Fresh sperm insemination after 72 hours of hormone injection intramuscularly (SE)	–16.4 (10.8)***	12.88 (12.8)
Fresh sperm insemination after 72 hours of hormone insemination into ovaries (SE)	–22.1 (10.8)*	13.59 (12.7)
Fresh sperm for dry external fertilization and hormone insemination (SE)	–11.3 (11.12)	17.98 (12.47)
I^2 (%)	81.12	93.64
R^2 (amount of heterogeneity accounted for) (%)	78.32	76.18
Overall	62.53	65.91

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.
SE, standard error; I^2, residual heterogeneity/unaccounted variability.

This study found that using cryopreserved sperm reduced fertilization and hatching rates. Cryopreservation can harm sperm, causing DNA damage or differences in environmental variables such as osmolality during activation in the fertilization medium. These factors contribute to lower fertilization rates and more cases of larval deformity (Müller et al., 2018c). Ac-

cording to this review, Müller et al. (2018a) provided the earliest research findings in *C. carpio* Linnaeus (1758) from Hungary, followed by the same research group in *C. gariepinus* Müller et al. (2018b). Following these preliminary experiments, the same research team from Hungary conducted the majority of subsequent investigations. The only exception to this pattern is a single article from Nigeria (Okomoda et al., 2023) and a sole article from Iran (Nargesi & Gorouhi, 2024), representing the most recent addition to the literature. This study found that the overall average fertilization and hatching rates were 62.53% and 65.91%, respectively.

Statistical tests confirmed that there was no significant difference between OLSAI and dry external fertilization, despite numerical inconsistencies found. This finding indicates that those two artificial fish propagation methods are comparably effective in terms of fertilization and subsequent hatching success. According to Nargesi & Gorouhi (2024), it can serve as a viable alternative to external fertilization. Additionally, Ittzés et al. (2020) report that ovarian insemination can serve as an alternative hormone introduction method.

Furthermore, as it is relevant to both live-bearing and external fertilization fish species, OLSAI procedures play an important role in fish propagation. This approach serves a dual purpose by facilitating both sperm insemination and hormone delivery. In different types of fish, scientists and fish farmers might use ovarian lavage for sperm insemination and hormone delivery to better control reproduction, which can help improve breeding success rates (Alebachew et al., 2022). Table 7 demonstrates the qualitative comparative analysis of OLSAI and dry external fertilization techniques in fish reproduction.

The results section explains that there was a significant increase in variability between studies. This inconsistency could be due to differences in parameters such as sperm treatment types, sperm application procedures, or the diversity of fish species tested. Based on the meta-regression outcomes, substantial effect size estimates were found in the types of sperm treatments used on the fertilization rate, especially in cryopreserved sperm insemination, cryopreserved sperm insemination mixed with fresh sperm for dry external fertilization, sperm from transgenic males and males separated by a transparent plastic screen in aquaria, fresh sperm insemination after 72 hours of hormone injection, and sperm insemination after 72 hours of hormone insemination into ovaries. Similarly, the hatching rate was significantly affected by the use of cryopreserved sperm, cryopreserved sperm mixed with fresh sperm for dry external fertilization, and fresh sperm hormone mix.

Table 7. Comparative analysis of ovarian lavage sperm artificial insemination (OLSAI) and dry external fertilization techniques for fish reproduction

	Dry external fertilization	OLSAI
Description	A method where fertilization occurs outside the female's body without the need for sperm injection. The process involves stripping sperm and eggs into separate dry containers. The milt and eggs then mix comprehensively using dry feathers before adding culture water (Abdissa et al., 2020; Mylonas et al., 2010). Used only in externally fertilizing fish species for reproduction and hybridization.	It involves collecting sperm and artificially inseminating it into the ovarian cavity of the female reproductive tract using a catheter tube, which is inserted 5–15 cm deep into the oviduct, depending on the fish size (Ittzés et al., 2020; Müller et al., 2018a, 2018b, 2019, 2020b; Quyến et al., 2022). It is used in both external fertilization fish species (Gazsi et al., 2021a; Müller et al., 2018a, 2018b) and live-bearing fish species (Gasparini et al., 2018) for reproduction and hybridization.
Merits	A simple and cost-effective method for fertilizing fish eggs outside the female's body. It allows hybridization and simplifies the application of manipulated sperm (cryopreserved or genetically modified) and eggs for further applications. Historical usage: This established method has a long history of use in pisciculture and experimental embryology for various external fertilization fish species.	It enables precise control over the fertilization process, resulting in higher success rates in fish reproduction. It allows for spontaneous spawning, which eliminates the need for stressful stripping. It also enables hybridization (Quyến et al., 2022). The delivery of the sperm is less time-dependent than in conventional in vitro fertilization (Müller et al., 2018a). The advancement of the process suggests a reduction in stress indicators (Okomoda et al., 2023). It also allows administration of hormones without the need for needle injections, which have the potential to induce harm and stress (Watson et al., 2009).
Demerits	Less control over the fertilization process compared to artificial insemination, potentially leading to lower reproductive efficiency. It is challenging to forecast the time of ovulation following hormone injection (Müller et al., 2018a).	The process requires additional equipment, such as a catheter tube, and expertise for sperm collection and insemination (Müller et al., 2018a), making it more intricate. A relatively new technique needs to be explored for various species.

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The publication bias may influence the meta-analysis's overall findings. The statistical test and the funnel plots showed visible asymmetry. The evidence suggests that smaller studies with unimportant results are less likely to be published, meaning that studies showing better rates of fertilization and hatching are more often shared, leading to biased overall results. Furthermore, the relatively small number of studies in the meta-analysis may exacerbate the effect of publication bias, as the limited sample size may not capture the full spectrum of the research output. Fortunately, a favorable number of observations were included. Lastly, a substantial variation in the included treatment types across the studies may increase the heterogeneity of this study.

Conclusion

This systematic review and meta-analysis revealed that there was no significant difference between dry external fertilization and OLSAI in effectiveness in terms of fertilization and hatching rates. This evidence indicates that both methods can be used interchangeably or are equally viable for promoting successful fertilization, hatching, and embryonic development, although OLSAI offers specific benefits over gamete stripping followed by dry external fertilization. These benefits include making it easier to figure out when to induce ovulation after hormone treatment, simplifying the creation of hybrid seeds, and reducing the risk of gamete contamination from urine and water. Additionally, it permits practicing spontaneous spawning conditions and reduces the stress levels in the treated species when compared to ovarian stripping. Combining these features, OLSAI makes a better option than the dry external fertilization procedures that require gamete stripping. However, in some cases, the application of a dry external fertilization method may be necessary, such as manipulation of fish eggs before fertilization. To optimize its benefits and advance scientific knowledge in the field, the study of OLSAI in various fish species is vital. Therefore, research across different species discloses insights into the procedure, effectiveness, and further applications. Such knowledge improves our compassion and gives us the room to improve and refine the techniques.

Competing interests

No potential conflict of interest relevant to this article was reported.

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Availability of data and materials

Upon reasonable request, the datasets used in this study can be made available from the corresponding author.

Ethics approval and consent to participate

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