REVIEW ARTICLE

Sperm DNA Extraction in Livestock: Structural Challenges, Methodological Advances, and Applications in Assisted Reproductive Technologies

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Abstract

Assisted reproductive technologies (ART), including artificial insemination, *in-vitro* fertilization, intra-cytoplasmic sperm injection, and embryo transfer, are central to genetic improvement and reproductive management in livestock. Beyond conventional semen parameters, sperm DNA integrity has emerged as a critical determinant of fertilization success, embryo development, pregnancy establishment, and offspring viability. However, the extraction of high-quality DNA from spermatozoa remains technically demanding due to the highly compact chromatin structure formed by protamine-rich DNA packaging and extensive disulfide cross-linking, which renders sperm nuclei resistant to standard lysis procedures. This review synthesizes current knowledge on sperm DNA extraction methodologies across livestock species, with emphasis on the structural and biochemical challenges unique to spermatozoa. Conventional extraction approaches, including organic solvent- and salt-based methods, are evaluated alongside modern and modified techniques incorporating strong reducing agents, optimized enzymatic digestion, chaotropic lysis buffers, and solid-phase purification systems. Recent advances, such as TCEP-based reduction, magnetic bead- and silica column–based platforms, and automated high-throughput workflows, have improved DNA yield, purity, integrity, and reproducibility, making them increasingly suitable for downstream molecular applications. Species-specific differences in chromatin organization among bovine, buffalo, ovine, caprine, porcine, and equine spermatozoa are highlighted, underscoring the need for tailored extraction protocols. The review also emphasizes the importance of rigorous DNA quality assessment for applications in genomic selection, epigenetic profiling, fertility prediction, and ART outcome optimization. Finally, key research gaps are identified, including the need for protocol harmonization, inter-laboratory validation, and integration with high-throughput omics and data-driven analytical approaches to support sustainable livestock breeding.

Key words: Assisted reproductive technologies, DNA integrity, Genomic selection, Sperm chromatin structure, Sperm DNA extraction

Ind J Vet Sci and Biotech (2026): 10.48165/ijvsbt.22.3.01

Introduction

Assisted reproductive technologies (ART) such as artificial insemination (AI), *In-Vitro* Fertilization (IVF), intra-cytoplasmic sperm injection (ICSI), and embryo transfer have significantly reshaped animal breeding practices. These tools accelerate genetic improvement, help conserve valuable or endangered germplasm, and enhance reproductive efficiency across livestock species. By enabling rapid dissemination of superior genetics and improving fertility outcomes, ART has become indispensable in modern breeding systems. At the molecular level, however, spermatozoal integrity -particularly DNA quality- is critical for the success of these technologies because it directly influences fertilization, embryo development, and progeny viability (Khezri *et al*., 2019; Abah *et al*., 2025).

Progress in genomics, proteomics, and bioinformatics has further boosted livestock reproductive performance by facilitating genetic evaluation, fertility prediction, and trait selection (Crowe *et al*., 2018; Cai *et al*., 2019). Nutritional strategies designed to enhance fertility have also advanced substantially (Crowe *et al*., 2018). Since the paternal

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How to cite this article: Khirbat, R., Dinesh, Virmani, M., & Kumar, A. (2026). Sperm DNA Extraction in Livestock: Structural Challenges, Methodological Advances, and Applications in Assisted Reproductive Technologies. Ind J Vet Sci and Biotech, 22(3), 1-8.

Source of support: Nil

Conflict of interest: None

Submitted 17/03/2026 **Accepted** 24/03/2026 **Published** 10/05/2026

genome plays a central role in early embryogenesis and offspring development, obtaining high-quality sperm DNA is fundamental for studies involving molecular markers, epigenetics, and genomic selection (Robertson *et al.*, 2024). Animal tissues such as semen, blood, hair, and saliva all serve as DNA sources, but spermatozoa present unique challenges due to their specialized chromatin configuration.

DNA extracted from sperm is highly essential for research that explores the genetic and epigenetic factors shaping reproductive success and long-term health outcomes. Numerous sperm-based studies depend on DNA extraction, and various methods have been distinguished address the biochemical complexity of sperm cells (Denis *et al.*, 2024). Preventing disease transmission through semen was one of the early motivations behind Artificial Insemination in livestock, yet extracting pure DNA from semen remains difficult due to contaminants present in the ejaculate, diluents, and cryopreservation additives (Kumar *et al.*, 2014).

As evaluation of sperm DNA integrity becomes more mainstream in breeding programs, its significance as an indicator of fertility potential is widely recognized. DNA fragmentation, oxidative stress, or molecular damage negatively influences fertilization success, embryo competence, and pregnancy establishment (Robertson *et al.*, 2024). Consequently, obtaining intact and high-purity DNA is indispensable. Molecular techniques such as PCR, SNP genotyping, methylation profiling, and next-generation sequencing increasingly rely on sperm DNA for cattle, buffalo, sheep, goat, and pig breeding programs (Chandaka *et al.*, 2024; Parmar *et al.*, 2024).

Genomic selection programs also depend heavily on accurate genotyping, which requires pure, high-quality DNA. PCR remains foundational for analyzing genetic markers, but its success is influenced by template purity and the absence of inhibitors (Manuja *et al.*, 2010). Recent attention has also turned to sperm epigenetics, including methylation studies that explore paternal contributions to development and transgenerational inheritance. These applications demand DNA of exceptional integrity (Khezri *et al.*, 2019).

High-quality sperm DNA is critical for ART outcomes, influencing fertilization, embryo development, and pregnancy success in IVF and ICSI (Robertson *et al.*, 2024; Abah *et al.*, 2025). It is also essential in conservation for parentage testing and genetic diversity assessment. Sperm DNA extraction is challenging due to protamine-rich, highly condensed chromatin stabilized by disulfide cross-links, along with resistant membrane structures and minimal cytoplasm (Aitken and De Iuliis, 2007). Efficient extraction therefore requires strong reducing agents and proteolytic treatments to release DNA (Miller *et al.*, 2010).

While commercial kits are rapid but costly, traditional phenol–chloroform methods remain common despite hazards and labor intensity. Additives such as proteinase K and DTT improve DNA yield (Fjelstrup *et al.*, 2017). Modern approaches, including magnetic-bead and automated systems, vary in efficiency and applicability, and no universal protocol exists (Montjean *et al.*, 2023). This review evaluates these methods to guide development of standardized, species-specific protocols.

Spermatozoa Structure and Challenges in DNA Extraction

Unique Morphological and Biochemical Features of Sperm Cells

Spermatozoa are specialized haploid cells delivering paternal DNA to oocytes. The head contains an acrosome with enzymes for zona pellucida penetration and a compact nucleus, while the tail and mid-piece with helically arranged mitochondria enable motility and ATP production (Leung *et al.,* 2023). Their membranes, rich in polyunsaturated fatty acids, sterols, and glycoproteins, are essential for capacitation, sperm oocyte recognition, and fusion, with composition varying across cattle species and affecting cryotolerance and fertility (Singh *et al.*, 2025). Mature sperm are transcriptionally inactive due to dense chromatin and minimal cytoplasm, which safeguards DNA during transit (Arévalo *et al.,* 2022; Björndahl and Kvist, 2014). They also carry regulatory RNAs and epigenetic marks that influence early embryonic development and inheritance (Abah *et al.*, 2025; Champroux *et al.*, 2016).

Protamine–Histone Exchange and Chromatin Compaction

During spermiogenesis, histones are gradually replaced by transition proteins and, eventually, protamines, which are small, arginine-rich nuclear proteins. This protamine-histone exchange results in up to six times more condensation than nucleosomal chromatin, allowing for tight DNA packing into a toroidal shape (Arévalo *et al.,* 2022). Hypercondensation protects sperm DNA from enzymatic, chemical, and physical damage during transit.

Different livestock species have different levels of protamine (P1 and P2 ratios), which affects sperm fertility and chromatin stability. Precise control of this exchange is essential; sperm integrity can be compromised by inadequate protamination or residual histones, which increases vulnerability to DNA fragmentation and reduces fertilization potential. This unique packaging limits the accessibility of lysis buffers, enzymes, and chaotropic agents, meaning that standard extraction techniques often fail to efficiently disrupt sperm chromatin (Björndahl and Kvist, 2014; Champroux *et al.*, 2016).

Disulfide Bond Stabilization and its Implications in DNA Release

During epididymal maturation, protamines form disulfide bonds between cysteines, stabilizing sperm chromatin and making it highly resistant to denaturation and enzymatic digestion. Ooplasmic glutathione reduces these bonds after fertilization, enabling chromatin decondensation and paternal genome activation (Champroux *et al*., 2016; Leung *et al*., 2023).

The sperm nuclear membrane's dense disulfide cross-links require strong reducing agents like DTT or β-mercaptoethanol for effective lysis (Ward, 2020; Khirbat *et al*., 2024). Disulfide stabilization varies by species, with livestock sperm (e.g., bovine, equine) more heavily cross-linked than human sperm (Bouhadana *et al*., 2025). These features necessitate specialized protocols that reduce disulfide bonds, remove protamines, and preserve DNA integrity during chromatin decondensation.

Species-Specific Variations among Livestock (bovine, ovine, caprine, porcine, equine, etc)

Bovine spermatozoa have strong disulphide cross-linking and highly compact nuclei, which improve DNA stability (Leung *et al*., 2023). There are small differences in protamine ratios between caprine and ovine sperm that affect cryotolerance and chromatin packaging (Singh *et al*., 2025). During cryopreservation, pig sperm have a less compacted chromatin structure and higher histone retention, which is related with increased DNA susceptibility (Singh *et al*., 2025). Equine sperm's different chromatin structure and greater protamine heterogeneity may be linked to species-specific variation in fertilization (Champroux *et al*., 2016). Understanding interspecies differences is critical for optimizing reproductive biotechnologies, semen preservation methods, and the outcomes of aided fertilization in livestock.

Influence of Semen Preservation Methods (Fresh, Extended, or Frozen)

Semen processing and storage methods significantly affect DNA integrity and extraction success. Cryopreservation, widely used in livestock breeding, induces oxidative stress, membrane damage, and ice crystal formation, leading to increased DNA fragmentation during freeze–thaw cycles (Paoli *et al*., 2014). Post-thaw sperm exhibit higher DNA fragmentation indices than fresh samples, complicating genomic and epigenetic analyses (Amidi *et al*., 2020; Ledesma *et al*., 2021; Virmani *et al*., 2020). Cryoprotectants and extenders may also introduce inhibitors, while extended storage increases oxidative damage and contamination risks (Kern and Liu, 2025; Kanwar *et al*., 2026).

Contamination by Somatic Cells and Inhibitors

Semen often contains leukocytes and epithelial cells that release excess genomic DNA, masking sperm-derived DNA if not removed (Sakkas and Alvarez, 2010). Co-extracted extender components can inhibit PCR and purification processes (Fouladvandi *et al*., 2025). Additionally, seminal DNases may degrade DNA unless inhibited by agents such as EDTA or proteinase K (Gosálvez *et al*., 2021). Effective pre-processing, including somatic cell removal and washing, is therefore essential.

DNA Damage during Extraction

Following chromatin decondensation, sperm DNA becomes highly vulnerable to damage. Mechanical stress (e.g., pipetting, vortexing) and oxidative factors can cause strand breaks (Lee *et al*., 2022). As sperm lack DNA repair mechanisms, such damage is irreversible and affects downstream analyses (Bouhadana *et al*., 2025). DNase activation during lysis may further degrade DNA, emphasizing the need for gentle handling and nuclease inhibition.

Conventional DNA Extraction Methods

Organic Solvent Based Extraction (Phenol-Chloroform, CTAB, SDS)

Organic solvent-based methods remain widely used for genomic DNA isolation. Phenol–chloroform extraction employs detergents to lyse cells and denature proteins, followed by phase separation to yield highly purified DNA by removing lipid and protein contaminants (Barbier *et al*., 2019). CTAB (cetyltrimethylammonium bromide) extraction is particularly effective for samples rich in polysaccharides, where CTAB–nucleic acid complexes enable separation from impurities during chloroform extraction (Turaki *et al*., 2017). SDS is commonly used to disrupt membranes and solubilize proteins, facilitating DNA release (Arakawa *et al*., 2024). Despite their efficiency, these methods are labor-intensive, involve hazardous chemicals, and require multiple centrifugation steps, limiting their suitability for high-throughput or routine clinical applications.

Salt Precipitation and Alcohol Precipitation Methods

The salting-out method and other salt-based precipitation processes provide a safer and easier substitute for organic solvents. Proteins are precipitated by high salt concentrations like sodium chloride or ammonium acetate, leaving DNA in solution. After that, ethanol or isopropanol precipitation is used to retrieve DNA. These are simple, economical, and appropriate techniques for handling big sample sizes (Hernández *et al*., 2018). Ethanol precipitation is a widely used method for the last stage of purification or concentration. It is quite effective at concentrating DNA after enzymatic or detergent-based lysis, even if it cannot eliminate all impurities on its own (Green and Sambrook, 2016). Salt precipitation techniques are less dangerous and more cost-effective as compared to organic solvent-based methods. But while working with samples that are high in proteins, lipids, or PCR inhibitors, they may generate DNA that is less pure.

Limitations of Conventional Methods for Sperm DNA

Sperm chromatin's unique structure makes conventional DNA extraction methods largely ineffective. DNA is tightly packed by protamines and stabilized by disulfide bonds, resisting standard lysis like phenol–chloroform or salting-out (Bouhadana *et al*., 2025). Organic solvents cannot fully remove protamine-associated proteins, often yielding damaged or low-quality DNA (Lee *et al*., 2022), and SDS alone is insufficient, requiring strong reducing agents such as DTT or β-mercaptoethanol.

Multiple pipetting, incubation, and centrifugation steps in traditional protocols can mechanically shear DNA during chromatin decondensation (Gosálvez *et al*., 2021), and damage is irreversible due to lack of repair mechanisms. Somatic cell contamination can further mask sperm DNA if not removed (Sakkas and Alvarez, 2010). These limitations highlight the need for targeted, species-specific extraction methods for forensic, epigenetic, and ART applications.

Modern and Modified DNA Extraction Techniques

Use of Reducing Agents (DTT, β-Mercaptoethanol, TCEP)

The compact structure of sperm chromatin, stabilized by protamines and disulfide bonds, makes DNA extraction challenging. Efficient release requires strong reducing agents to break these bonds and partially decondense chromatin. Traditionally, thiol-based reducers like DTT and β-mercaptoethanol have been used, but they have limitations including toxicity, odor, instability, and reduced efficiency under certain pH conditions.

Tris 2-carboxyethyl phosphine (TCEP) has emerged as a more reliable alternative. Odourless, stable, and active across broad conditions, TCEP (50 mM) in guanidinium thiocyanate lysis buffers with bead homogenization enables rapid sperm disruption, often eliminating extended proteinase K digestion and achieving >90% DNA recovery via spin columns (Wu *et al*., 2015). TCEP also improves sperm RNA extraction under acidic conditions and enhances DNA yields from challenging forensic samples when combined with stabilizers like iodoacetamide (Roszkowski and Mansuy, 2021).The comparative characteristics of different reducing agents used for sperm DNA extraction are mentioned in Table 1. Although cost and buffer compatibility may limit widespread use, TCEP's efficiency, stability, and versatility make it increasingly preferred for high-quality sperm DNA extraction in research and applied settings.

Enzymatic Digestion Approaches (Proteinase K, Pronase, Lysozyme)

The introduction of strong reducing agents has substantially improved approaches to sperm chromatin decondensation, and tris 2-carboxyethyl phosphine (TCEP) has emerged as one of the most effective among them. By efficiently breaking disulphide bonds within the protamine-stabilised sperm nucleus, TCEP promotes loosening of the highly compact chromatin structure and improves access to nucleic acids. Nevertheless, even with efficient chemical reduction, complete chromatin relaxation is not always achieved, as residual nuclear and membrane-associated proteins may continue to restrict DNA release.

For this reason, enzymatic digestion remains an important supporting step in many extraction protocols. Proteinase K is still the most commonly used protease because of its broad activity range and stability under the harsh conditions typically required for sperm lysis. That said, recent work has shown that prolonged enzymatic digestion may not be necessary when strong reducing agents such as TCEP are used in combination with mechanical disruption. In such cases, brief or minimal proteinase K treatment has been reported to yield DNA quantities comparable to those obtained with extended digestion, allowing for faster and more practical extraction workflows (Wu *et al*., 2015).

Table 1: Comparative characteristics of reducing agents used for sperm DNA extraction

Feature	Dithiothreitol (DTT)	β-Mercaptoethanol (β-ME)	Tris(2-carboxyethyl) phosphine (TCEP)
Chemical class	Thiol-based reducer	Thiol-based reducer	Phosphine-based reducer
Reducing strength	Moderate to strong	Moderate	Strong
Stability	Limited; prone to oxidation	Low; volatile and unstable	High; resistant to oxidation
Odour	Mild but noticeable	Very strong, unpleasant	Odourless
Toxicity	Moderate	High	Lower than thiol-based agents
pH tolerance	Reduced activity at extreme pH	Reduced activity at extreme pH	Active over a wide pH range
Compatibility with acidic conditions	Limited	Limited	High
Effectiveness in sperm chromatin decondensation	Effective; often requires long incubation	Effective but less consistent	Highly effective and rapid
Requirement for proteinase K digestion	Usually required	Usually required	Often reduced or eliminated
Application in RNA extraction	Limited	Limited	Highly suitable
Use in forensic samples	Moderate	Moderate	High
Cost	Low	Low	Higher
Overall suitability for sperm DNA extraction	Widely used but with limitations	Less preferred due to toxicity	Increasingly preferred

Additional enzymes may be employed in specific sample types or experimental settings. For example, collagenase or DNase treatment can facilitate sperm recovery or remove extracellular DNA contamination in testicular or tissue-derived sperm preparations. Pronase, a broad-spectrum protease, has been explored for particularly resistant samples, although careful optimization is required to avoid over-digestion and potential DNA fragmentation.

Taken together, modern sperm DNA extraction strategies increasingly favour an integrated approach in which efficient chemical reduction, particularly with TCEP, is paired with controlled enzymatic digestion. This balanced methodology minimises enzyme exposure while ensuring effective chromatin decondensation, resulting in improved DNA yield, integrity, and overall workflow efficiency.

Detergent- and Chaotropic Agent-Based Lysis Buffers

Efficient DNA extraction is strongly influenced by the chemical composition of the lysis buffer. Chaotropic salts such as guanidinium thiocyanate are key components of modern protocols, as they disrupt protein–protein and protein–DNA interactions, promote chromatin expansion, and enhance DNA binding to silica or magnetic matrices. Detergents including SDS, Triton X-100, or NP-40 further assist by solubilising lipid membranes and denaturing proteins. Careful optimisation of chaotrope–detergent combinations is essential to avoid DNA shearing or inhibition of solid-phase DNA binding. This approach is well illustrated by the method described by Wu *et al.* (2015), which integrates a guanidinium-based buffer with TCEP and bead homogenization to achieve complete sperm lysis at room temperature. Nevertheless, thorough washing steps are required, as residual chaotropic salts and detergents can interfere with enzymatic applications such as PCR and sequencing.

Commercial Extraction Kits Tailored for Sperm DNA

Commercial DNA extraction kits have evolved to address sperm chromatin's compact structure, incorporating strong reducing agents like TCEP or DTT, enhanced proteases, and specialized lysis buffers for effective chromatin decondensation, often combined with silica- or magnetic bead purification for reliable DNA recovery. Although comprehensive comparisons are limited, modern kits generally provide better standardization and reproducibility, which is crucial in clinical, forensic, and ART settings. Limitations include higher cost, less flexibility for protocol customization, and occasionally lower DNA yields than lab-optimized methods. Choosing between commercial kits and custom protocols therefore balances convenience and consistency against maximal DNA recovery.

Magnetic Beads and Silica Column Based Extraction Systems

Solid-phase extraction technologies dominate modern DNA purification due to their efficiency and suitability for automation.

Silica Spin Columns

Due to their affordability, efficiency, and compatibility with numerous lysis chemicals, silica column systems are still in widespread use. The flexibility of silica processes is demonstrated by the high efficiency (Wu *et al.,* 2015), where TCEP-based homogenates produced >90% recovery on commercial silica columns.

Magnetic Beads

Magnetic bead-based extraction employs solid-phase reversible immobilization (SPRI) to capture DNA. These systems offer gentle handling, scalability, and easy integration with automated platforms. A 2024 study showed that lengthy DNA fragments appropriate for nanopore sequencing were maintained using a magnetic bead-based extraction technique, with read-length N50 values of about 9 kb and accurate repeat expansion detection. Additionally, in diagnostic applications, bead-based systems have demonstrated higher sensitivity, surpassing silica columns in the detection of infections like *Trypanosoma cruzi* (Farani *et al*., 2025). Despite their benefits, magnetic bead systems necessitate meticulous optimisation of elution volumes, washing conditions, and bead chemistry to guarantee optimal DNA integrity.

Automated and High-Throughput Extraction Platforms

Automation is now central to molecular labs, enabling fast and reproducible DNA extraction. Recent systems include magnetic bead–based platforms for large-scale pathogen detection and benchtop instruments like the T-Prep24, which process 24 samples in ~30 min using silica beads, as well as automated workflows for high-molecular-weight DNA suitable for long-read sequencing. A 2024 study showed that Fire Monkey chemistry on a liquid-handling robot produced ultra-long DNA (N50 ~44 kb) without post-extraction size selection. Comparative analyses of platforms such as QIA Symphony, Maxwell RSC, and KingFisher Apex highlight that performance depends on sample type, storage, buffer compatibility, and pre-treatment. Automated systems therefore require application-specific validation rather than assuming universal efficiency.

Comparative Evaluation of Extraction Protocols

Sperm chromatin's tight protamine packing and disulfide cross-links make conventional extraction methods, like phenol–chloroform or salting-out, largely ineffective (Oliveira *et al*., 2007). These approaches often leave protamine-associated proteins, reducing yield and damaging DNA (Lee *et al.*, 2022), while SDS alone cannot break disulfide bonds, necessitating reducing agents such as DTT or β-mercaptoethanol. Multiple processing steps can mechanically shear DNA, and since sperm lack repair mechanisms, damage is permanent (Gosálvez *et al*., 2021). Somatic cell contamination further masks sperm DNA if not removed (Sakkas and Alvarez, 2010). Species-specific, sperm-targeted protocols are therefore essential. Comparative evaluations are summarized in Table 2.

Criteria for Evaluation: Yield, Purity, Integrity, Cost, and Reproducibility

A thorough comparative assessment of extraction protocols mainly focus on various performance metrics:

Yield: The absolute quantity of DNA recovered (e.g., in ng or µg), as measured by spectrophotometry, fluorometry, or gel quantification.

Purity: The purity of extracted DNA is commonly assessed using the absorbance ratio (A_{260}/A_{280}), which indicates protein, phenol and other contaminants.

Integrity: The integrity of sperm DNA is evaluated by analyzing its molecular weight profile and fragmentation pattern, commonly assessed using agarose gel electrophoresis.

Cost: It includes labor, time, and reagent expenses, which are particularly important in breeding or genetic marker laboratories.

Reproducibility: It mainly depends on consistency across laboratories, including inter-laboratory, batch-to-batch, and operator-related variations.

These factors enable the evaluation of raw DNA recovery as well as practical applicability for labs with varying resource restrictions and downstream applications (PCR, sequencing, and diagnostics).

Applications in Assisted Reproductive Technologies

Sperm DNA integrity is crucial for ART, affecting fertilization, embryo development, and pregnancy outcomes (Simon *et al*., 2014). Oocytes have limited repair capacity, so high DNA fragmentation can cause failed fertilization or early embryonic loss, often appearing in later stages in cattle despite normal motility (Fatehi *et al*., 2006; Kumar *et al*., 2014).

Intact sperm DNA is also essential for genomic selection and marker-assisted breeding, ensuring accurate SNP genotyping, whole-genome sequencing, and reliable predictions, while fragmented DNA can reduce efficiency and skew results in large-scale breeding (Tutt *et al*., 2000; Hayes *et al*., 2009; Kumari *et al*., 2019; Khirbat *et al*., 2025).

For parentage verification and conservation, high-quality DNA enables accurate microsatellite or SNP testing, preserves pedigree integrity, and assesses genetic diversity in endangered breeds (Blackburn, 2012; Oldenbroek, 2023). Cryopreservation exposes sperm to stress that can damage DNA undetected by motility checks (McClure *et al.,* 2018). Adding DNA integrity tests in AI centers improves quality control, identifies poor cryotolerance, and optimizes freezing thawing protocols for consistent ART outcomes.

Table 2: Comparative evaluation of commonly used sperm DNA extraction protocols in livestock

Extraction Method	Yield	Purity (A_{260}/A_{280})	DNA Integrity	Cost & Labor	Reproducibility	Major Advantages	Key Limitations	Typical Applications
Phenol–chloroform method	High	Moderate–High (risk of phenol conta-mination)	Moderate (risk of shearing)	Low reagent cost; labor-intensive and time-consuming	Moderate (operator dependent)	High DNA yield; effective protein removal	Toxic reagents; residual inhibitors; not ideal for high-throughput	Conventional PCR, microsatellite analysis
Salting-out method	Moderate	Moderate	Moderate	Low cost; simple workflow	Moderate	No organic solvents; safer handling	Incomplete protein removal; lower purity	Routine genotyping, basic PCR
Chelex-based extraction	Low–Moderate	Low–Moderate	Low	Very low cost; rapid	Low	Fast and simple; minimal reagents	Poor DNA integrity; not suitable for long-term storage	Forensic screening, quick PCR
Silica column–based commercial kits	Moderate	High	High	Higher reagent cost; low labor	High	Standardized, clean DNA; minimal inhibitors	May underperform with highly compact sperm chromatin	PCR, qPCR, SNP genotyping
Magnetic bead–based kits	Moderate–High	High	High	High cost; automation-friendly	Very high	Scalable; high reproducibility; automation compatible	Expensive equipment and consumables	High-throughput genomics, sequencing
Modified sperm-specific protocols (DTT + Proteinase K)	High	High	Very high	Moderate cost; moderate labor	High	Efficient chromatin decondensation; preserves integrity	Protocol complexity; longer processing time	NGS, epigenetic studies, ART research
Forensic differential extraction methods	Variable	Moderate–High	Moderate	High labor; specialized	Moderate	Effective separation from epithelial cells	Not optimized for livestock sperm	Mixed-sample analysis

Conclusion

In conclusion, sperm DNA extraction has evolved into a critical component of modern reproductive biotechnology in livestock, driven by the unique structural complexity of sperm chromatin and the growing demands of advanced molecular and reproductive applications. Technological improvements in lysis strategies, reducing agents, enzymatic digestion, and purification systems have significantly enhanced the recovery and quality of sperm DNA, enabling more reliable use in assisted reproductive technologies and genomic analyses. High-quality sperm DNA is now recognized as a key determinant of reproductive success, influencing fertilization efficiency, embryo development, and the genetic potential of offspring. However, variability in extraction protocols and quality assessment practices across laboratories continues to limit reproducibility and comparative research. Therefore, the development of standardized, species-specific, and application-oriented protocols, combined with integration of high-throughput omics technologies and collaborative multidisciplinary efforts, will be essential for maximizing the diagnostic, reproductive, and genetic improvement potential of sperm DNA analysis in livestock breeding programs.

Future Perspectives and Research Gaps

Sperm DNA extraction and analysis face several challenges that limit their broad application in reproduction and livestock breeding, as species-specific chromatin differences including variations in protamine content, disulfide bonding, and membrane stability mean that human- or model-based protocols often yield inconsistent DNA (Aitken and De Iuliis, 2009). Standardization remains lacking, as variability in lysis buffers, reducing agents, and purification methods hinders comparisons and downstream analyses, highlighting the need for validated protocols with defined quality controls and multicenter testing (Barratt *et al.,* 2010). Furthermore, integration with high-throughput omics and artificial intelligence offers opportunities to expand analyses to epigenetics and genome-wide variation, enabling the linkage of molecular data with fertility and embryo outcomes (Kumar *et al.,* 2021). Ultimately, accurate sperm DNA assessment can refine sire selection, reduce reproductive inefficiency, enhance genetic gain, and improve animal welfare by lowering embryonic loss, underscoring the importance of developing field-adapted and practical applications for livestock breeding (Diskin and Kenny, 2016).

Acknowledgments

The authors sincerely acknowledge LUVAS administration for providing the necessary facilities and support to carry out this work. No external funding was received for this study.

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