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REVIEW ARTICLE

Effects of Electroejaculation on Semen Quality in Domestic Ruminants: A Systematic Review, Meta-Analysis and Meta-Regression

Rehardus Ricco Pantecostoma[1], Saroch Ngarmkum[1], Thanis Damrongwatanapokin[1], Watcharapong Mitsuwan[1], Dian Meididewi Nuraini[2], Morsid Andityas[3] and Chaithep Poolkhet[1*]

[1]Akkhraratchakumari Veterinary College, Walailak University, Nakhon Si Thammarat, 80161, Thailand; [2]Department of Animal Science, Faculty of Animal Science, Universitas Sebelas Maret, Surakarta, 57126, Indonesia; [3]Veterinary Technology Study Program, Department of Bioresources Technology and Veterinary, Vocational College, Universitas Gadjah Mada, 55281, Indonesia.
*Corresponding author: chaithep.po@wu.ac.th

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ABSTRACT

Electroejaculation (EE) is an alternative technique for semen collection from untrained males or those with sexual behavior issues. However, its effects on semen quality in domestic ruminants remain inconsistent across studies. This study aimed to quantitatively evaluate the effects of EE on selected fresh and post-thaw semen quality parameters in the bull, male goat and ram, using a systematic review, meta-analysis and meta-regression. The study followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. A systematic literature search was conducted in PubMed, Scopus, EBSCOhost, ProQuest, ScienceDirect, and Google Scholar. Based on predefined inclusion and exclusion criteria, 18 studies during the period from 1990 to 2025 were included. Seven outcomes were evaluated including: semen volume, sperm concentration, total sperm motility, sperm morphology, and viability in fresh semen, as well as post-thaw total sperm motility and DNA integrity. Standardized mean differences (SMD) were calculated using random-effects models. Heterogeneity was assessed using Cochran's Q test and the I^2 statistics. Publication bias was evaluated using funnel plots, Egger's test and trim-and-fill analysis, while robustness was assessed through leave-one-out sensitivity analysis. Results showed that EE was positively associated with increased semen volume, while total sperm motility, sperm morphology, and post-thaw DNA integrity were not significantly affected. On the other hand, EE was significantly associated with decreased sperm concentration, sperm viability and post-thaw total motility. Meta-regression analysis showed that the overall effect size of EE compared with the artificial vagina (AV) method showed non-significant temporal change over the past three and a half decades. These findings indicate that EE remains a useful method for semen collection under specific circumstances.

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INTRODUCTION

Semen collection and evaluation is the basic procedure for breeding soundness examination (BSE) in domestic ruminants. The quality of collected semen is assessed, along with physical condition of the male to determine its reproductive ability (Harighi *et al.*, 2022; Diaz-Miranda *et al.*, 2024). Sperm motility and morphology are the most commonly considered parameters of the bull semen quality (Koziol and Armstrong, 2018; King and Hopper, 2024). While BSE guidelines for rams and male goats vary, sperm morphology, motility, and concentration are most commonly evaluated (Tibary *et al.*, 2018; Harighi *et al.*, 2022). Besides BSE, semen collection is also the initial step for assisted reproductive technologies (ARTs), followed by semen preservation in either liquid form or through cryopreservation for artificial insemination (Bashar *et al.*, 2025; Mulyati *et al.*, 2025). The semen collection method is often overlooked, despite its potential influence on the interpretation of BSE in breeder selection or the success rate of ARTs.

In domestic ruminants, semen is collected through artificial vagina (AV) or electroejaculation (EE) procedures. The AV method mimics natural service, allowing the male to ejaculate voluntarily. Typically, AV requires prior training of up to 3 weeks in rams (Wulster-Radcliffe *et al.*, 2001). Prior adaptation and training for 3 months successfully enabled semen collection using AV from all 3 Creole bulls (Argudo *et al.*, 2019), whereas 4 weeks of training in 20 South African indigenous male goats resulted in successful semen collection from only 8 males using AV (Bopape *et al.*, 2015). Although prior training is conducted, this does not guarantee that the male will be able to donate semen (Malejane *et al.*, 2014; Bopape *et al.*, 2015). This technique may be challenging when assessing large populations, such as male beef cattle for BSE. In contrast, EE offers several advantages, including the ability to collect semen from untrained males or those with sexual behavior issues and to obtain semen from a large number of animals rapidly and efficiently (Wulster-Radcliffe *et al.*, 2001; Yotov *et al.*, 2020). However, EE is considered as an invasive procedure that may induce stress to the male (Khonmee *et al.*, 2023; Kaka *et al.*, 2025), which may affect quality of the collected semen. Although there are limited studies confirming this hypothesis by comparing EE and AV to measure both stress levels and sperm quality under controlled experimental conditions, most of the evidence instead focuses on sperm quality parameters (Argudo *et al.*, 2019; Yotov *et al.*, 2020; Lv *et al.*, 2025). However, no consensus has been reached across studies in rams (Ledesma *et al.*, 2014; Jiménez-Rabadán *et al.*, 2016), male goats (Bopape *et al.*, 2015; Lv *et al.*, 2025), and bulls (León *et al.*, 1991; Argudo *et al.*, 2019). Understanding the potential influence of semen collection methods on sperm quality is necessary for improving breeding soundness examination and optimizing the success of reproductive technologies in livestock.

To our knowledge, no quantitative study has comprehensively evaluated the overall effect of EE on semen quality in domestic ruminants. This inconsistency may lead to varying perspectives on the application of EE due to concerns regarding semen quality, beyond animal welfare issues. Hence, the main purpose of this study was to conduct a meta-analysis to assess the effects of EE technique on ejaculatory volume, sperm concentration, total motility, morphology, and viability of sperm in fresh semen, as well as total motility in frozen–thawed semen in bulls, male goats, and rams. In addition, the effect sizes of AV and EE across publication years were evaluated using meta-regression. The AV method was selected as the comparator because it is widely used and accepted as a non-invasive, and yields natural ejaculation.

MATERIALS AND METHODS

The study protocol was registered on the Open Science Framework (OSF; https://osf.io/xv8ej). The protocol and reporting of this study followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Page *et al.*, 2021). The search for relevant articles was conducted during the period from December 27, 2025 to January 24, 2026.

Search strategy: The literature search was conducted in EBSCOhost, Google Scholar, ProQuest, PubMed, ScienceDirect, and Scopus. The following terms were combined using the Boolean operators "AND" and "OR": "artificial vagina", "digital manipulation", "hand glove", "electroejaculation", "electro ejaculation", "electroejaculator", and "electro ejaculator". Additional studies were identified by screening the references lists in the related studies (Page *et al.*, 2021).

Eligibility criteria: The inclusion criteria were based on: (1) population–bulls, male goats, and rams; (2) intervention–semen collection using electroejaculation; (3) comparison–between electroejaculation and the artificial vagina method; and (4) outcomes–at least one of the following parameters (ejaculatory volume, sperm concentration, total motility, viability, morphology, post-thaw total motility, and DNA fragmentation or integrity of sperm). Studies published in languages other than English were excluded. There was no restriction on journal indexing to avoid overestimation of the true effects (McAuley *et al.*, 2000). However, individual study appraisal was performed to assess study eligibility. Only studies published in or after 1990 were considered eligible for inclusion. Duplicate removal and title–abstract screening were conducted using the Rayyan Intelligent Systematic Review platform (www.rayyan.ai) independently. In cases of disagreement between the first and second reviewers, the issue was resolved through discussion until a consensus was reached or consultation with a third reviewer as an independent adjudicator, if needed.

Data extraction: Data from the included studies were extracted into a Microsoft Excel worksheet and subsequently reviewed. The following data were recorded: author(s), year of publication, number of animals, sample sizes of both AV and EE groups, sperm concentration, semen volume, total motility, viability, normal morphology, frozen–thawed total motility, and DNA integrity, country, electro-ejaculator probe type and stimulation protocols. The mean and SD of all outcomes were recorded. Results presented in graphical form were extracted using Plot Digitizer (https://plotdigitizer.com/). Transformation and missing outcome values were carefully handled following the Cochrane Handbook for Systematic Reviews of Interventions (Higgins *et al.*, 2019). Further details of the data extraction strategies are provided in the registered protocol (https://osf.io/xv8ej).

Quality appraisal of studies: The Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE's) tool was used to assess individual studies across the domains of selection bias, performance bias, detection bias, attrition bias, reporting bias, and other bias, comprising 10 indicators (Hooijmans *et al.*, 2014). Studies with four or more high-risk judgments were excluded from the meta-analysis. To enhance data visualization, the results were presented as summary graphs using R (version 4.5.2; Comprehensive R Archive Network, Vienna, Austria), RStudio (version 2026.1.1.403), and the ggplot2 package (version 4.0.2; Wickham, 2016; Posit team, 2026).

Publication bias and sensitivity analysis: Sensitivity analysis was performed using the leave-one-out method to assess the influence of individual studies on the overall effect (Meng *et al.*, 2024). If a study was found to exert an extreme influence on the overall analysis, exclusion was considered following comprehensive evaluation. Egger's test and a funnel plot were performed to evaluate publication bias. A P-value less than 0.1 ($P<0.1$) indicated a significant presence of bias (Egger *et al.*, 1997). Considering the small sample size of our meta-analysis (n=18 studies), a significance threshold of $P<0.1$ was used to increase the sensitivity of the test in detecting potential publication bias. This approach is also consistent with the original publication of Egger's test (Egger *et al.,* 1997). The trim-and-fill method was applied to address the potential presence of missing studies and to estimate the adjusted standardized mean differences (Duval and Tweedie, 2000).

Statistical analysis: The effect size was calculated using a random-effects model with Hedges standardized mean difference (SMD) and a 95% confidence interval. Cochran's Q test P-value and I^2 were used to assess heterogeneity (Borenstein *et al.*, 2009). I^2 represents the percentage of variability in effect estimates that is attributable to heterogeneity. According to Higgins *et al.* (2003), heterogeneity was classified as high (75–100%), moderate (50–75%), low (25–50%), and no heterogeneity (0–25%). Significant heterogeneity was indicated when the P-value was less than 0.05 ($P<0.05$). Subgroup analysis was applied to explore the effects of EE on each related ruminant species (male goat, bull and ram). The trend over time was further assessed through meta-regression. However, only outcome domains with at least 10 studies were analyzed. Leave-one-out analysis, Egger's test, funnel plot, trim-and-fill method, meta-analysis, meta-regression, and subgroup analysis were conducted in RStudio using the "meta" packages (version 8.2.1; Schwarzer, 2015; Harrer *et al.*, 2021).

RESULTS

Characteristics of the included studies: The online database search resulted in 375 records, with an additional 15 records identified through reference list screening. The database search yielding 375 records was performed on December 27, 2025, whereas the snowballing search identifying 15 records was conducted on January 20-24, 2026. After duplicate removal and screening of titles, abstracts, and full texts, 18 studies consisting of 19 experiments (with one study reporting data for both male goats and rams) published between 1990 and 2025 were eligible for inclusion in the systematic review and meta-analysis (Fig. 1). Two full-text articles were excluded because their study design did not meet the inclusion criteria, specifically due to exhausted semen collection procedures and mixing of seminal plasma from EE and AV. One additional article was excluded due to duplication, as it involved the same experimental animals and reported similar authorship, sample size, primary outcomes, and study period across multiple publications. Nine studies published before 1990 were excluded. These twelve studies were not included in 18 studies described above. The included studies involved male goats (n=7), bulls (n=4), and rams (n=8), with one study reporting data for both male goats and rams. A total of 285 biological units were evaluated across semen volume, sperm concentration, total motility, viability, normal morphology, post-thaw total motility, and DNA integrity outcomes (Table 1).

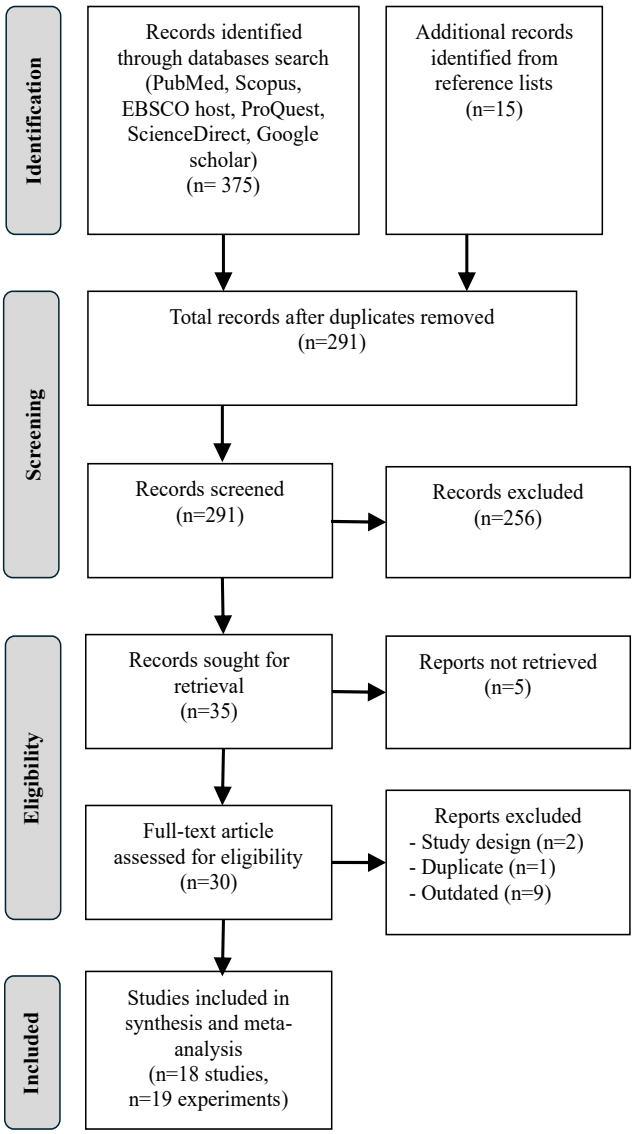

Fig. 1: The PRISMA flow diagram.

Quality appraisal: All of 18 studies were considered eligible for inclusion in the meta-analysis based on the SYRCLE risk-of-bias assessment. No study was judged to have a high risk of bias across in more than four domains (Fig. 2). For the overall assessment, all studies provided sufficient baseline characteristics for all groups, explanations for incomplete outcome data, and showed no indication of selective outcome reporting or other bias. Blinding of outcome assessors and blinding of caregivers and/or investigators were not clearly stated in all studies. Only five studies (27.78%) reported random sequence generation, three studies (16.67%) employed allocation concealment, and two studies (11.11%) applied random selection for outcome assessment (Fig. 3).

Table 1: Detail about included studies investigating the effects of EE on semen quality parameters

Animal	N	Age (Years)	Parameters	Probe	Protocol	Country	Study
Male goat	10	2-3	Volume, concentration, total motility	Not reported	Voltage: increasing, max 5Volt; Stimulation: 5s; Rest: 5s; Series: 4×	China	Lv *et al.* (2025)
Male goat	8	1-2	Morphology, post-thaw total motility, post-thaw DNA integrity	Diameter: 2cm; length 28cm	Voltage: increasing, max 5Volt; Stimulation: 4-6s; Rest: 4-5s; Series: 4×	South Africa	Lukusa and Kabuba (2021)
Male goat	20	1-2	Volume, concentration, total motility, viability, morphology, post-thaw total motility, post-thaw DNA integrity	Diameter: 2cm; length 28cm	Voltage: increasing, max 5Volt; Stimulation: 4-6s; Rest: 4-5s; Series: 4×	South Africa	Lukusa and Kabuba (2020)
Male goat	7	>1.5	Volume, concentration, total motility, post-thaw total motility, post-thaw DNA integrity	A 3-electrodes probe. Diameter: 3.2cm; length 35cm	Voltage: increasing, max 5Volt; Stimulation: 5s; Rest: 5s; Series: 4×	Spain	Jiménez-Rabadán *et al.* (2016)a
Male goat	8	1-2	Volume, concentration, total motility, viability	Not reported	3-5Volt	South Africa	Bopape *et al.* (2015)
Male goat	6	>1.5	Volume, concentration, total motility, viability, post-thaw total motility, post-thaw DNA integrity	A 3-electrodes probe. Diameter: 3.2cm; length 35cm	Voltage: increasing, max 5Volt; Stimulation: 5s; Rest: 5s; Series: 4×	Spain	Jiménez-Rabadán *et al.* (2013)
Male goat	7	>1.5	Volume, concentration, total motility, post-thaw total motility, post-thaw DNA integrity	A 3-electrodes probe. Diameter: 3.2cm; length 35cm	Voltage: increasing, max 5Volt; Stimulation: 5s; Rest: 5s; Series: 4×	Spain	Jiménez-Rabadán *et al.* (2012)
Bull	3	2-3	Volume, concentration, viability, morphology, post-thaw total motility,	Not reported	Not reported	Ecuador	Argudo *et al.* (2019)
Bull	3	2-3	Volume, concentration, total motility, morphology	Not reported	Voltage: increasing; Stimulation: 2s; Rest: 2s	Australia	Rêgo *et al.* (2015)
Bull	96	2-7	Volume, viability	Not reported	Not reported	USA	Barth *et al.* (2004)
Bull	40	3-7	Volume, concentration, total motility, post-thaw total motility	Not reported	Not reported	Mexico	León *et al.* (1991)
Ram	18	1-2	Volume, concentration	Not reported	Not reported	Bulgaria	Yotov *et al.* (2020)
Ram	5	>1.5	Volume, concentration, total motility, post-thaw total motility, post-thaw DNA integrity	A 3-electrodes probe. Diameter: 3.2cm; length 35cm	Voltage: increasing, max 5Volt; Stimulation: 5s; Rest: 5s; Series: 4×	Spain	Jiménez-Rabadán *et al.* (2016)b
Ram	11	1-2	Volume, concentration, total motility, viability, morphology	Not reported	Stimulation: 3-5s	South Africa	Malejane *et al.* (2014)
Ram	5	5.0	Volume, total motility	A 3-electrodes probe. Diameter: 2.5cm; length 22cm	Voltage: increasing by 0.5Volt; Stimulation: 2s; Rest: 2s	Argentina	Ledesma *et al.* (2014)
Ram	8	5-7	Total motility, post-thaw total motility,	A 3-electrodes probe. Diameter: 3cm; length 25cm	Voltage: 4Volt (average)	Spain	Álvarez *et al.* (2012)
Ram	4	1-3	Volume, concentration,	Diameter: 2cm; length 17.5cm	Voltage: 6Volt; Stimulation: 4s; Rest: 4s; Series: 4×	Chile	Robayo *et al.* (2008)
Ram	8	Mature	Volume, concentration, total motility, post-thaw total motility	A 3-electrodes probe. Diameter: 2.5cm; length: 22cm	Voltage: increasing by 1Volt; Stimulation: 2s; Rest: 2s	Spain	Marco-Jiménez *et al.* (2005)
Ram	18	1-2	Volume, concentration, total motility, viability, morphology	Not reported	Not reported	South Africa	Matthews *et al.* (2003)

Letters (a, b) following the year in citations denote independent experiments reported from the same study when multiple species were included. Specifically, Jiménez-Rabadán *et al.* (2016)a and Jiménez-Rabadán *et al.* (2016)b represent data from male goats and rams, respectively.

Sensitivity and publication bias: The leave-one-out sensitivity analysis revealed that no individual experiment exerted an extreme influence on the overall pooled estimates. Funnel plot visualization demonstrated a symmetrical distribution for semen volume, sperm viability, normal morphology, and post-thaw total motility, while the sperm concentration, total sperm motility, and post-thaw DNA integrity appeared asymmetrical (Table 2). Further assessment using Egger's test indicated non-significant publication bias for semen volume, sperm viability, normal morphology, and post-thaw total sperm motility ($P>0.1$). Statistically significant publication bias was recorded for sperm concentration, total sperm motility, and post-thaw DNA integrity ($P<0.1$). Despite the corresponding P-value not being substantially changed, trim-and-fill analysis suggested an adjustment of the sperm normal morphology SMD from −0.10 (Table 3) to 0.13 (Table 2), due to two potentially missing studies. No substantial adjustments in SMD or P-value were suggested for ejaculate volume, sperm concentration, sperm total motility, sperm viability, post-thaw total motility, or post-thaw DNA integrity (Table 2) compared to the main model (Table 3).

Table 2: Publication bias assessment of included studies investigating the effects of EE on semen quality parameters

Parameter	Funnel plot	Egger's test	Trim and fill		
			Missing study	SMD (95% CI)	P-value
Semen volume	Symmetry	0.511	2	0.58 [0.08, 1.08).	0.026
Sperm concentration	Asymmetry	0.003	6	-0.94 [-1.36, -0.51)	<0.001
Sperm total motility	Asymmetry	0.075	2	0.08 [-0.27, 0.44)	0.622
Sperm viability	Symmetry	0.882	0	-0.30 (-0.53, -0.06)	0.020
Sperm normal morphology	Symmetry	0.109	2	0.13 [-0.64, 0.91)	0.692
Post-thaw total motility	Symmetry	0.376	1	-0.78 [-1.18, -0.38)	0.001
Post-thaw DNA integrity	Asymmetry	0.077	2	-0.39 [-1.78, 1.00)	0.531

Meta-analysis of fresh and cryopreserved semen outcomes: Pooled estimates on semen volume from 17 experiments demonstrated that electroejaculation significantly increased semen volume (SMD=0.71; P=0.004; Table 3), with moderate heterogeneity observed among experiments (I^2=52.1%). Conversely, sperm concentration (SMD=-1.15; P<0.001, I^2=20.3%) and sperm viability (SMD=-0.30; P=0.046, I^2=0.0%) were consistently observed as significantly negatively affected. Small but statistically non-significant effect sizes were observed for sperm total motility (SMD=0.05; P=0.768) and normal morphology (SMD=-0.10; P=0.724), with no (I^2=24.6%; I^2=20.7%) heterogeneity, respectively. Ten pooled experiments on post-thaw total motility revealed a significant negative effect of EE on post-thaw total motility (SMD=-0.82; P<0.001; Table 3), with no heterogeneity detected (I^2=0.0%). Statistically non-significant effect of EE was observed on post-thaw sperm DNA integrity (SMD=-0.93; P=0.124), although moderate heterogeneity was detected (I^2=66.7%).

Subgroup analysis: As shown in Table 3, semen volume was significantly increased by EE in bulls (SMD=0.72; P=0.007) subgroups, with no heterogeneity (I^2=0.0). However, EE had non-significant effect on semen volume in male goats and rams. In contrast, EE demonstrated a significantly negative effect on sperm concentration in male goats and rams (P<0.05), with negative but non-significant effects in bulls. Despite a significant negative effect of EE on overall sperm viability, none of the species showed significant effects, with no heterogeneity detected (I^2=0.0%). A plausible explanation is that the effect sizes for all species were negative, and most confidence intervals overlapped zero while showing a slight tendency toward negative values. Thus, when pooled into a larger sample size, a significant effect was detected. All species showed non-significant effects of EE on sperm total motility, normal sperm morphology, and post-thaw sperm DNA integrity (Table 3). However, male goat semen showed a significant negative effect of EE on post-thaw total sperm motility (SMD=-1.15; P=0.010), with no heterogeneity observed (I^2=0.0%).

Meta-regression: The meta-regression analysis revealed non-significant temporal trend in the effect size of EE on semen volume across 35 years of publication (β=0.029; 95% CI: -0.023 to 0.080, P=0.251; Fig. 4A). Similarly, statistically non-significant association was observed between publication year and sperm concentration (β=-0.009; 95% CI: -0.047 to 0.028, P=0.603; Fig. 4B). The analysis also showed non-significant temporal trend in total motility (β=-0.021; 95% CI: -0.052 to 0.010, P=0.171; Fig. 4C). Overall, statistically non-significant temporal change in the standardized mean difference between EE and AV was observed over time. Similarly, post-thaw total motility also showed non-significant temporal trend over the past three and a half decades (β=-0.005; 95% CI: -0.041 to 0.031, P=0.754; Fig. 4D).

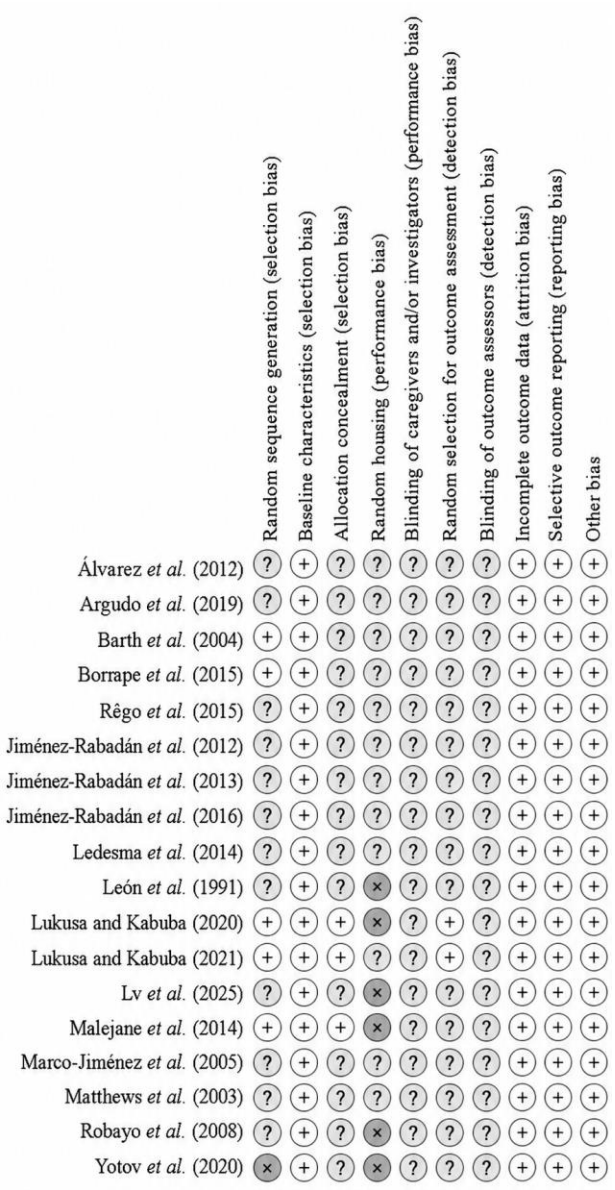

Fig. 2: Risk of bias summary based on SYRCLE's RoB, categorized as low (+), unclear (?) or high (×) risk of bias for each item.

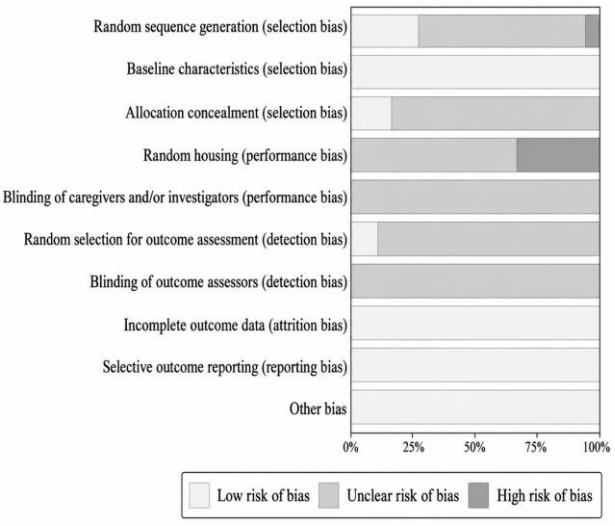

Fig. 3: Risk of bias graph summary presenting the percentage distribution of each risk-of-bias item across included studies.

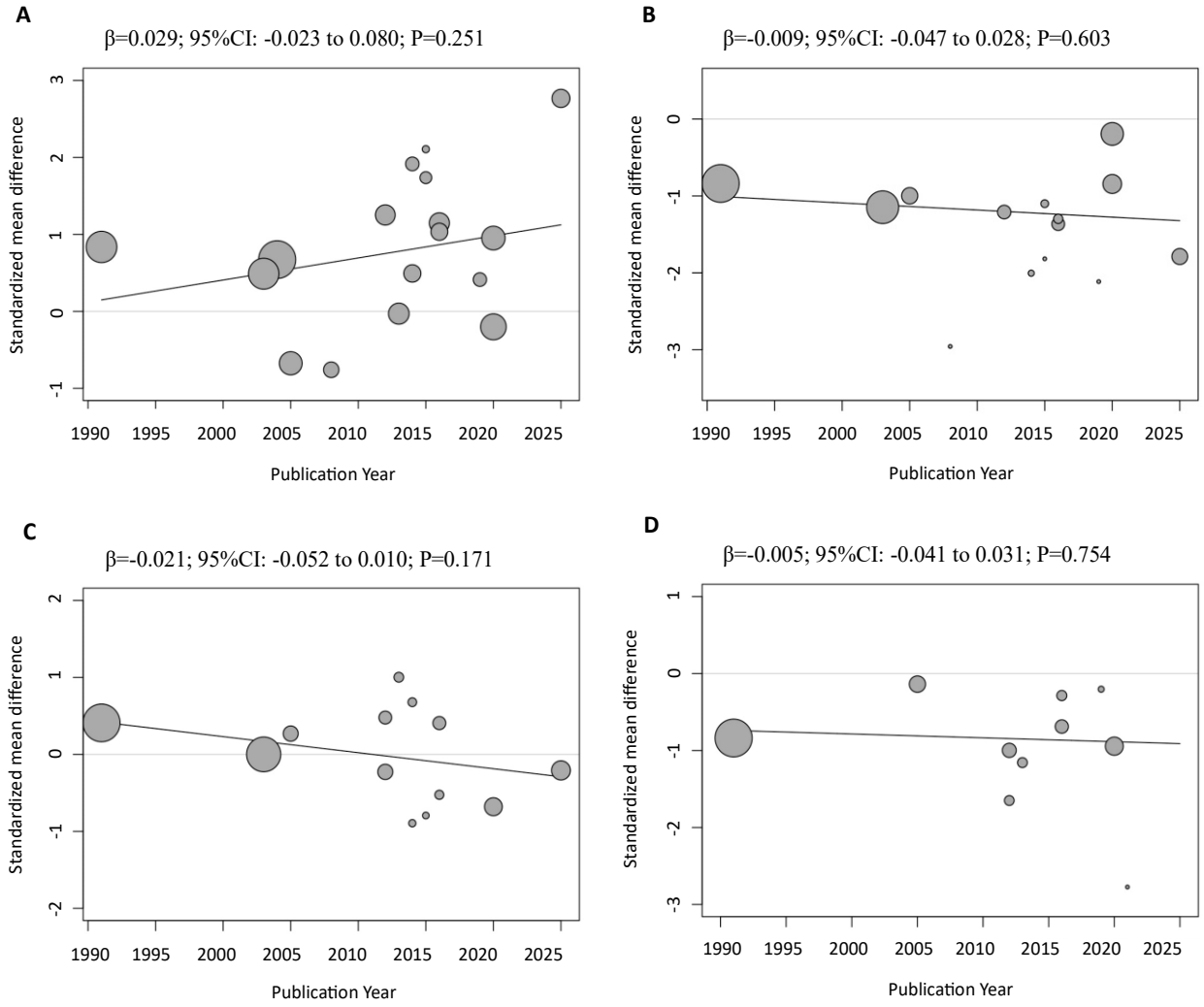

Fig. 4: Temporal trends in effect sizes of EE compared with AV for (A) semen volume, (B) sperm concentration, (C) total sperm motility, and (D) post-thaw total sperm motility.

Table 3: Pooled standardized mean difference and heterogeneity of semen quality parameters

Parameter	Subgroup	Trials		SMD (95% CI)	P-value	Heterogeneity	
		EE	AV			I^2 (%)	P-value (Q)
Semen volume	Overall	220	175	0.71 (0.26, 1.16)	0.004	52.1	0.006
	Male goat	44	44	1.03 (-0.15, 2.21)	0.075	72.0	0.003
	Bull	122	78	0.72 (0.37, 1.06)	0.007	0.0	0.678
	Ram	54	53	0.44 (-0.37, 1.25)	0.230	50.4	0.060
Sperm concentration	Overall	119	118	-1.15 (-1.52, -0.79)	<0.001	20.3	0.227
	Male goat	44	44	-1.35 (-2.47, -0.23)	0.027	59.2	0.031
	Bull	26	26	-1.01 (-2.24, 0.23)	0.072	0.0	0.476
	Ram	49	48	-1.19 (-1.68, -0.69)	0.002	0.0	0.623
Sperm total motility	Overall	116	115	0.05 (-0.29, 0.39)	0.768	24.6	0.189
	Male goat	44	44	0.02 (-0.61, 0.56)	0.949	32.1	0.195
	Bull	23	23	-10.23 (-177.43, 156.96)	0.579	79.0	0.029
	Ram	49	49	-0.05 (-0.51, 0.40)	0.779	0.0	0.600
Sperm viability	Overall	142	97	-0.30 (-0.60, -0.01)	0.046	0.0	0.569
	Male goat	20	20	-0.36 (-1.57, 0.85)	0.332	0.0	0.469
	Bull	99	55	-0.16 (-1.41, 1.10)	0.358	0.0	0.559
	Ram	23	22	-0.75 (-1.80, 0.29)	0.069	0.0	0.792
Normal morphology	Overall	43	42	-0.10 (-0.80, 0.59)	0.724	20.7	0.278
	Male goat	14	14	-0.50 (-5.67, 4.67)	0.434	3.5	0.309
	Bull	6	6	-0.42 (-11.65, 10.81)	0.719	43.5	0.184
	Ram	23	22	0.33 (-2.27, 2.94)	0.350	0.0	0.497
Post-thaw total motility	Overall	78	78	-0.82 (-1.19, -0.46)	<0.001	0.0	0.533
	Male goat	34	34	-1.15 (-1.85, -0.45)	0.010	0.0	0.499
	Bull	23	23	-0.75 (-3.54, 2.04)	0.182	0.0	0.474
	Ram	21	21	-0.47 (-1.65, 0.70)	0.226	0.0	0.533
Post-thaw DNA integrity	Overall	39	39	-0.93 (-2.22, 0.36)	0.124	66.7	0.010
	Male goat	34	34	-1.15 (-2.74, 0.45)	0.116	70.9	0.008
	Ram	5	5	-0.00 (-1.24, 1.24)	0.995	-	-

EE=Electroejaculation; AV=Artificial vagina; P-value (Q)=Cochran's Q test P-value

DISCUSSION

The use of EE for semen collection remains controversial due to animal welfare concerns (da Silva *et al.*, 2024; Kaka *et al.*, 2025). Despite these concerns, there is no clear consensus regarding the effects of EE on semen quality parameters following collection, as well as cryopreservation. This review provides a comprehensive synthesis of available evidence comparing EE and AV in domestic ruminants, including bulls, rams and male goats. The pooled evidence suggested that semen volume was significantly higher with EE than with AV, likely due to an increased proportion of seminal plasma (SP). This may be explained by electrical stimulation during EE, which activates local pelvic musculature and stimulates the secretory activity of accessory sex glands (Groh *et al.*, 2018). This overstimulation of accessory sex glands may result in increased SP secretion. This may also relate to prolonged stimulation pulses, which can last for several minutes, with an average duration of approximately 3 minutes (180 seconds) in rams (Álvarez *et al.*, 2012), 109.1 seconds in male goats (Abril-Sánchez *et al.,* 2017) and from 90 up to 214 seconds in bulls (Whitlock *et al.,* 2012; Pagliosa *et al.,* 2015). According to Lv *et al.* (2024), variations in SP proportion in semen may influence protein abundance associated with sperm quality in goat semen. In contrast, sperm concentration was significantly lower with EE than with AV. Most studies report that total sperm count per ejaculate obtained with EE is similar to, or slightly lower than, that obtained with AV, resulting in reduced sperm concentration per unit semen volume due to increased SP volume (Argudo *et al.*, 2019; Lukusa and Kabuba, 2020; Yotov *et al.*, 2020). Interestingly, the overall total sperm motility and morphology of fresh semen were not significantly different between EE and AV methods. This suggests that semen collected using EE may adequately reflect individual sperm quality, as these parameters are routinely evaluated in breeding soundness examination (BSE) of domestic ruminants (Koziol and Armstrong, 2018; Harighi *et al.*, 2022; King and Hopper, 2024).

Sperm motility does not appear to be highly sensitive to electrical stimulation. In a study using human sperm, direct exposure to sinusoidal alternating electrical stimulation maintained sperm motility even under extremely high voltages of 80 V/2 mm (400 V/cm) for 30 seconds (Saito *et al.*, 1999). These findings suggest that the electrical stimulation applied during rectal electroejaculation is unlikely to impair sperm motility during semen collection. On the other hand, overall sperm viability showed a significant negative association with EE. The effect of EE on sperm viability remains poorly understood. Sperm viability is commonly assessed based on plasma membrane integrity (Odinius *et al.*, 2024; Xue *et al.*, 2025). Most alterations in sperm membranes are commonly associated with oxidative or osmotic stress during cryopreservation (Loetjettanarom *et al.*, 2025), sperm sorting (Reese *et al.*, 2021) and liquid preservation (Pantecostoma *et al.*, 2025). Differences in seminal plasma protein composition have been suggested to contribute to plasma membrane regulation, as proteins in AV-collected ejaculates are primarily derived from the epididymis, whereas those in EE-collected ejaculates mainly originate from the accessory sex glands (Rêgo *et al.*, 2015). For instance, albumin and serotransferrin, which are more abundant in AV-collected semen, have been associated with the protection of sperm membranes against reactive oxygen species (ROS). Similarly, arachidonic acid, a metabolite involved in maintaining membrane fluidity, was also reported at higher abundance in AV-collected semen (Lv *et al.*, 2025). Further proteomic and metabolomic investigations are required to clarify the mechanisms underlying the effects of EE on sperm viability.

A significantly negative effect of EE was observed on post-thaw total sperm motility in the male goat semen, whereas such effect was not observed in bulls or rams. However, post-thaw sperm DNA integrity was not affected by EE in any species. Further optimization of cryopreservation protocols for semen obtained through EE could be explored, as has been previously performed (Jiménez-Rabadán *et al.*, 2013; Toker *et al.*, 2024).

Despite the use of various EE procedures and devices over this period, there have been no significant changes compared to AV since its initial development. No significant temporal trends were detected for semen volume, sperm concentration, and total sperm motility. Similarly, post-thaw total sperm motility did not demonstrate a significant temporal trend. In this study, AV was considered the reference standard for semen collection, with no substantial changes over past decades (Rich and Orbach, 2025). Most studies have compared the effects of EE and AV under different conditions, combined with or without other factors (season, breed, cryopreservation protocol, extender), by evaluating parameters such as semen quality, fertility, seminal plasma profile, and post-thaw quality. We could found limited studies that specifically aimed to improve semen quality obtained through EE by modifying the procedure, cryopreservation protocol, or through animal-based interventions. Nevertheless, a recent experiment investigated the administration of oral selenium to enhance the quality of cryopreserved semen obtained by EE (Lukusa and Kabuba, 2021). In addition, another study was aimed to optimize the cryopreservation protocol for semen collected through EE (Jiménez-Rabadán *et al.*, 2013). Other strategies that have not been compared with AV include brushing (Ungerfeld *et al.*, 2021; Orihuela *et al.*, 2024a), administration of cloprostenol and oxytocin (Ungerfeld *et al.*, 2018), carbetocin (Orihuela *et al.*, 2024b), butorphanol (Ungerfeld *et al.*, 2022), fasting prior to EE (Romano *et al.*, 2021), and habituation (O'Neill *et al.*, 2024). Sedation with 2% xylazine was the most consistently applied procedure across several studies.

The results of this meta-analysis suggest that EE performs comparably to AV for selected parameters and species. These findings should be carefully considered when utilizing EE in BSE, particularly with respect to sperm concentration (Koziol and Armstrong, 2018; Tibary *et al.*, 2018). For cryopreservation, the observed effect sizes suggest that semen collected using EE may be acceptable, particularly in bulls and rams. Further investigation of seminal plasma profiles, oxidative stress levels, and sperm structural integrity is required to better understand the implications of EE on sperm quality. Furthermore, strategies to cryopreserve semen obtained by

EE through modification of protocols may improve the likelihood of successfully preserving semen from untrained or low-libido animals.

The limitations of our study are as follows: (1) a small number of studies (18 only) could be included in the review; (2) semen quality assessment was not standardized, given the wide publication range over 35 years; (3) the estimation of missing values in some studies could slightly affect the effect size; (4) the species, region, and breeding management may introduce heterogeneity; and (5) language restrictions and unretrieved studies may introduce publication bias due to limitations in language and data sources. Statistical approaches, including subgroup analysis, trim-and-fill, sensitivity analysis, Egger's test, and funnel plots, were employed to overcome these limitations.

Conclusions: This systematic review and meta-analysis summarize comparative studies of EE and AV in domestic ruminants from 1990 up to recent publications (2025), highlighting differential effects on selected fresh and frozen-thawed semen quality parameters. The functional and structural characteristics of fresh sperm used for routine assessment were generally not affected. However, EE may be associated with reduced post-thaw semen quality under certain conditions. These findings reflect the current progress of alternative semen collection methods, which highlight the need for further optimization and mechanistic investigation.

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REFERENCES

Abril-Sánchez S, Freitas-de-Melo A, Beracochea F, *et al.*, 2017. Sperm collection by transrectal ultrasound-guided massage of the accessory sex glands is less stressful than electroejaculation without altering sperm characteristics in conscious goat bucks. Theriogenology 98:82–87.

Álvarez M, Tamayo-Canul J, Martínez-Rodríguez C, *et al.*, 2012. Specificity of the extender used for freezing ram sperm depends of the spermatozoa source (ejaculate, electroejaculate or epididymis). Animal Reproduction Science 132(3-4):145–154.

Argudo DE, Galarza DA, Bueno P, *et al.*, 2019. Methods of collection, extender type, and freezability of semen collected from creole bulls raised in the tropical highlands of Ecuador. Tropical Animal Health and Production 51(7):1839–1845.

Barth AD, Arteaga AA, Brito LFC, *et al.*, 2004. Use of internal artificial vaginas for breeding soundness evaluation in range bulls: an alternative for electroejaculation allowing observation of sex drive and mating ability. Animal Reproduction Science 84(3-4):315–325.

Bashar MA, Akhtar MM, Shejuty SF, *et al.*, 2025. Effect of glutathione, ascorbic acid, and their combination on the quality and fertility of cryopreserved spermatozoa of Black Bengal buck. Veterinary and Animal Science 30:100549. doi: 10.1016/j.vas.2025.100549.

Bopape MA, Lehloenya KC, Chokoe TC, *et al.*, 2015. Comparison of electro ejaculator and artificial vagina on semen collection from South African indigenous goat following assessment by computer aided sperm analysis. Open Journal of Animal Sciences 5(2):210–218. doi: 10.4236/ojas.2015.52023.

Borenstein M, Hedges LV, Higgins JPT, *et al.*, 2009. Introduction to Meta-Analysis. John Wiley & Sons; Chichester, UK.

Diaz-Miranda EA, Penitente-Filho JM, Gomez-Leon VE, *et al.*, 2024. Selection based on the breeding soundness evaluation is associated with the improvement of the reproductive quality of young Nellore bulls. Theriogenology 226:369–377.

Duval S and Tweedie R, 2000. A nonparametric "Trim and Fill" method of accounting for publication bias in meta-analysis. Journal of the American Statistical Association 95(449):89–98. https://doi.org/10.1080/01621459.2000.10473905.

Egger M, Smith GD, Schneider M, *et al.*, 1997. Bias in meta-analysis detected by a simple, graphical test. BMJ 315:629–634. doi:10.1136/bmj.315.7109.629.

Groh AMR, Moore CW, El-Warrak A, *et al.*, 2018. Electroejaculation functions primarily by direct activation of pelvic musculature: Perspectives from a porcine model. Translational Research in Anatomy 10:10–17. doi:10.1016/j.tria.2018.01.001.

Harighi MF, Wahid H, Khumran AM, *et al.*, 2022. Breeding soundness examination (BSE): A decision-making tool that requires a particular guideline for male goats. Tropical Animal Health and Production 54(3):174. doi: 10.1007/s11250-022-03178-8.

Harrer M, Cuijpers P, Furukawa TA, *et al.*, 2021. Doing meta-analysis with R: A hands-on guide. Boca Raton, FL and London: Chapman & Hall/CRC Press. ISBN 978-0-367-61007-4.

Higgins JPT, Thomas J, Chandler J, *et al.*, 2019. Cochrane Handbook for Systematic Reviews of Interventions, 1st Ed, John Wiley & Sons, Inc. doi:10.1002/9781119536604.

Higgins JPT, Thompson SG, Deeks JJ, *et al.*, 2003. Measuring inconsistency in meta-analyses. BMJ 327(7414):557–560. doi: 10.1136/bmj.327.7414.557.

Hooijmans CR, Rovers MM, de Vries RB, *et al.*, 2014. SYRCLE's risk of bias tool for animal studies. BMC Medical Research Methodology 14:43. doi: 10.1186/1471-2288-14-43.

Jiménez-Rabadán P, Ramón M, García-Álvarez O, *et al.*, 2012. Effect of semen collection method (artificial vagina vs. electroejaculation), extender and centrifugation on post-thaw sperm quality of Blanca-Celtibérica buck ejaculates. Animal Reproduction Science 132(1-2):88–95.

Jiménez-Rabadán P, Ramón M, García-Álvarez O, *et al.*, 2013. Improved cryopreservation protocol for Blanca-Celtibérica buck semen collected by electroejaculation. Cryobiology 67(3):251–257.

Jiménez-Rabadán P, Soler AJ, Ramón M, *et al.*, 2016. Influence of semen collection method on sperm cryoresistance in small ruminants. Animal Reproduction Science 167:103–108.

Kaka U, Degu NY, Kumar P, *et al.*, 2025. Do bulls experience pain or stress during electroejaculation? Evidence from electroencephalography, behavioral, hormonal, and metabolite profiling. Veterinary World 18(4): 763–772. doi: 10.14202/vetworld.2025.763-772.

Khonmee J, Brown JL, Pérez AL, *et al.*, 2023. Effect of electroejaculation protocols on semen quality and concentrations of testosterone, cortisol, malondialdehyde, and creatine kinase in captive Bengal tigers. Animals 13(12):1893. doi: 10.3390/ani13121893.

King EH and Hopper RM, 2024. The bull breeding soundness examination and its application in the production setting. Veterinary Clinics of North America: Food Animal Practice 40(1):19–27. doi: 10.1016/j.cvfa.2023.08.001.

Koziol JH and Armstrong CL, 2018. Society for Theriogenology Manual for Breeding Soundness Examination of Bulls, 2nd Ed, Society for Theriogenology, Pike Road, Alabama, USA.

Ledesma A, Manes J, Cesari A, *et al.*, 2014. Electroejaculation increases low molecular weight proteins in seminal plasma modifying sperm quality in Corriedale rams. Reproduction in Domestic Animal 49(2):324–332. doi: 10.1111/rda.12279.

León H, Porras AA, Galina CS, *et al.*, 1991. Effect of the collection method on semen characteristics of Zebu and European type cattle in the tropics. Theriogenology 36(3):349–355. doi: 10.1016/0093-691x(91)90463-n.

Loetjettanarom T, Authaida S, Boonkum W, *et al.*, 2025. Effect of *Kaempferia parviflora* supplementation in semen extenders on post-thaw sperm quality in Thai Native bulls. Animals 15(7):962. doi: 10.3390/ani15070962.

Lukusa K and Kabuba J, 2020. Semen collection methods and cooling rates affect post-thaw sperm motility and kinematic parameters of Saanen goat. Asian Pacific Journal of Reproduction 9(5):239–246. doi: 10.4103/2305-0500.294666.

Lukusa K and Kabuba J, 2021. Oral supplementation of selenium improves post-thaw sperm quality in Saanen bucks. Asian Pacific Journal of Reproduction 10(4):187–194. doi: 10.4103/2305-0500.321126.

Lv C, Larbi A, Liang J, *et al.*, 2025. Effects of semen collection methods on sperm quality and metabolite profile in goat seminal plasma: Comparing between artificial vagina and electro-ejaculator techniques. Animal Reproduction Science 279:107885.

Lv C, Larbi A, Li C, *et al.*, 2024. Decoding the influence of semen collection processes on goat sperm quality from a perspective of seminal plasma proteomics. Journal of Proteomics 298:105141. doi: 10.1016/j.jprot.2024.105141.

Malejane CM, Greyling JPC and Raito MB, 2014. Seasonal variation in semen quality of Dorper rams using different collection techniques. South African Journal of Animal Science 44(1):26.

Marco-Jiménez F, Puchades S, Gadea J, *et al.*, 2005. Effect of semen collection method on pre- and post-thaw Guirra ram spermatozoa. Theriogenology 64(8):1756–1765.

Matthews N, Bester N and Schwalbach LMJ, 2003. A comparison of ram semen collected by artificial vagina and electro-ejaculation. South African Journal of Animal Science 4(1):28–30.

McAuley L, Pham B, Tugwell P, *et al.*, 2000. Does the inclusion of grey literature influence estimates of intervention effectiveness reported in meta-analyses? The Lancet 356(9237):1228–1231. doi: 10.1016/S0140-6736(00)02786-0.

Meng Z, Wang J, Lin L, *et al.*, 2024. Sensitivity analysis with iterative outlier detection for systematic reviews and meta-analyses. Statistics in Medicine 43(8):1549–1563. doi: 10.1002/sim.10008.

Mulyati S, Mustofa I, Susilowati S, *et al.*, 2025. Increasing the fertility of Sapudi sheep semen at 5°C storage temperature with the addition of oxytocin in a diluent of skim milk, egg yolk, and citrate. Open Veterinary Journal 15(9):4700–4708.

Odinius TS, Siuda M, Lautner M, *et al.*, 2024. Sperm functional status: A multiparametric assessment of the fertilizing potential of bovine sperm. Veterinary Sciences 11(12):678.

O'Neill HA, Scholtz J, Kruger LP, *et al.*, 2024. Short communication: Impact of rest intervals and habituation on electro-ejaculated semen quality in merino-type rams. South African Journal of Animal Science 54(6):685–690.

Orihuela JC, Freitas-de-Melo A, Pinto-Santini L, *et al.*, 2024a. Brushing rams before and during electroejaculation improves sperm motility and kinetics with slight changes in stress biomarkers. Animal Reproduction Science 268:107565. doi: 10.1016/j.anireprosci.2024.107565.

Orihuela JC, Freitas-de-Melo A, Pinto-Santini L, *et al.*, 2024b. A single administration of carbetocin before electroejaculation increases the insemination doses produced from each ejaculate in rams. Theriogenology 221:1–8. doi: 10.1016/j.theriogenology.2024.03.008

Page MJ, McKenzie JE, Bossuyt PM, *et al.*, 2021. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 372: n71. doi: 10.1136/bmj.n71.

Pagliosa RC, Derossi R, Costa DS, *et al.*, 2015. Efficacy of caudal epidural injection of lidocaine, xylazine and xylazine plus hyaluronidase in reducing discomfort produced by electroejaculation in bulls. Journal of Veterinary Medical Science 77(11):1339–1345. doi:10.1292/jvms.14-0369.

Pantecostoma RR, Jiwakanon J and Sirisathien S, 2025. Effect of idebenone supplementation in a semen extender on boar spermatozoa quality during liquid storage. Veterinary World 18(6):1479–1486.

Posit team, 2026. RStudio: Integrated Development Environment for R. Posit Software, PBC: Boston, MA. http://www.posit.co/

Rêgo JPA, Moura AA, Nouwens AS, *et al.*, 2015. Seminal plasma protein profiles of ejaculates obtained by internal artificial vagina and electroejaculation in Brahman bulls. Animal Reproduction Science 160:126–137.

Reese S, Pirez MC, Steele H, *et al.*, 2021. The reproductive success of bovine sperm after sex-sorting: A meta-analysis. Scientific Reports 11(1):17366. doi: 10.1038/s41598-021-96834-2.

Rich J and Orbach DN, 2025. Artificial vagina conformation and composition for semen collection. Reproduction and Fertility 6(4): e250061. Doi: org/10.1530/RAF-25-0061.

Robayo I, Montenegro V, Valdés C, *et al.*, 2008. CASA assessment of kinematic parameters of ram spermatozoa and their relationship to migration efficiency in ruminant cervical mucus. Reproduction in Domestic Animal 43(4):393–399.

Romano JE, Mari G, Stradaioli G, *et al.*, 2021. Effect of fasting prior to electroejaculation on behavioral responses and reproductive parameters in young Simmental bulls. Theriogenology 173:19–22.

Saito K, Kinoshita Y and Hosaka M, 1999. Direct and indirect effects of electrical stimulation on the motility of human sperm. International Journal of Urology 6(4):196–199. doi: 10.1046/j.1442-2042.1999.06444.x.

Schwarzer G, Carpenter JR and Rücker G, 2015. Meta-Analysis with R. Springer International Publishing: Cham.

da Silva MKF, de Almeida Gélio L, Oba E, *et al.*, 2024. Evaluation of pharmacological alternatives to reduce the pain and discomfort produced by electroejaculation in rams. Reproduction in Domestic Animals 59(1):e14528. doi: 10.1111/rda.14528.

Tibary A, Boukhliq R and Allali KE, 2018. Ram and buck breeding soundness examination. Revue Marocaine des Sciences Agronomiques et Vétérinaires 6(2):241–255.

Toker MB, Sabancı AÜ, Avcı G, *et al.*, 2024. Evaluation of cryopreserved ram sperm with nano-ozone solution and post-thaw life span by flow cytometric analysis. Biopreservation and Biobanking 22(4):312–320. doi: 10.1089/bio.2023.0073.

Ungerfeld R, Casuriaga D, Giriboni J, *et al.*, 2018. Administration of cloprostenol and oxytocin before electroejaculation in goat bucks reduces the needed amount of electrical stimulation without affecting seminal quality. Theriogenology 107:1–5.

Ungerfeld R, Pinto-Santini L, Chaumont S, *et al.*, 2021. Is the ram that is more receptive to brushing, less reactive to electroejaculation? Livestock Science 254:104764.

Ungerfeld R, De Moura Fernandes DA, Balaro MFA, *et al.*, 2022. Administration of butorphanol with ketamine/xylazine sedation reduces the negative responses to electroejaculation in rams. Theriogenology 191:96–101.

Whitlock BK, Coffman EA, Coetzee JF, *et al.*, 2012. Electroejaculation increased vocalization and plasma concentrations of cortisol and progesterone, but not substance P, in beef bulls. Theriogenology 78(4):737–746. doi: 10.1016/j.theriogenology.2012.03.020.

Wickham H, 2016. ggplot2: Elegant Graphics for Data Analysis. 2nd Ed, Springer: Switzerland.

Wulster-Radcliffe MC, Williams MA, Stellflug JN, *et al.*, 2001. Technical note: Artificial vagina vs a vaginal collection vial for collecting semen from rams. Journal of Animal Science 79(12):2964-2967. doi: 10.2527/2001.79122964x.

Xue S-H, Xu B-B, Yan X-C, *et al.*, 2025. Sperm membrane stability: In-depth analysis from structural basis to functional regulation. Veterinary Sciences 12(7):658. doi: 10.3390/vetsci12070658.

Yotov S, Atanasov A, Fasulkov I, *et al.*, 2020. Sperm motility and viability of chilled ram semen collected by artificial vagina and electroejaculation. Veterinarija ir Zootechnika 78(100):45–49.