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Association between sperm DNA fragmentation and fertility parameters in farm animals: a systematic review and meta-analysis

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Abstract

Background Accurately predicting male fertility is crucial for the animal breeding industry due to its significant economic implications. Existing literature suggests that mammalian fertility is partially dependent on sperm DNA integrity. However, routine semen analysis often fails to detect DNA damage and does not consistently correlate with field fertility outcomes. While assessing sperm DNA integrity provides valuable biological insights, its role in diagnosing animal infertility remains uncertain.

Results This meta-analysis evaluated the association between sperm DNA fragmentation (SDF) and fertility in farm animals. Comprehensive searches were conducted using PubMed, Google Scholar, and Springer Link Library, with results stratified by animal species and SDF detection methods. Across 30 studies, the overall correlation coefficient (COR) between SDF and male fertility was -0.46 (95% confidence interval [CI]: -0.54 to -0.37; Z = -8.97; $p < 0.001$). A significant association was observed in bulls (COR = -0.47; 95% CI: -0.54 to -0.40; Z = -11.13; $p < 0.001$) and stallions (COR = -0.54; 95% CI: -0.72 to -0.29; Z = -3.83; $p < 0.001$), whereas no significant relationship was found in boars (COR = -0.19; 95% CI: -0.37 to 0.01; Z = -1.84; $p = 0.07$). The effect of SDF on male fertility was analyzed in 15 studies, demonstrating significantly higher SDF values in low-fertility animals compared to high-fertility groups (SMD = 0.85; 95% CI: 0.68 to 1.01; Z = 10.07; $p < 0.001$). This pattern was observed in both bulls (SMD = 1.21; 95% CI: 0.85 to 1.57; Z = 6.59; $p < 0.001$) and stallions (SMD = 0.64; 95% CI: 0.44 to 0.85; Z = 6.14; $p < 0.001$) subgroups.

Conclusions These findings suggest that incorporating SDF assays into breeding soundness evaluations could enhance the accuracy of selecting high-quality breeding males for artificial breeding programs. However, further research with adequately powered studies, standardized methodologies, and appropriate sample sizes is necessary to fully elucidate the impact of elevated SDF on fertility in farm animals.

Keywords Subfertility, Meta-analysis, Semen quality, Sperm DNA damage, Animal reproduction

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Background

Ensuring male fertility is essential for the profitability, sustainability, and advancement of the livestock industry [1]. Subfertility can lead to prolonged calving or farrowing intervals, increased culling rates, and the need to maintain surplus males on the farm, all of which negatively impact economic viability. Studies have shown that pregnancy rates per cycle in mares bred using cooled semen are approximately 67% [2–4], meaning that around 33% of mares fail to conceive following insemination in each cycle. In dairy cattle, the first-service conception rate has reportedly declined from 70 to 40% over the past 30 to 50 years [5, 6]. Both male and female factors influence the likelihood of a successful pregnancy. Sperm DNA fragmentation, defined as the breakage of sperm DNA into single- or double-strand breaks [7], is proposed as one of the contributing factors to male subfertility.

The traditional assessment of sperm quality and male fertility typically relies on parameters such as sperm concentration, motility, and morphology across various species [8–11]. However, certain sperm characteristics that influence fertility, particularly molecular-level factors such as sperm DNA fragmentation, may not be detectable through conventional semen analysis but could serve as potential biomarkers for fertility [12–14]. Furthermore, research indicates that predicting male fertility based on a single sperm attribute alone is unlikely to be accurate. The fertilization process is highly complex, requiring a multifactorial approach for reliable evaluation [1, 9, 15–17].

In men, sperm DNA damage has been linked to impaired fertilization [18, 19], suboptimal embryo quality [20–22], reduced pregnancy rates [23, 24], and an increased risk of spontaneous abortion following IVF [7, 20, 25]. Similarly, in farm animals, sperm DNA fragmentation (SDF) has been associated with fertility differences between high- and low-fertility males [1, 25–28]. However, conflicting findings exist, as some studies have failed to establish a clear relationship between SDF and fertility parameters in animals [10, 29–31]. Therefore, the objective of this meta-analysis was to perform a comprehensive evaluation using published data to assess the correlation between SDF and fertility parameters across various farm animal species, including cattle, horses, pigs, and sheep. We sought to determine the impact of sperm DNA integrity and assay type on fertility outcomes.

Results

Literature search and selection of studies

The search strategy yielded 1528 citations. After reviewing the titles or abstracts, 1448 articles were deemed irrelevant and excluded. Full papers were obtained for the remaining 80 citations. Of these, 33 were excluded as they did not meet the inclusion criteria of SDF and fertility parameters. Seventeen studies were further excluded because the researchers either did not provide the value of the correlation coefficient or did not analyze the correlation of SDF to fertility: (i) Bull ($n=8$) [28, 32–38]; (ii) Stallion ($n=1$) [30]; (iii) Boar ($n=5$) [39–43]; (iv) Ram ($n=3$) [27, 44, 45].

Based on the criteria outlined, the total number of included studies examining the linear association between SDF and fertility was thirty (Fig. 1). Fifteen studies grouped animals into high or low fertility categories and provided the mean and SD of SDF. Five studies that did not provide a correlation coefficient but met this inclusion criteria were included in this category [27, 28, 30, 34, 39].

Study design characteristics

The main characteristics of the studies are presented in Tables 1 and 2. Thirty (30) studies with 943 animals, meeting all inclusion criteria, were considered for analysis in the first part (Table 1). Sperm Chromatin Structure Assay (SCSA) was used to evaluate sperm SDF in eighteen studies, terminal deoxynucleotidyl transferase dUTP nicked-end labeling (TUNEL) was used in four studies, sperm chromatin dispersion test (SCD)/Halomax in 10 studies, and acridine orange test (AO) test in two studies. No study utilizing the Comet assay (neutral or alkaline) met the inclusion criteria, as the available studies were conducted on buffalo bulls [46, 47] or assessed fertility in vitro (IVF) [48, 49] and were therefore excluded.

Nineteen studies focused on bulls, six on stallions, and five on boars. Fertility was assessed using various parameters across the studies: non-return to estrus rate (NRR) in thirteen studies, pregnancy rate (PR) in eight, conception rate (CR) in five, farrowing rate (FR) in three, and direct boar effect (DBE) in one study. In the second part of the analysis (Table 2), a total of 15 studies were included in the review, comprising 311 animals in the high-fertility group and 213 in the low-fertility group. Fourteen of these studies were retrospective, while one was prospective. Various methods were employed to categorize subjects into high- and low-fertility groups (Table 2). Most studies used males of breeding age.

Meta-analysis

In the first part of this meta-analysis, a significant negative correlation between SDF and fertility was observed across the thirty studies analyzed, with a correlation coefficient (COR) of −0.46 (random effects model, 95% confidence interval: −0.54 to −0.37; Z-test = −8.97; $p<0.001$) (Fig. 2). Statistical heterogeneity was present, with a

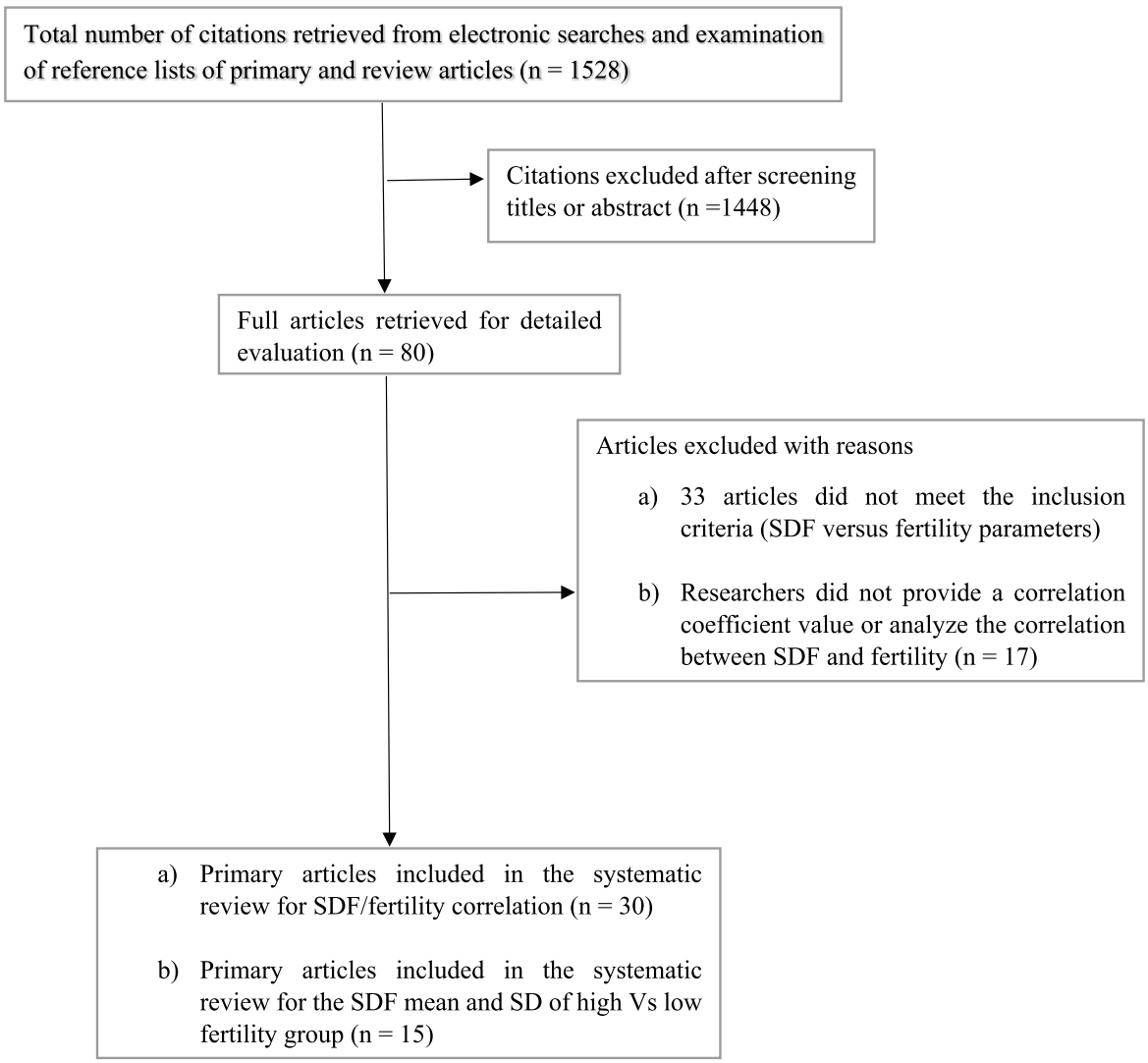

Fig. 1 Preferred reporting items for systematic reviews and meta-analyses (PRISMA) flowchart used in the selection of eligible studies

heterogeneity value of 60.5% ($p < 0.001$). In the subgroup analysis, a significant negative correlation between SDF and fertility was found in bulls, with a correlation coefficient of −0.47 (random effects model, 95% confidence interval: −0.54 to −0.40; Z-test = −11.13; $p < 0.001$). Similarly, a significant correlation was observed in stallions, with a correlation coefficient of −0.54 (random effects model, 95% CI: −0.72 to −0.29; Z-test = −3.83; $p < 0.001$). However, no significant correlation was found between SDF and fertility in boars, where the correlation coefficient was −0.19 (random effects model, 95% confidence interval: −0.37 to 0.01; Z-test = −1.84; $p = 0.07$) (Fig. 3). Heterogeneity levels varied across subgroups: moderate heterogeneity was observed in bulls (heterogeneity = 39.9%; $p = 0.02$), whereas stallions exhibited high heterogeneity (heterogeneity = 74.0%; $p < 0.001$). In contrast, no statistical heterogeneity was found in the boar subgroup (heterogeneity = 48.8%; $p = 0.06$). A combined assay-type analysis showed a significant correlation for both the SCSA (random effects model, COR = −0.43, 95% confidence interval: −0.53 to −0.33; Z-test = −8.54; $p < 0.001$) and the (SCD)/Halomax method (random effects model, COR = −0.51, 95% confidence interval: −0.77 to −0.24; Z-test = −3.78; $p < 0.001$). However, no significant correlation was found for the TUNEL assay (random effects model, COR = −0.41, 95% confidence interval: −0.90 to 0.08; Z-test = −1.66; $p = 0.098$). Further analysis of specific assays by species revealed significant correlations for the SCSA method in bulls ($n = 12$) (random effects model, COR = −0.40, 95% confidence

Table 1 Characteristics of included studies addressing the correlation between sperm DNA fragmentation and fertility parameters

Author & year	Assay	Design	Number of animals	COR	Species	Fertility measure
Narud et al. [9]	SCSA	Retrospective	37	−0.57	Bull	NRR56
Zoca et al. [31]	SCSA	Prospective	5	−0.41	Bull	PR30-90d
Dogan et al. [29]	TUNEL	Retrospective	10	−0.02	Bull	CR
Zahan et al. [50]	Halomax	Retrospective	10	0.53	Bull	NRR
Dogan et al. [51]	Halomax	Retrospective	20	−0.69	Bull	CR
Anzar et al. [52]	TUNEL	Retrospective	5	−0.90	Bull	NRR56
Ballachey et al. [53]	SCSA	Retrospective	49	−0.40	Bull	NRR
	SCSA	Retrospective	18	−0.53	Bull	NRR59
Bollwein et al. [54]	SCSA	Prospective	20	−0.58	Bull	NRR56
Erickson et al. [55]	TUNEL	Retrospective	43	−0.62	Bull	NNR56
Gliozzi et al. [56]	SCSA	Retrospective	18	−0.17	Bull	NRR
Garcia-Macias et al. [57]	Halomax	Retrospective	60	−0.42	Bull	NRR96
	SCSA	Retrospective	60	−0.29	Bull	
Januskaukas et al. [58]	SCSA	Retrospective	20	−0.53	Bull	NRR56
Januskaukas et al. [59]	SCSA	Retrospective	18	−0.51	Bull	NRR56
Ballachey et al. [60]	SCSA	Prospective	9	−0.94	Bull	CR
Karoui et al. [61]	Halomax	Retrospective	201	−0.45, −0.49	Bull	CR
Morrel et al. [17]	SCSA	Retrospective	31	−0.56	Bull	NRR56
Nagy et al. [62]	SCSA	Retrospective	43	−0.05	Bull	NRR
Kumaresan et al. [1]	SCSA	Retrospective	20	−0.61	Bull	NRR56
Pardede et al. [63]	AO	Retrospective	9	−0.78, −0.73, −0.83	Bull	CR
	Halomax	Retrospective	9	−0.69, −0.67, −0.77	Bull	
Love and Kenney [26]	SCSA	Retrospective	84	−0.27, −0.42, −0.41	Stallion	SPR, FCP, PC14-40d
Al-Kass et al. [2]	SCSA	Prospective	12	−0.35	Stallion	SPR
Morrel et al. [8]	SCSA	Retrospective	11	−0.63	Stallion	SPR
Atroshchenko et al. [64]	Halomax	Prospective	17	−0.94	Stallion	PR14-18d
Crespo et al. [65]	SCD	Prospective	11	−0.62	Stallion	PC15d
Kenney et al. [66]	SCSA	Retrospective	51	−0.40	Stallion	SPR
Estrada et al. [67]	SCD	Prospective	12	−0.16, −0.39	Boar	PR30d
Batista et al. [68]	Halomax	Prospective	5	0.38	Boar	FR
Ausejo et al. [10]	TUNEL	Retrospective	58	−0.06, −0.09, 0.02	Boar	DBE
Didion et al. [12]	SCSA	Retrospective	18	−0.55	Boar	FR
Tsakmakidis et al. [69]	AO	Prospective	7	−0.90	Boar	FR

COR Correlation coefficient, *NRR* Nonreturn to estrous rate after 56, 59, or 96 days; *PR* Pregnancy rate and time of detection after AI, *CR* Conception rate, *SPR* Seasonal pregnancy rate, *FR* Farrowing rate, *DBE* Fertility direct boar index, *FCP* Percentage pregnant per first cycle, *PC* Percentage pregnant per cycle

interval: −0.51 to −0.29; Z-test = −6.15; $p < 0.001$) and stallions ($n = 4$) (random effects model, COR = −0.51, 95% confidence interval: −0.67 to −0.34; Z-test = −7.04; $p < 0.001$). Additionally, significant correlations were observed for the TUNEL method in bulls (n = 3) (random effects model, COR = −0.61, 95% confidence interval: −1.20 to −0.01; Z-test = −2.00; $p = 0.046$) and the Halomax/SCD method in bulls ($n = 5$) (random effects model, COR = −0.51, 95% confidence interval: −0.59 to −0.42; Z-test = −11.8; $p < 0.001$). However, no significant correlation was found in studies utilizing the Halomax/SCD assay in boars ($n = 3$) (random effects model, COR = −0.04, 95% confidence interval: −0.27 to 0.18; Z-test = −0.38; $p = 0.707$) (Table 3).

In the second part of this meta-analysis, sperm DNA fragmentation (SDF) was significantly higher in the low fertility (LF) group compared to the high fertility (HF) group, with a standardized mean difference (SMD) of 0.85 (random effects model, 95% confidence interval: 0.68 to 1.01; Z-test = 10.07; $p < 0.001$). Moderate statistical heterogeneity was observed (heterogeneity = 40.3%; $p = 0.02$) (Fig. 4). A subgroup analysis was conducted based on species and the method of SDF detection. In species-specific analysis, a significantly higher SDF was

Table 2 Characteristics of included studies addressing the comparison of sperm DNA fragmentation between high and low fertility groups

Author & year	High fertility (n)	Low fertility (n)	Assay	Design	Definition of high and low fertility
Narud et al. [9]	19	18	SCSA	Retrospective	LSmean 0.76 to 0.78 vs. 0.46 to 0.65, NRR56
Puglisi et al. [28]	90	15	SCSA	Retrospective	ERCR > −2.6 vs. ERCR < −2.6
			TUNEL		
Dogan et al. [29]	5	5	TUNEL	Retrospective	6.14% vs. −9.94% deviation from average CR
Rosyada et al. [34]	4	4	AO	Retrospective	79.04% vs. 65.84%, FSCR
Dogan et al. [51]	10	10	Halomax	Retrospective	+2 vs. −2 deviation from mean CR
Gliozzi et al. [58]	9	9	SCSA	Retrospective	ERCR ≥ 1 vs. ERCR ≤ 1
Garcia-Macias et al. [57]	20	20	Halomax	Retrospective	NRR ≥ 80 vs. NRR 70–40%
			SCSA		
Morrel et al. [17]	13	8	SCSA	Retrospective	Mean NRR > 100 vs. Mean NRR < 100
Kumaresan et al. [1]	6	5	SCSA	Retrospective	NRR > +1 SD vs. NRR < −1 SD
Pardede et al. [63]	3	3	AO	Retrospective	%FSCR > 70% vs. %FSCR < 70%
	3	3	AO		
	3	3	AO		
	3	3	Halomax		
	3	3	Halomax		
	3	3	Halomax		
Paradowska-Dogan et al. [30]	14	14	SCSA	Retrospective	NRR 28% = 76–100% vs. 20–50%
Crespo et al. [65]	3	8	SCD	Prospective	PR ≥ 50% vs. PR < 50%
Love and Kenney [26]	42	42	SCSA	Retrospective	SPR > 80% vs. SPR ≤ 80%
Vicente-Fiel et al. [27]	4	4	SCD	Retrospective	OR 1.4 and 1.7 vs. 0.6 and 0.9
Kenney et al. [66]	54	33	SCSA	Retrospective	SPR: No history of subfertility vs. history of subfertility

LSmean least square mean, *ERCR* Estimated relative conception rate, *FSCR* First service conception rate, *OR* Odd ratio, *NRR* Nonreturn to estrous rate, *SPR* Seasonal pregnancy rate *CR* Conception rate

found in the LF group compared to the HF group in both bulls (random effects model, SMD = 1.21, 95% confidence interval: 0.85 to 1.57; Z-test = 6.59; $p < 0.001$) and stallions (random effects model, SMD = 0.64, 95% confidence interval: 0.44 to 0.85; Z-test = 6.14; $p < 0.001$). Statistical heterogeneity was present in the bull subgroup (heterogeneity = 46.5%; $p = 0.02$), whereas no significant heterogeneity was observed in the stallion subgroup (heterogeneity = 0%; $p = 0.52$) (Fig. 5). One study in rams, which contained two results, was excluded from the subgroup analysis due to statistical limitations. Regarding the detection methods, SDF was significantly higher in the LF group compared to the HF group in studies employing the SCSA method in bulls ($n = 6$) (random effects model, SMD = 0.99, 95% confidence interval: 0.61 to 1.38; Z-test = 5.03; $p < 0.001$) and stallions ($n = 3$) (random effects model, SMD = 0.64, 95% confidence interval: 0.43 to 0.86; Z-test = 5.95; $p < 0.001$). A significant difference was also observed in studies utilizing the Halomax/SCD method in bulls ($n = 3$) (random effects model, SMD = 1.85, 95% confidence interval: 0.68 to 3.01; Z-test = 3.10; $p = 0.002$) (Table 4). Two studies employed the TUNEL assay method; however, they were not included in the subgroup analysis due to statistical constraints.

Publication bias

The funnel plots and their corresponding bias coefficient (Begg and Mazumdar rank) for estimating the overall correlation between SDF and fertility for published studies (−0.165, $p = 0.123$) provide no evidence for publication bias among eligible studies. However, significant publication bias was observed in the overall standardized mean difference (SMD) between high and low fertility groups (0.434, $p < 0.001$) and in the subgroup analyses for the SMD in bulls (0.456, $p = 0.01$).

Discussion

This meta-analysis demonstrates the influence of sperm DNA fragmentation on male fertility in farm animals. The combined results reveal a significant negative correlation between SDF and fertility. However, considering the differences in the reproductive biology across various farm animal species, the overall finding may have limited

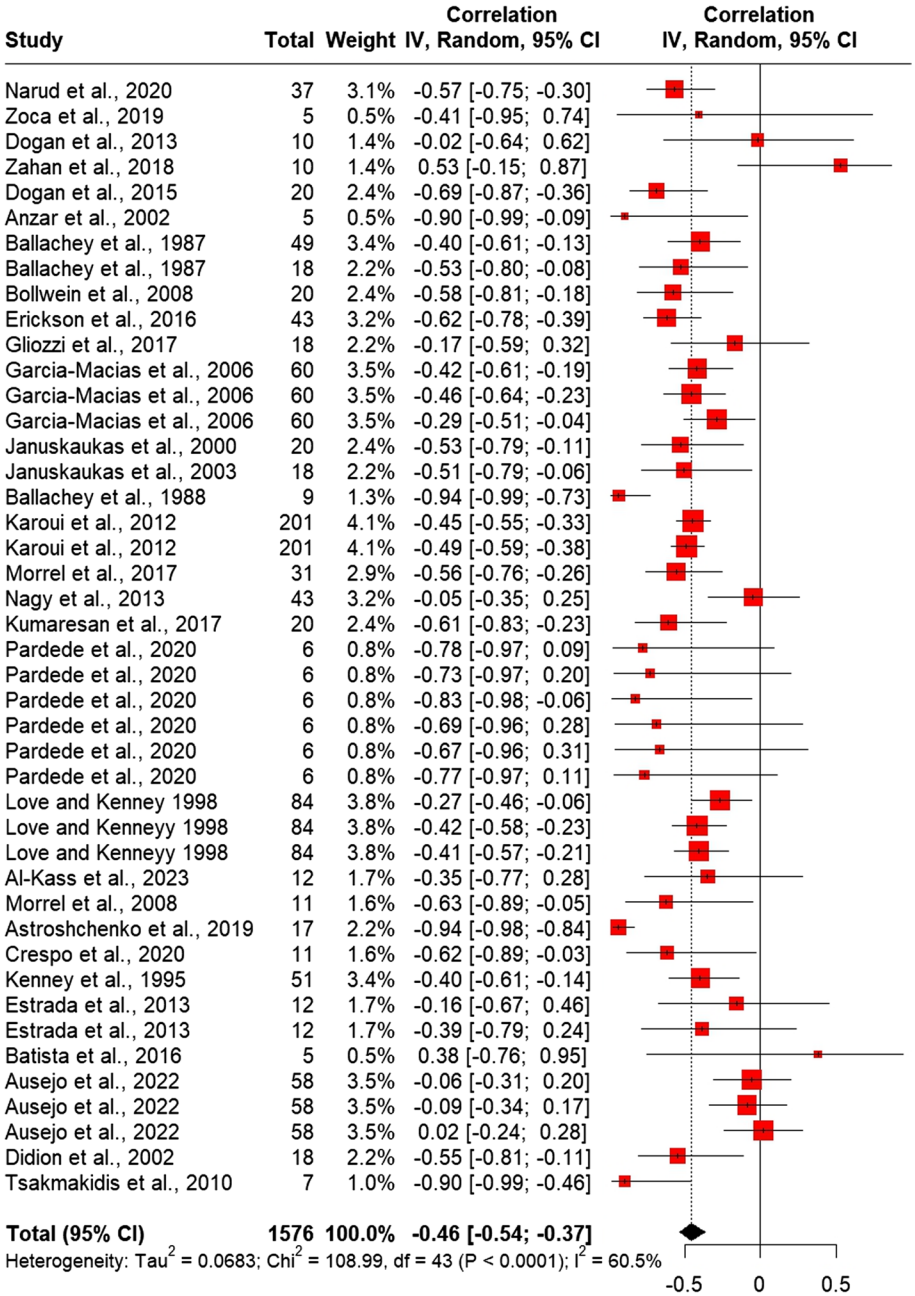

Fig. 2 Forest plot showing the results of the overall correlation coefficient between SDF and fertility parameters in farm animals. COR = −0.46, $p < 0.001$, Z-test = −8.97, heterogeneity = 60.5% ($p < 0.001$)

significance. Consequently, species-specific analyses were conducted. Our findings revealed a significant negative correlation between SDF and fertility in bulls and stallions, while no such relationship was observed in boars. When a combined analysis based on different assay types was analyzed, the results demonstrated a significant correlation between SDF and fertility using the SCSA and Halomax/SCD tests. In contrast, no such correlation was observed with the TUNEL assay. To address potential measurement bias arising from the use of multiple assays, breeds, or semen incubation times in some studies, the correlation values for each method were individually analyzed in this meta-analysis.

The results revealed high heterogeneity in the stallion subgroup analysis and moderate heterogeneity in the bull subgroup in the first part. This finding highlights

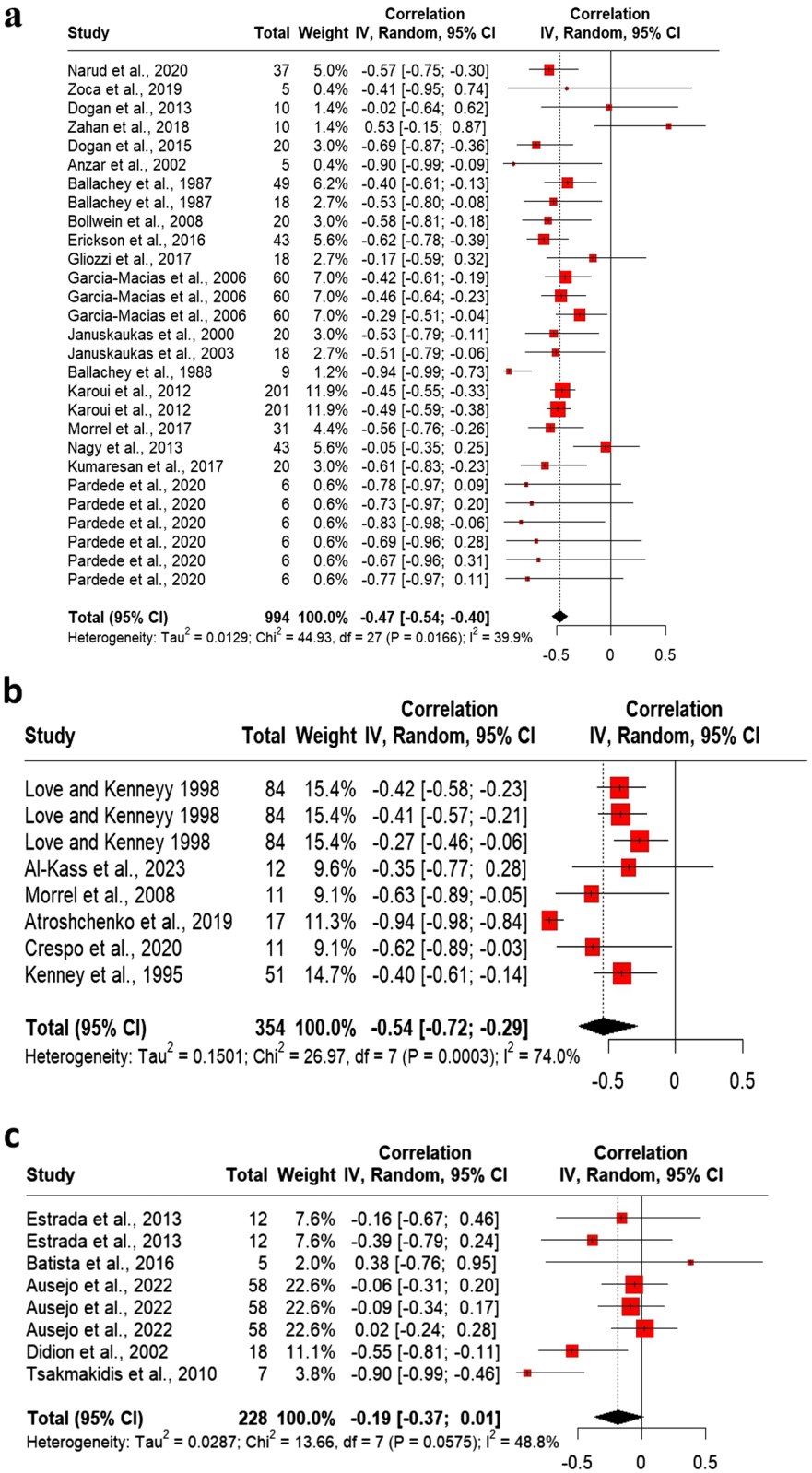

Fig. 3 Forest plot showing the correlation coefficient between SDF and fertility in (**a**) bull (**b**) stallion and (**c**) boar. COR = −0.47, p < 0.001, Z-test = −11.13, heterogeneity = 39.9% (p = 0.017). COR = −0.54, p < 0.001, Z-test = −3.83, heterogeneity = 74.0% (p < 0.001). COR = −0.19, p = 0.065, Z-test = −1.84, heterogeneity = 48.8% (p = 0.058)

Table 3 Random-effects model for the correlation between SDF and fertility using (a) SCSA in bulls (b) SCSA in stallions (c) TUNEL in bulls (d) Halomax in bulls (e) Halomax in boars

a Random-effects model (k = 13)						
	Estimate	**se**	**Z**	***p***	**CI Lower Bound**	**CI Upper Bound**
Intercept	−0.506	0.0823	−6.15	< .001	−0.667	−0.345
b Random-effects model (k = 6)						
	Estimate	**se**	**Z**	***p***	**CI Lower Bound**	**CI Upper Bound**
Intercept	−0.401	0.0570	−7.04	< .001	−0.513	−0.289
c Random-effects model (k = 3)						
	Estimate	**se**	**Z**	***p***	**CI Lower Bound**	**CI Upper Bound**
Intercept	−0.607	0.304	−2.00	0.046	−1.203	−0.011
d Random-effects model (k = 9)						
	Estimate	**se**	**Z**	***p***	**CI Lower Bound**	**CI Upper Bound**
Intercept	−0.505	0.0429	−11.8	< .001	−0.589	−0.421
e Random-effects model (k = 4)						
	Estimate	**se**	**Z**	***p***	**CI Lower Bound**	**CI Upper Bound**
Intercept	−0.0434	0.115	−0.376	0.707	−0.270	0.183

the study's limitations and necessitates cautious interpretation. The substantial heterogeneity observed among stallions may be attributed to the different fertility measures used across studies. Various fertility parameters, including seasonal pregnancy rate (SPR), pregnancy per cycle (PC), and pregnancy per first cycle (FCP), were employed. SPR is defined as the percentage of mares that become pregnant by a single stallion within the same breeding season, calculated as the number of pregnant mares divided by the total number of mares bred by that stallion in that season. PC is determined by dividing the total number of pregnancies by the total number of estrous cycles bred, expressed as a percentage. This measure includes pregnancies diagnosed between 14 and 40 days of gestation that subsequently resulted in abortion. FCP is the percentage of mares diagnosed as pregnant following their first breeding of the season, calculated as the number of mares pregnant after their first cycle divided by the total number of mares confirmed pregnant during the breeding season [26]. PC is considered a more precise measure of fertility than SPR [66]. The use of multiple fertility parameters may have contributed to variability in study outcomes, as certain measures do not account for intrinsic subfertility in mares. Additional factors, including stallion breed, sample size, semen storage method (cooled vs. frozen-thawed), and assay type (SCSA vs. Halomax), may also explain the observed heterogeneity. The studies included multiple breeds, such as Thoroughbred, Warmblood, Standardbred, Trotter, and Arabian. This breed variation, along with individual stallion and ejaculate differences, likely influenced SDF and, consequently, fertility outcomes. Sperm quality has been shown to vary across breeds, individual stallions, and ejaculates, contributing to discrepancies in fertility results [8].

Although the correlation between SDF and fertility measures in pigs was not statistically significant, a trend toward significance was observed, given the proximity of the *p*-value to the predefined threshold. Therefore, this result may not accurately reflect the true relationship due to variability in study design, differences in DNA fragmentation assessment methods, and relatively small sample sizes in some studies. Thus, we consider this finding inconclusive. The lack of a significant difference in combined TUNEL results compared to SCSA and SCD is unsurprising. The TUNEL assay is primarily designed for somatic cells, and due to the highly compacted nature of sperm chromatin, requires potentiators such as dithiothreitol (DTT) to improve accessibility. Furthermore, the large TdT enzyme used in the TUNEL assay can access and label only a limited portion of in situ DNA, which may constrain its sensitivity and accuracy in sperm DNA fragmentation analysis [70, 71].

The studies in this meta-analysis employed various sperm DNA integrity tests, each utilizing different compounds to assess sperm DNA fragmentation. The levels of DNA fragmentation detected by these assays are not directly comparable, as they rely on distinct principles and exhibit varying sensitivities and specificities [70, 71]. This methodological variability may partially explain the inconsistencies observed among the studies across different species. The SCSA evaluates sperm nuclear DNA susceptibility to acid-induced denaturation, which correlates with DNA strand breaks. This is achieved by exposing spermatozoa to an acid–detergent solution, leading to DNA denaturation at the sites of single-strand

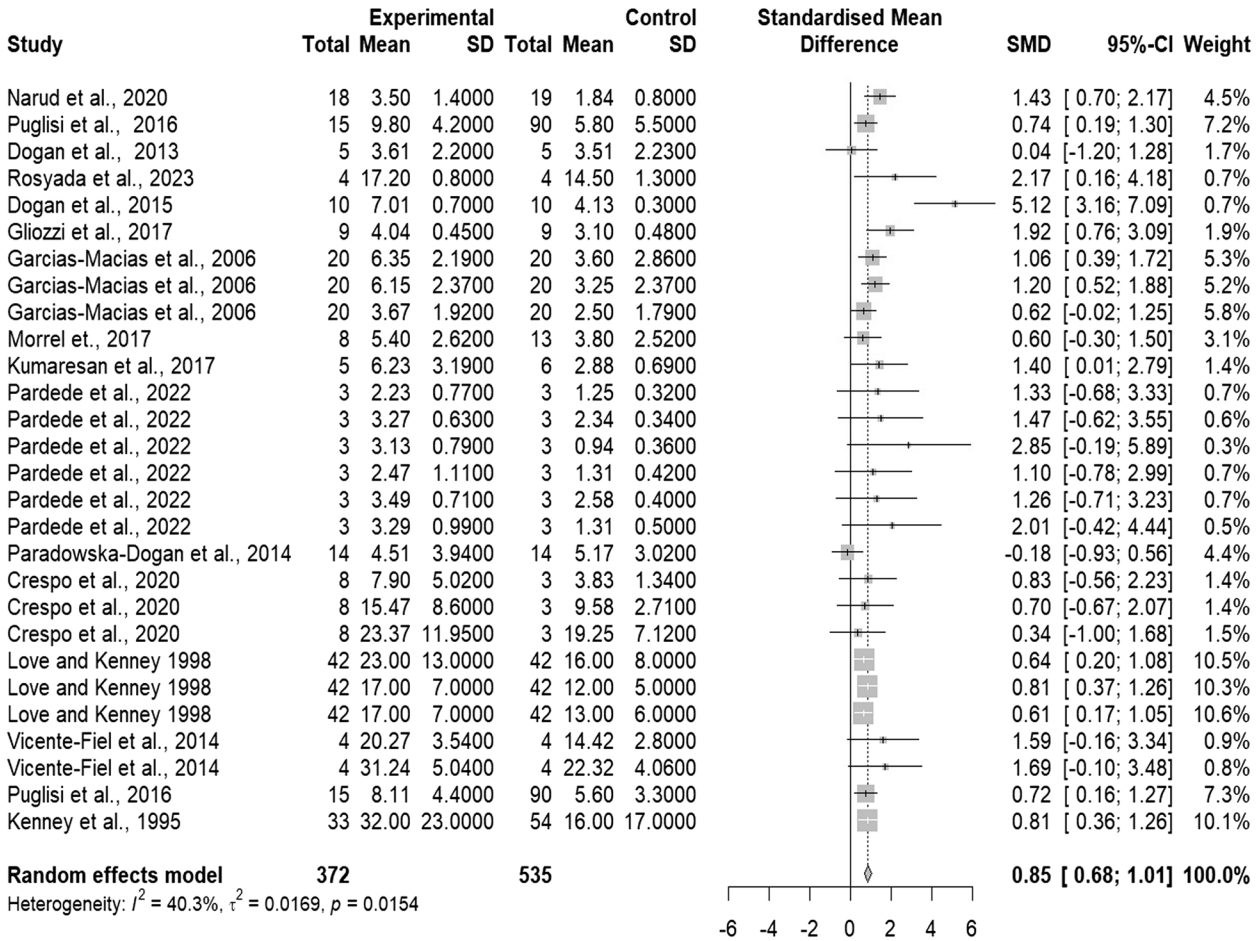

Fig. 4 Forest plot showing the results of a meta-analysis of studies comparing the effect of high and low SDF on the fertility parameters in farm animals. SMD = 0.85, $p < 0.001$, Z-test = 10.07, heterogeneity = 40.3% ($p = 0.015$)

or double-strand breaks. AO, a metachromatic dye, is then applied, with red fluorescence indicating single-stranded, denatured DNA and green fluorescence representing double-stranded, intact DNA [71, 72]. The TUNEL assay detects DNA strand breaks by labeling free 3′-OH termini on single- or double-stranded DNA. TUNEL employs terminal deoxynucleotidyl transferase, which catalyzes the addition of deoxyuridine triphosphate (dUTP) to the 3′-OH termini of fragmented DNA. The degree of fluorescent or chemical labeling attached to dUTP is directly proportional to the number of DNA strand breaks [72]. The SCD or Halo assay evaluates sperm DNA integrity through a protein depletion treatment, which induces lysis and allows the differentiation of fragmented versus intact DNA. Sperm with minimal DNA denaturation form large halos of dispersed chromatin, indicating intact DNA, whereas fragmented DNA exhibits no or minimal halo formation [72]. Despite the long-term use of some of these assays, there remains debate regarding their predictive value for fertility and pregnancy outcomes in mammals, including humans [71]. A comparative study in men, bulls, stallions, and rams reported a correlation between SCSA and TUNEL but indicated that SCSA demonstrated higher sensitivity [73]. Another study comparing SCD and SCSA reported that SCD exhibited lower repeatability than SCSA [74]. Additionally, a study in pigs comparing SCSA, TUNEL, and SCD reported a correlation between SCSA and TUNEL but not SCD [10]. Each assay presents specific advantages and limitations [6]. The present study highlights the need for standardization and the adoption of a single, validated assay for assessing sperm DNA integrity within or across species. The lack of standardization across different sperm DNA fragmentation tests can lead to false interpretations of outcomes and affect conclusions. Additionally, there is a concern that the tests themselves could potentially induce artificial DNA damage [13, 75]. Establishing a standardized method would facilitate the determination of a definitive SDF threshold, above which an animal may be classified as sub fertile.

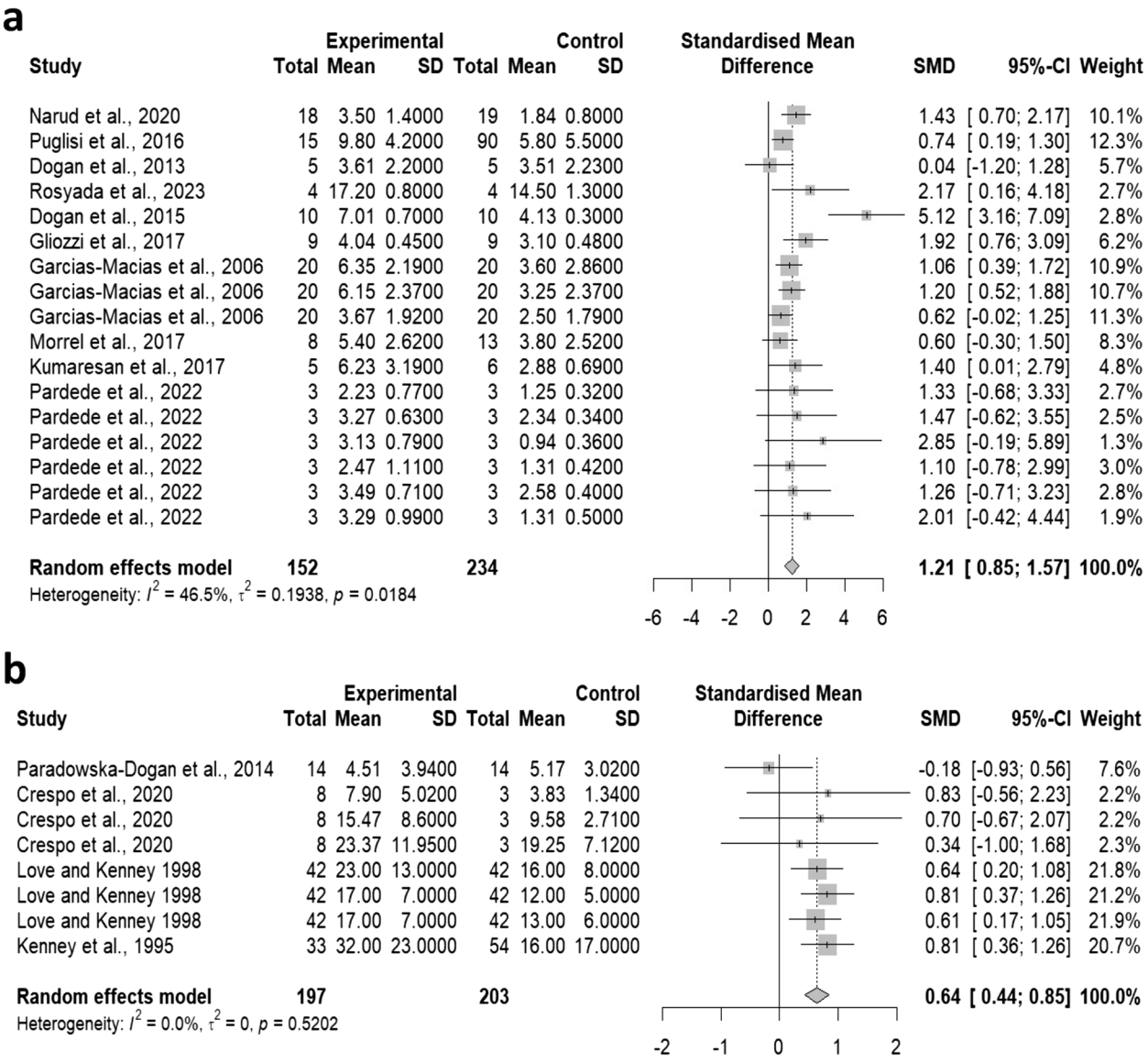

Fig. 5 Forest plot showing the results of a meta-analysis of studies comparing the effect of high and low SDF on the fertility parameters in (**a**) bulls and (**b**) stallions. SMD = 1.21, $p < 0.001$, Z-test = 6.59, heterogeneity = 46.5% ($p = 0.018$). SMD = 0.64, $p < 0.001$, Z-test = 6.14, heterogeneity = 0% ($p = 0.520$)

Table 4 Random-effects model for the results of the meta-analysis of studies comparing the effect of high and low SDF on the fertility parameters using (a) SCSA in bulls (b) SCSA in stallions (c) Halomax in bulls

a Random-effects model (k = 6)						
	Estimate	**se**	**Z**	***p***	**CI Lower Bound**	**CI Upper Bound**
Intercept	0.995	0.198	5.03	< .001	0.607	1.382
b Random-effects model (k = 5)						
	Estimate	**se**	**Z**	**p**	**CI Lower Bound**	**CI Upper Bound**
Intercept	0.644	0.108	5.95	< .001	0.432	0.856
c Random-effects model (k = 6)						
	Estimate	**se**	**Z**	**p**	**CI Lower Bound**	**CI Upper Bound**
Intercept	1.85	0.595	3.10	0.002	0.680	3.012

Tau^2 Estimator: Restricted Maximum-Likelihood

In this study, we present the first systematic analysis of the impact of sperm DNA fragmentation (SDF) on male subfertility in farm animals. Our results demonstrate a significantly higher proportion of SDF in the low-fertility group compared to the high-fertility group, based on the combined data as well as within specific subgroups, including bulls, stallions, and assessments using SCSA and Halomax/SCD. Several previous original studies have reported significant differences in mean SDF levels between high- and low-fertility animals [28, 34, 65, 66]. While some causes of subfertility and infertility have been identified [2, 76], others remain unknown [2, 77]. The inability to accurately identify subfertile males and remove them from breeding programs has considerable economic implications for livestock production and horse breeding [2]. Our findings suggest that assessing SDF could serve as a valuable tool for identifying subfertile males, thereby enabling the exclusion of low-quality ejaculates from breeding programs and improving reproductive efficiency [2]. It is of great interest to note that the bull subgroup analysis revealed a high standardized mean difference, indicating a substantial distinction between high- and low-fertility groups. This finding highlights the potential value of incorporating sperm DNA integrity testing as a pre-screening tool for bulls. With improvements in methodological consistency, standardization, and increased sample sizes, sperm DNA integrity testing in cattle holds significant promise for reducing the use of suboptimal sires, thereby enhancing the overall success of assisted reproductive technologies (ART) in this species.

One of the strengths of systematic reviews is the improved precision of the summary outcomes compared to individual studies. Subgroup estimates in bulls and stallions indicate that sperm DNA damage affects fertility. Evaluating the effect in bull, nine cases showed a standard mean difference greater than unity. Five out of eight cases in stallions were greater than the combined mean difference. Additionally, the combined SDF value in the low-fertility group was significantly higher than in the high-fertility group, suggesting that a higher proportion of DNA fragmentation negatively impacts male fertility. However, this meta-analysis has several limitations due to the heterogeneous characteristics of the included studies. The studies in cattle utilized various fertility measures, such as 56-day nonreturn to estrus (NRR56), conception rate, pregnancy rate, and estimated relative conception rate to classify bulls. Similarly, studies in pigs used different methods, including farrowing rate, pregnancy rate, and fertility direct boar index. The NRR56 refers to the percentage of cows or heifers that do not return to estrus within 56 days after AI or natural breeding. Conception rate is the percentage of cows that become pregnant after being bred, while pregnancy rate is the percentage of cows that become pregnant within a specific breeding period, considering both the frequency of breeding and the success rate of those breedings. Although these parameters may be related, they are not equivalent, and some may be subject to bias. This variability may account for discrepancies among the studies. Another limitation of this study is the use of diverse study designs within and between species. The majority of studies were retrospective, which may not have accurately captured the true DFI of an individual animal at the time of service or insemination. Genotoxic agents may influence spermatogenesis, causing fluctuations in DFI over time. Many studies assessed SDF in a limited number of males, using only a few ejaculates from a single individual. This may affect the statistical robustness of those studies. Moreover, some studies reported a correlation between SDF and fertility based on SDF results after incubating the semen for several hours. The SDF measured after chilling or immediately post-thaw may differ significantly from that measured after, for example, 6 h of incubation. The SDF in fresh, chilled, or frozen-thawed semen may also vary within species. Furthermore, the previously mentioned variability in SDF detection methods and fertility measures remains a limitation of this study.

Based on the findings of this study, it is evident that the impact of sperm DNA fragmentation on livestock fertility may only be fully recognized and accepted by livestock farmers when future research focuses on defining species-specific fertility thresholds, standardizing SDF assays, and identifying complementary fertility markers. To date, and to the best of our knowledge, no established fertility threshold for SDF has been reported for any livestock species using the TUNEL, SCD, or AO assays. One review suggested an estimated threshold above which the SCSA DNA fragmentation index (DFI) has a detrimental effect on fertility, bulls: 10–20%, horses: ~ 28%, and pigs: 6% [71]. However, no specific reference to a primary research paper was provided. Establishing a fertility threshold for SDF will be critical for integrating sperm DNA integrity testing into routine animal semen evaluation. The need for assay standardization cannot be overemphasized, as there remains an ongoing debate regarding the precise definition of DNA integrity and the optimal methods for its assessment [72]. Consistency in methodology will be essential for ensuring reliable comparisons across studies and facilitating the practical application of SDF testing in livestock breeding programs.

The present study highlights the importance of evaluating additional sperm quality parameters beyond the traditional measures of motility, concentration, and morphology for artificial insemination (AI) or selecting males

for AI. While a spermiogram can identify and exclude clear cases of male infertility or subfertility and assist in determining specific ejaculates for processing [27, 78], more subtle changes in sperm quality require advanced testing. These advanced tests assess various aspects of sperm biology, physiology, and kinematics. In addition to SDF, key parameters include velocity metrics [59, 79], capacitation status [80, 81], acrosome reaction status [82, 83], chromatin integrity [2, 59], lipid peroxidation status [84], and mitochondrial function [85]. Furthermore, recent research has begun to explore additional variables such as semen and sperm metabolites, as well as genes and proteins potentially involved in subfertility, including heat shock proteins [9, 34].

Conclusion

In conclusion, elevated sperm DNA fragmentation appears to negatively impact male fertility in bulls and stallions. Despite variability in study design characteristics and some relatively small sample sizes, the findings of this meta-analysis suggest that incorporating sperm DNA fragmentation assays into breeding soundness evaluations could enhance the accuracy of selecting high-quality males for artificial breeding programs. However, the impact of elevated sperm DNA fragmentation on fertility in farm animals warrants further investigation through well-powered studies with robust sample sizes and standardized methodologies.

Materials and methods

Identification of the literature

A comprehensive computer literature search was conducted to identify articles dealing with farm animal sperm DNA fragmentation to assess male fertility. The PUBMED, Google Scholar, and Springer Link databases were searched without time restrictions, with a December 31, 2023 cutoff date. Key operators used in the systematic search included "sperm DNA fragmentation," "fertility," "bull," "boar," "ram," "buck," "stallion," "semen" and "animal." These key terms were employed in combination with the "AND" operator. Additionally, references from the acquired studies and a review article were manually extracted.

Inclusion and exclusion criteria

When assessing sperm DNA damage, various abnormalities such as defective protamination, chromatin decompaction, sperm DNA fragmentation, and impaired DNA cross-linking are typically measured. In this systematic review, only studies that looked into sperm DNA fragmentation in farm animals were included. Studies dealing with sperm chromatin compaction impairment were excluded from the meta-analysis. Studies were included if they measured the relationship between sperm DNA fragmentation and fertility in the bull, stallion, boar, ram, and goats. The outcomes of interest were non-return to estrus rate (NRR), pregnancy rate (PR), or farrowing rate.

The inclusion criteria comprised studies that assessed sperm DNA damage using the most commonly employed methods, including SCSA, TUNEL, SCD/Halomax, Comet assay, and AO assays; studies reporting natural or artificial insemination in females; articles published in English; and both retrospective and prospective study designs. Studies evaluating the effect of sperm DNA fragmentation in vitro (e.g., on IVF or ICSI outcomes), those using sorted sperm, and studies with unspecified designs were excluded.

Data extraction

Eligible studies were selected through a two-step process. Initially, the titles and abstracts of articles identified through the electronic search were reviewed. Full articles of citations that met the predetermined selection criteria were then obtained. Subsequently, the full articles were examined, and relevant studies were selected. For each publication, the extracted data included the name of the first author, species of the animal, year of publication, sperm DNA assay type, sample size (control, experimental, and total), study design (prospective, retrospective, or unknown), and semen type (fresh, frozen-thawed, chilled, or liquid-stored). To assess the association between sperm DNA fragmentation and male fertility, Pearson or Spearman correlation coefficient values were recorded for each study. The mean and standard deviation (SD) SDF values in the high and low fertility groups were also recorded.

Statistical analysis

The correlation coefficient between SDF and fertility parameters was analyzed using Jamovi software (version 2.3.21, The Jamovi Project) and R (version 4.4.3). The mean and standard deviation (SD) of SDF in studies comparing high and low fertility groups were also entered into the software programs. A meta-analysis was conducted using a random-effects model where appropriate. R software generated forest plots, while Jamovi was employed for random-effects model tables. Heterogeneity across studies was assessed using the I^2 statistic, which quantifies variability in effect sizes [86]. Potential sources of heterogeneity were explored by examining variations in population characteristics and exposure factors. Studies were subgrouped based on animal species and the assays used for SDF measurement, including SCSA, TUNEL, SCD, Comet, and AO. Publication bias was assessed through funnel plot analysis, and asymmetry in the primary outcome was visually evaluated [87]. A significance level of $p < 0.05$ was applied to all statistical tests.

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Authors' contributions
Conceptualization, K.O.A.; methodology, K.O.A. and J.I.I.; validation, K.O.A., Z.L.K., G.A., A.P. and W.N.; data curation, K.O.A., Z.L.K., G.A., A.P. and W.N.; writing—original draft preparation, K.O.A.; writing—review and editing, J.I.I., G.A., A.P and W.N.; visualization K.O.A. and J.I.I.; supervision, W.N.; All authors have read and agreed to the published version of the manuscript.

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Data available on request from the authors.

Declarations

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Competing interests
The authors declare no competing interests.

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