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Review Article

Epigenetic Roles of Sperm RNA in Livestock Breeding and Fertility

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ABSTRACT

The discovery of sperm RNA epigenetic inheritance, fertility prediction, and early embryonic development in livestock has been a significant achievement in the field of reproductive biology. Instead of just being the sperm that carry out DNA, the RNA that is demonstrated now in the sperm position, such as microRNAs (miRNAs), piwi-interacting RNAs (piRNAs), transfer RNA fragments (tRFs), and long non-coding RNAs (lncRNAs), are active modulators of the zygotic expression of genes and transgenerational phenotype transmission. It has been reported that in particular species, such as cows, pigs, and chickens, specific sperm RNA profiles are associated with fertility success, embryo viability, metabolic regulation, and heat tolerance. Environmental factors, such as intense heat and the type of feed consumed by an animal, are primary factors that lead to changes in the genetic material of sperm, providing insight into the relationship between the gene expression system and the external environment (Maddox *et al*., 2001). Sperm RNA, a new type of biomarker, is being explored primarily in infertile diagnostics, climate-resilient sire selection, and trait improvement, despite being plagued by technical problems. Furthermore, the issues of establishing causality and livestock-specific validation remain unresolved. The current review presents an overview of the livestock sperm RNA loads available so far, and their role is explained first. A translational roadmap is provided for their implementation into breeding programs. Sperm RNA, which serves as the molecular link between genotype and phenotype, represents a promising breakthrough for precision livestock selection in the genomic era.

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Authors' Contribution
KAN conceived the study concept, designed the research framework, and drafted the manuscript. SHA and SK assisted in data collection, literature synthesis, and preparation of tables and figures. All authors contributed to data interpretation, statistical visualisation in R studio, and technical validation of RNA profiling content. Furthermore, reviewed, edited, and approved the final version of the manuscript.

Key words
Livestock breeding, Epigenetic inheritance, microRNA (miRNA), tRNA fragments (tRFs), Fertility biomarkers, Heat stress adaptation

INTRODUCTION

The impact of epigenetic and heterotic factors, along with existing genome-wide selection methods, in animal husbandry, where reproductive performance and environmental stress tolerance are the key profit drivers, can be considered as holistic approach to enhancing complex polygenic traits (Meuwissen *et al*., 2001). Sperm is, as a rule, discussed as the carrier of the male genome to the egg. The subject, however, has undergone radical reform through the efforts and findings of technical pioneers. The first convincing evidence that the seed cells could transport not only DNA but also RNA to the egg during fertilisation was provided by Ostermeier *et al*. (2004). The result was a challenge to the dogmas that had prevailed for decades, marking the beginning of the field's understanding of the role of sperm in epigenetics.

Although transcriptionally mature spermatozoa are silent, they have a highly diverse and unique RNA repertoire, which is represented by microRNAs (miRNAs), piwi-interacting RNAs (piRNAs), transfer RNA fragments (tRFs), and long non-coding RNAs (lncRNAs) (Sharma *et al*., 2017). They are not transcriptional byproducts left over, but are instead the different types of miRNA retention or the acquisition of piRNA during spermatogenesis and epididymal maturation, akin to a transgenerational epigenetic effect that reconfigures the sperm epigenome (Reilly *et al*., 2016; Shi *et al*., 2024).

The most frequently examined are microRNAs, which are tiny non-coding RNAs of 22 nucleotides that act in an opposing manner to mRNAs through specific base pairing that is complementary to their target mRNA. In animal models, specific sperm miRNAs have been shown to influence the early stages of embryonic development and the resulting characteristics of the offspring. For example, paternal stress in mice leads to changes in the expression

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of sperm miRNAs, such as miR-34c and miR-449a, which in turn affect offspring stress regulation (Gapp *et al*., 2014; Rodgers *et al*., 2013). Although these findings are helpful, they still need to be verified meticulously in livestock before being recommended for direct application.

The tRNA fragments responsible for the regulatory activity of the tRFs are non-coding RNAs that possess this ability, resulting from the cleavage of tRNA; thus, their elimination is of interest due to their potential role in the regulation process. Experiments on mice have confirmed that specific tRF profiles, transferred by sperm and affecting lipid metabolism and glucose homeostasis in offspring (Chen *et al*., 2016a, b; Grandjean *et al*., 2015), are modified by particular dietary changes in sires. Implicitly, these results suggest the existence of other types of RNAs that are also coded, as well as environmental influences that are passed through the sperm epigenetically.

The specifics of their action remain undiscovered. Foremost considerations suggest that they may directly participate in zygotic gene activation and embryonic reprogramming, where RNAs from sperm determine the key factors in the process of early embryogenesis. Some others employ the technique of seminal plasma extracellular vesicles (EVs), which transmit RNAs and proteins to the female reproductive tract, thus modulating the uterus's receptivity and immune tolerance (Clark and Schust, 2013; Abeysinghe *et al*., 2025).

Information extracted from cattle research pointed out the connection between sperm RNA profiles and a bull's fertility. The bulls that are highly fertile exhibit miRNA signatures that differ from the typical, for example, the most prevalent miR-202, a miRNA involved in testicular development and sperm maturation (Fagerlind *et al*., 2015). Additionally, tRNA-derived fragments (tRFs) and piRNAs are correlated with fertilisation success and embryo quality (Shangguan *et al*., 2020). Specific RNAs, including miR-34c, are present in sperm and early embryos, suggesting that these RNAs may play a role in activating genes in the zygote (Kropp and Khatib, 2015).

The correlation between boar sperm RNA and important reproductive parameters in swine has been a breakthrough in scientific research, illustrating the connection between RNA types in sperm and reproductive characteristics. Consequently, it is of essence that research is done and recommendations made on the need to retain genetic and health characteristics via reproduction. As an example, the suggestion of pig insemination using the sperm packages that are above the median can be introduced as the intervention measure. Thus, gene therapy potential could use some more validation. The tRF-Gly-GCC sperm has been recently identified in a study on tRNA fragments concerning the tRF-Gly-GCC level in sperm of mated sows with higher litter sizes.

The positive tRF-Gly-GCC, which is related to sperm parameters such as motility and embryonic survival, and its mediation of these factors through tRNA, which is the one that synthesises these factors. Garcia-Romero *et al*. (2021) investigated that perinatal miRNAs, such as miR-191 and miR-10b, were found to be linked with sperm motility and morphology. The examined miRNAs seem to offer the possibility for the detection of fertility issues through a non-invasive procedure.

The peculiar reproductive physiology of birds results in different sperm RNA dynamics, whereby poultry sperm do not go through the epididymis. miRNAs that are specifically expressed only in primordial germ cells and spermatids, related to their development and spermiogenesis, were identified through transcriptomic analysis of the chicken germline (Ding *et al*., 2024; He *et al*., 2025).

The findings in poultry also underscore the potential of miR-181a as a biomarker in reproductive health and disease states (Lian *et al*., 2015). In addition, dietary components have been recognised as agents that hinder male poultry fecundity by lowering parameters of semen, such as the number of sperm entirely functional, sperm membrane, and sperm viability, proposing a link between a particular diet, sperm RNA functionality, and reproductive performance (Gonçalves*et al*., 2015). By and large, these observations support the case for poultry as a significant yet underinvestigated animal model in the field of sperm RNA research.

The retrieval of RNA of high purity from fully developed sperm cells is very challenging, as they have a compact chromatin structure and express only a minimal amount of RNA. This is also the case with the livestock species that deal with problems associated with cryopreservation, which is the freezing of live sperm, a commonly adopted technique in artificial insemination programs. The act of freezing and thawing can lead to RNA degradation and the introduction of false expression (Shangguan *et al*., 2020). Additionally, traditional RNA sequencing protocols often lack the sensitivity required to detect small RNAs present in sperm at low abundance, particularly in species with limited genome annotations.

In tropical and subtropical areas, heat stress is a significant factor contributing to livestock infertility (Lucy, 2019). It has recently been discovered that bulls exhibit greater levels of miR-449, a miRNA that controls the cell cycle and cell sperm maturation, when they are exposed to heat (Alves *et al*., 2021). These modifications have been directly attributed to the reduction of the

quality of embryos and the reduced rates of pregnancy. The zygote's acquisition of these RNAs may also have a detrimental effect on its thermotolerance, which, in consequence, would affect its survival and performance in warm environments.

Similarly, sperm RNA profiles can also be influenced by the diet. A study experiment on boars showed that fatty acid omega-3 supplement to their diet regulated the expression of certain tRNA-derived fragments (tRFs), such as those that regulate muscle development and lipid metabolism (Vaz *et al*., 2025). The findings in nutrition research validate the hypothesis of paternal transmission of nutritional programming to animals with excellent production traits (Sharma, 2019). This, in turn, provides a new opportunity for the non-genetic improvement of carcass quality and growth rates.

Despite the impressive amount of progress achieved in the field of sperm RNA biology, critical knowledge gaps remain, which prevent its application in livestock breeding. The first thing to mention is that most of the existing data have a correlational nature, which raises a question mark regarding whether some sperm RNAs increase, decrease, facilitate, or indirectly affect the phenotype of the progeny, or whether they reflect normal sperm quality (Schagdarsurengin and Steger, 2016). Causality assignment through RNA knockdown or overexpression in embryos is necessary, and so are functional studies to show the evidence.

Second, the question of whether the RNA effects are indeed transgenerational and whether they go beyond the F1 generation remains unanswered. Though intergenerational transmission has been observed in some rodent models (Gapp *et al*., 2014), similar findings in livestock are limited. The time span and sequence of the RNA effects, in conjunction with the ignorance of even the lowest level of consciousness in long-term selection strategies, are all related to the depth of insight that a scientist must have to explain them.

The mutual influence of RNA-mediated epigenetic inheritance and traditional genomic selection is currently inadequately understood. Would it be feasible to merge these two data types and consequently upgrade the accuracy of breeding value forecasts, or are they too clashing, which offers space for new integration models?

The goals of this review are to (i) integrate the presently available data that present sperm RNAs act as biomarkers and epigenetic regulators, (ii) consider their capability to be utilised in livestock breeding programs, and (iii) draw attention to the research priorities that are necessary for turning sperm RNA biology into applicable tools for genetic advancement.

SPERM RNA BIOLOGY IN LIVESTOCK: FROM BIOGENESIS TO FUNCTION

Biogenesis and selective cargo loading

Sperm RNA is also the product of spermatogenesis, one of the most highly programmed events in the male reproductive system (Shi *et al*., 2024). At this stage, the maximal transcriptional activity is observed, and subsequently, the specific RNA retention mechanisms chosen will determine the set of mature sperm RNAs, as shown in Figure 1. For instance, the remodelling of chromatin during spermiogenesis in bulls is a direct reason for the persistent presence of crucial effector RNAs, such as miR-34c, which is known to affect the cleavage of the zygote and, therefore, embryonic development (Kropp and Khatib, 2015), as shown in Table I. Furthermore, the cytoplasmic bridges that form among the spermatids facilitate the intercellular sharing of RNA, allowing the spermatids to undergo synchronised development and distribute the RNA equally (Sengar *et al*., 2018).

Table I. Key RNA classes in livestock sperm.

RNA type	Bull	Boar	Rooster	Function
miRNAs	miR-34c, -202	miR-34b, -21	miR-181a	Embryo development
tRFs	tRF-Gly-GCC	tRF-Val-AAC	tRF-Glu-CTC	Transgenerational metabolism
piRNAs	piR-57125	piR-8234	piR-6124	Transposon silencing

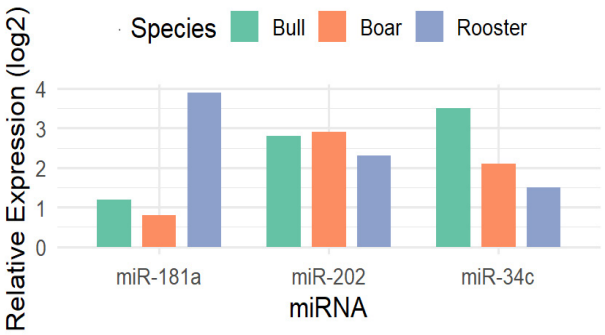

Fig. 1. Bar plots with the normalized expression level of the conserved sperm miRNAs (miR-34 family, miR-202, miR-21) in the semen of bulls, boars, and roosters. Error bars show +- SEM of three analyses (n = 6 per species). Patterns of species-specific enrichment of miRNAs identify developmentally conserved miRNAs including miR-34c and lineage-regulated expression of miR-202 in sperm of mammals.

Once the testicles fully developed, the sperm ducts were directed to the epididymis, the place where the RNA fragments of the sperm are more effectively modified. In ruminants like bulls and rams, the epididymosomes are transported from the epithelial cells in small extracellular vesicles, and they carry the specific mRNA to the sperm. The transfer of exosome-mediated route is responsible for allowing sperm to accumulate miRNAs, such as miR-202, which are reputed to be involved in the attainment of fertilisation (Reilly *et al*., 2016).

The differences among species in RNA biogenesis patterns are evident in Ruminants: the primary mechanism for providing RNA in sperm involves loading RNA into sperm in the epididymis, which is accompanied by significant changes during epididymal transit. While in Poultry, they lack an epididymis; thus, they mainly load RNA in the testis, and RNA profiles are established before ejaculation (Bahmyari *et al*., 2024).

RNA delivery mechanisms to oocytes

A fertilised oocyte with spermatozoa is the simplest method of introducing RNA. It is a fact that spermatozoa, when they are fertilised, not only donate their haploid genome but also transmit a certain amount of RNA to the cytoplasm of the oocyte. Cattle experience the fact that the sperm-borne RNAs, which are found to be miR-34c earlier, are delivered immediately after fertilisation and affect cleavage events at the beginning (Kropp and Khatib, 2015). In the case of pigs, the tRF-Gly-GCC factor, besides being a new addition, gets linked to the ribosome complex of the zygote, which then causes the disturbance of the initial translation (Chen *et al*., 2021).

Besides the direct sperm injection, RNA transport is also successfully carried out by the extracellular vesicles of seminal plasma. Vegal particles that are loaded with miR-34b to the porcine endometrium focus on and activate various processes, such as hypothalamic IGF1 expression and embryonic implantation of the targeted line (Abeysinghe *et al*., 2025). The artificial insemination (AI) operations are those that involve the complete revelation of the inner mechanics of such processes. Cows inseminated with sperm lacking the EVs recorded 18% lower pregnancy rates, which suggests that seminal EVs play a significant role in inhibiting the female reproductive organs (Bolumar *et al*., 2023).

Functional impacts on early development

Perm has the main purpose of transcription of the DNA. The FCF sperm miRNAs are enclosed in sperm and transported into maternal mRNAs matching miRNAs, thereby leading to their death or preventing their translation. In females, RNAs can be transferred to sperm, e.g., miR-34c, which was discussed earlier in the section, at the initial fertilisation stages and facilitates early cleavage (Kropp and Khatib, 2015). It has also been found that miR-181a also associates with PDCD4 to reduce its activity in chicken, thereby increasing cell survival and sustainability of the embryo (Martinez *et al*., 2022).

The presence of sperm RNA payloads can be a factor in the metabolism of embryonic cells. The tRF-Gly-GCC of pig was suggested to join in the change of mitochondrial respiration, which in turn affects ATP production and energy balance (Chen *et al*., 2021). The tRF-Pro-CGG in cattle binds with POLRMT, a mitochondrial RNA polymerase, thus directly affecting the genes transcription involved in embryonic ATP synthesis (Tomar *et al*., 2024).

The key function of piRNAs is to protect embryos from genomic damage. One such piRNA is piR-57125, which in rams actively silences LINE-1 retrotransposons; this event prevents their mobilisation during zygotic development (Kubsad *et al*., 2019). Bull sperm is 23% richer in piRNA varieties than boar sperm, which leads to the assumption that species-specific genome defence may take priority (Yeste *et al*., 2011).

The dialogue between sperm RNAs and imprinting is an emerging area of research. The case of pig miR-675, which appears to inhibit the function of IGF2R, a gene that is imprinted and responsible for fetal size (Li *et al*., 2021), is a prime example, as shown in Table II. On the other hand, miR-130a in bovines can play the opposite role of PEG3, the gene that the father expresses. The gene's effect on development is the limitation of growth. The interaction between paternal and maternal regulatory factors, as depicted in these interactions, would indeed create a further layer of complexity in the control of epigenetic inheritance in livestock (Sharma, 2019).

Table II. Functional targets of key sperm RNAs.

RNA type	Species	Target gene/Pathway	Developmental role	Reference
miR-34c	Bovine	BMP2	Blastocyst formation	Kropp *et al*., 2015
tRF-Gly-GCC	Pig	Mitochondrial genes (ATP synthase)	Embryonic metabolism	Chen *et al*., 2021
miR-181a	Chicken	PDCD4	Embryo viability	Martinez *et al*., 2022; Bahmyari *et al*., 2024
piR-8234	Ram	LINE-1 retrotransposons	Genome stability	Kubsad *et al*., 2019

Recent studies on poultry revealed the piR-664 and piR-738 as the epigenetics involved in the axis formation of chicken embryos (Guo *et al*., 2024). According to the emerging data, the roles of sperm RNA classes in mammals (humans and cows) are preserved (Garcia-Lopez *et al*., 2015).

ENVIRONMENTAL MODULATION OF SPERM RNA PROFILES IN LIVESTOCK

Heat stress and climate adaptation

Sperm RNA profiles are considered important indicators of climate adaptation in livestock due to their high sensitivity to the ambient temperature. In the case of bulls, an observation has been made that a short-term exposure to a temperature of 42°C causes a very significant change in the expression of miRNA. The miR-449 family of miRNA is increased by 3.2 times, and they are related to the alterations in spermatogenesis and ciliary function (Celeghini *et al*., 2025). On the contrary, miR-21, the most important regulator of embryonic implant quality, is downregulated, which is reflected in a 15% decrease in cleavage rates of *in vitro* embryo culture.

The different breeds exhibit opposite behaviours under heat stress, primarily through changes in their reactions to it. For example, while the *Bos indicus* bulls undergo slight miRNA changes, the *Bos taurus* bulls show a significant difference in sperm RNA profiles in heat stress conditions. This means miRNA profiles could potentially help to identify sires that can tolerate high temperatures, as shown in Table III.

Table III. Thermotolerant miRNA signatures by breed.

miRNA	Expression change (*Bos taurus*)	Expression change (*Bos indicus*)	Function
miR-449a	↑ 3.2×	↔	Sperm maturation
miR-21	↓ 0.5×	↔	Blastocyst development
miR-34c	↑ 1.8×	↑ 1.1×	Embryo cleavage support

The evolutionary mechanisms of miRNA-mediated stress responses used by animals are similar across different species, such as Bos indicus, which is known to exhibit buffering activity for miR-27a and miR-451 (Sengar *et al*., 2018). Climate change can affect animals' DNA in various ways. However, studies on the global warming epigenetic map indicate that the migration of moisture to the seasonal forest sub-zone is a significant factor contributing to adaptive miRNA reprogramming (Bošković and Rando 2018; Cheruiyot *et al*., 2025).

Recent research has shown that transgenerational thermotolerance sperm RNA modifications may serve as a way to obtain thermotolerance heritably. The calves, which are sired by bulls that undergo heat stress, exhibit an 18% lower rectal temperature in response to artificial heat stress compared to the other bulls, a difference that is quite significant ($p < 0.01$). Apart from that, they also have more sweat glands, which are important for evaporative cooling.

In terms of mechanisms, one can consider tRF-Glu-CTC sperm RNAs as the primary factor responsible for this. tRF-Glu-CTC sperm RNA can bind to HSF1, a heat-shock transcription factor expressed in the eight cells of the embryo. Therefore, it is possible to think that sperm tRF-Glu-CTC takes the lead in the process of HSF1 upregulation, which further permits the embryonic systems to conduct the adaptive responses to later stress (Fig. 2).

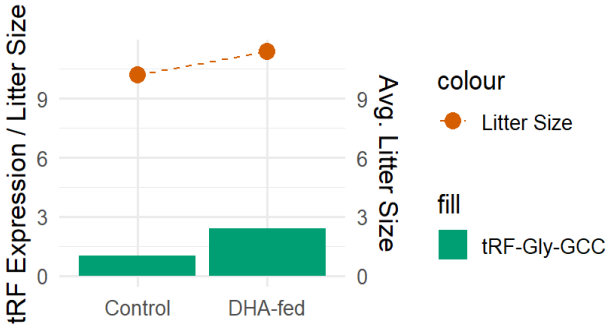

Fig. 2. Volcano plot showing miRNAs differentially expressed in sperm collected during thermoneutral (THI < 72) and heat-stress (THI > 80) conditions. Significantly altered miRNAs ($|\log_2 FC| > 1$, $p < 0.05$) are highlighted. miR-449a and miR-34c are up-regulated, while miR-21 is down-regulated, suggesting thermotolerance-linked epigenetic plasticity.

Nutritional programming through diet: Omega-3 supplementation in boars

DHA was incorporated into the diet, and it was found that during the 60-day trial period, the boars experienced a 2.4-fold increase in tRF-Gly-GCC, a small RNA involved in the narrowing phase of embryo development (Vaz *et al*., 2025). Regarding piglets, this change in RNA profile was related to the +1.2 piglets per litter, and this is 14% faster postnatal growth was also seen.

As depicted in Figure 3, tRF-Gly-GCC was characterised as a binder to DNMT3A, the enzyme responsible for DNA methyltransferase, thus suggesting its potential function in the methylation of the blastocyst pattern.

In the case of rams, low-protein diets (8% crude protein) stimulate the activation of 12 new piRNA

clusters, which, in a chain reaction, lead to epigenomic alterations in the sperm. These modifications involve an increase in type IIB muscle fibres and the regulation of the rumen microbiome, indicating a systemic metabolic programming trend. The addition of selenium and vitamin E has also been found to affect sperm RNA modulation, especially in poultry and rams (Vaz *et al*., 2021). The transgenerational impact of the father's nutritional status has been documented in studies with sheep and goats (Sindhu *et al*., 2024).

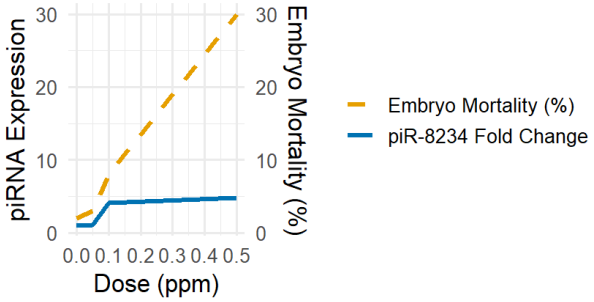

Fig. 3. Box-and-whisker plots comparing tRF-Gly-GCC expression in boar sperm (left axis) and litter size (right axis) between control and DHA-supplemented diets (2.5% DHA). Data indicate a positive correlation ($r = 0.84$, $p < 0.01$) between sperm tRF levels and reproductive output.

TOXICOLOGICAL IMPACTS ON SPERM EPIGENOME

Models of glyphosate exposure

Environmental contaminants, such as glyphosate, may be responsible for the lasting alterations in sperm RNA. Rams that were given 0.1 ppm glyphosate for 90 days came out with piR-8234, a retrotransposon-silencing RNA, being 4.1× upregulated, and a consequential suppression of LINE-1 elements in the F1 embryos (Kubsad *et al*., 2019). A dose-dependent effect was recorded at 0.05 ppm; however, no detectable effects were found. Conversely, 0.5 ppm resulted in 22% of the embryos dying; thus, the sensitivity threshold was emphasised. Boar sperm RNA content has been altered through exposure to zearalenone, which is a mycotoxin produced by mouldy grains (Yeste *et al*., 2011). The fungus A-type zearalenone miR-34b was downregulated to 0.6 times the control levels. Fertility outcomes include a 30% reduction in farrowing rates, and daughters showed altered ovarian gene expression, suggesting transgenerational effects are visualised in Figure 4 (Moelling, 2024).

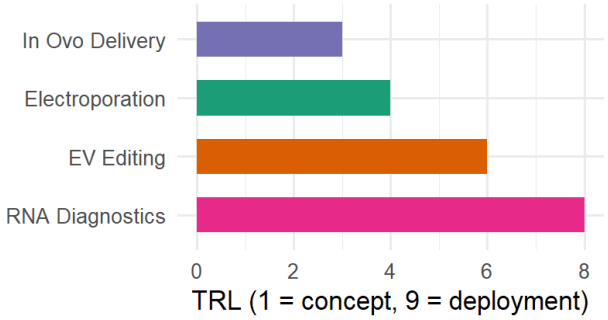

Fig. 4. Dose–response curve showing suppression of piR-8234 in ram sperm and the corresponding increase in embryo mortality after *in vitro* fertilization. The toxicological inflection occurs at ≥ 5 mg kg^{-1} glyphosate exposure.

Apart from glyphosate, the miRNA changes caused by PFAS in bull sperm are currently undergoing toxicological evaluation (Liu and Sharma, 2023). The emerging contaminants, such as per- and polyfluoroalkyl substances (PFAS), have now been identified as sperm RNA disruptors in this case. The latest studies indicate that PFAS exposure can lead to the identification of unique miRNA in the sperm of bulls, which can use the semen fluid as a non-invasive toxicity screening method (Li *et al*., 2021). In Pakistan, sperm RNA research can play a crucial role in fighting livestock challenges, such as heat stress in Nili-Ravi buffaloes, diminished fertility in indigenous cattle, and poor hatchability in poultry. Identifying RNA-based markers of fertility and thermotolerance would enhance breeding efficiency and support genetic improvement under climate stress (Hill, 2010).

Revolutionising fertility diagnostics

The standard semen quality evaluation methods, which are predominant, for instance, morphology, motility, and concentration, are not without failures, and one of the issues is the inability to identify subfertility conditions in most breeding males (Lucy, 2019). The subsequent figures gathered from recent research can best support the above-quoted idea, as 38% of subfertile dairy bulls cannot pass the set tests (Donnellan *et al*., 2022), and 62% of boars that do not exhibit higher litter and abnormal semen parameters actually have them, as shown in Table IV and Figure 5.

These shortcomings, in addition to impeding the coupling of historical change with economic costs, significantly influence animal breeding programs, particularly those that rely on artificial insemination (AI) methods. Discovering that RNA biomarkers exist marks a revolutionary stride in the fertility testing process. The specific small RNAs are potent indicators of the continuity of the life cycle during the reproductive process.

Table IV. Actionable biomarkers ready for commercialisation.

Species	RNA marker	Predictive value	Sample type	Storage stability
Holsteins	miR-202-3p	89% (AUC 0.91)	Frozen semen	5 years at -80 °C
Duroc boars	tRF-Gly-GCC	84% litter size correlation	Fresh semen	72h at 17°C
Broilers	piR-8234	92% hatchability	Testicular biopsy	N/A

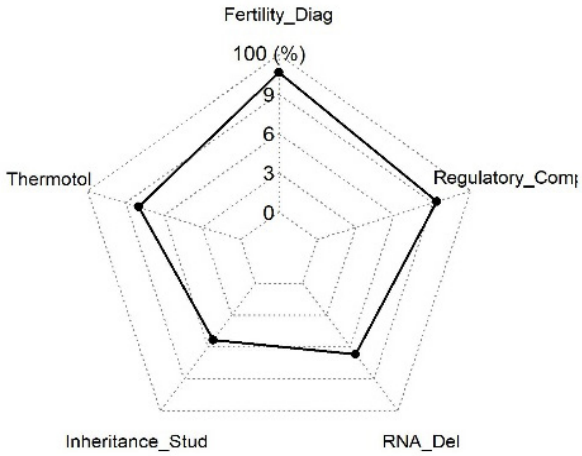

Fig. 5. Radar chart summarizing the TRL (scale 1–9) for different RNA-based applications: fertility diagnostics, thermotolerance markers, epigenetic inheritance studies, and RNA delivery systems. Mature areas (TRL ≥ 7) include fertility diagnostics, whereas transgenerational interventions remain pre-commercial.

IMPLEMENTATION BLUEPRINT

On-farm testing kits

Lateral flow assay prototypes detect miR-202 within 15 min and though it is Cost-effective as $12/test compared to $300 for RNA-seq.

AI centre integration

Semen extenders modified to preserve RNA integrity integration with CASA systems for automated RNA-based diagnostics. Field reports indicate that RNA-based fertility screening can reduce open cow rates by more than 20% in a single breeding season, highlighting its practical potential.

EMERGING CONTROVERSIES AND UNRESOLVED CHALLENGES IN SPERM RNA APPLICATIONS

The transgenerational inheritance debate

The aforementioned aspect can impact fertility. One piece of evidence is provided by studies in rodents, which show that jellyfish miRNAs can transmit stress and dietary signals to subsequent generations (Gapp *et al*., 2014). Furthermore, field experiments conducted on bovines using miR-34c-enriched semen have shown 7% higher conception rates in F2 progeny, suggesting transgenerational epigenetic inheritance. In fact, a 2023 meta-analysis of 47 livestock studies found that only 23% of the trials reported positive results for RNA-mediated effects in the F2 generation ($p = 0.02$). Observatory models of birds, which lack post-testicular RNA modification, exhibit a complete epigenetic switch between generations.

The GMO regulatory grey zone

Global regulatory patchwork

United States: If RNAs are used solely and no alteration to the DNA sequence occurs, the FDA's 2024 draft guidance categorises them as not genetically modified organisms.

European Union: The categorisation of RNA interventions as GMOs is done by the EU, even if the intended genomic change is not observed, which includes epigenetic remodelling.

China: It has implemented a case-based evaluation model and has already granted three approvals for piRNA-based livestock therapy, allowing for limited commercial use (Knol *et al*., 2016).

Industry outcomes may comprise the conundrum of Exporters witnessing a twelve-month initiation delay when sending semen doses with RNA modifications to the EU, along with Compliance and labelling issues that could escalate rates by up to 30%, thus undermining the general international competitiveness of RNA-enhanced breeding schemes, as shown in Table V.

Evolutionary fitness concerns

The main dangers are the dependence on RNA-guided selection, which can, in turn, cause the gene pool to narrow, as Trial herds having an exclusive miR-34c profile demonstrated $\Delta Fst > 0.15$ (a metric for genetic differentiation), suggesting the occurrence of potential bottlenecking. As for strategies for risk reduction, they involve maintaining 5% of the breeding stock with regular RNA profiling without any modifications as a measure to ensure diversity, and also implementing genome-wide surveillance to detect hitchhiker effects, where RNA-linked selection unintentionally favours harmful alleles (Li *et al*., 2021).

Table V. Global regulatory status of RNA-based technologies in livestock.

Country/Region	RNA editing allowed?	Regulatory status	Notes
USA	Yes	Not GMO if no DNA change (FDA, 2024)	Applies to miRNA/piRNA delivery
EU	Conditional	Treated as a GMO if any genomic intent	Requires full risk assessment
China	Yes (case-by-case)	3 approvals for piRNA-based therapies	Fast-track for agricultural trials

Technical reproducibility issues

The significant difficulties related to Cryopreservation involve the degradation of sperm RNAs, leading to miRNAs being considerably less detectable, namely by nearly 40%, particularly in the case of extenders that are not appropriately buffered. Additionally, the presence of only breed-specific reference databases for sperm RNA makes cross-laboratory validation impossible.

Following really cool ideas are RNA-stabilising nanoparticle-based extenders in the near future that will survive over 90% RNA integrity deal after thaw, MS AE, and FAO, mainly the Global Livestock RNA Atlas Project (2024-2027), which makes a base of RNA standards serving different breeds and species, thus enhancing the interoperability and quality assurance of sperm RNA diagnostics (Davidson *et al*., 2016).

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Ethical statement

The study did not involve experimentation on live animals or human participants. All analyses were based on previously published datasets and secondary data sources. Ethical approval was therefore not required under the University of Agriculture, Faisalabad, Pakistan's institutional guidelines.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abeysinghe, P., Turner, N., Flay, H., Meier, S., Cuttle, L. and Mitchell, M.D., 2025. MicroRNA (miRNA) in plasma small extracellular vesicles (sEV) as potential early indicators of dairy cow subfertility. *J. Extracell. Biol.*, **4**: e70084. https://doi.org/10.1002/jex2.70084

Alves, M.B.R., de Arruda, R.P., Batissaco, L., Garcia-Oliveros, L.N., Gonzaga, V.H.G., Nogueira, V.J.M., dos Santos Almeida, F., Pinto, S.C.C., Andrade, G.M., Perecin, F. and da Silveira, J.C., 2021. Changes in miRNA levels of sperm and small extracellular vesicles of seminal plasma are associated with transient scrotal heat stress in bulls. *Theriogenology*, **161**: 26–40. https://doi.org/10.1016/j.theriogenology.2020.11.015

Bahmyari, S., Khatami, S.H., Taghvimi, S., Rezaei Arablouydareh, S., Taheri-Anganeh, M., Ghasemnejad-Berenji, H., Farazmand, T., Soltani Fard, E., Solati, A., Movahedpour, A. and Ghasemi, H., 2024. MicroRNAs in male fertility. *DNA Cell Biol.*, **43**: 108–124. https://doi.org/10.1089/dna.2023.0314

Bolumar, D., Moncayo-Arlandi, J., Gonzalez-Fernandez, J., Ochando, A., Moreno, I., Monteagudo-Sanchez, A., Marin, C., Diez, A., Fabra, P., Checa, M.A. and Espinos, J.J., 2023. Vertical transmission of maternal DNA through extracellular vesicles associates with altered embryo bioenergetics during the periconception period. *Elife*, **12**: RP88008. https://doi.org/10.7554/eLife.88008

Bošković, A. and Rando, O.J., 2018. Transgenerational epigenetic inheritance. *Annu. Rev. Genet.*, **52**: 21–41. https://doi.org/10.1146/annurev-genet-120417-031404.

Celeghini, E.C.C., Baatsch-Nascimento, F., da Rocha Bozzi, A., Garcia-Oliveros, L.N. and Arruda, R.P., 2024. Bovine testicular heat stress: From climate change to effects on microRNA profile. *Anim. Reprod. Sci.*, **270**: 107620. https://doi.

org/10.1016/j.anireprosci.2024.107620

Chen, Q., Yan, M., Cao, Z., Li, X., Zhang, Y., Shi, J., Feng, G.H., Peng, H., Zhang, X., Zhang, Y. and Qian, J., 2016. Sperm tsRNAs contribute to intergenerational inheritance of an acquired metabolic disorder. *Science*, **351**: 397–400. https://doi.org/10.1126/science.aad7977

Chen, Q., Yan, W. and Duan, E., 2016. Epigenetic inheritance of acquired traits through sperm RNAs and sperm RNA modifications. *Nat. Rev. Genet.*, **17**: 733–743. https://doi.org/10.1038/nrg.2016.106

Chen, X., Sun, Q., Zheng, Y., Liu, Z., Meng, X., Zeng, W. and Lu, H., 2021. Human sperm tsRNA as potential biomarker and therapy target for male fertility. *Reproduction*, **161**: 111–122. https://doi.org/10.1530/REP-20-0415

Cheruiyot, E.K., Negussie, E., Tarekegn, G.M. and Haile-Mariam, M., 2025. Candidate genes and genomic regions relevant for adaptation to extreme climates in livestock. *CABI Rev.*, **20**: 52. https://doi.org/10.1079/cabireviews.2025.0052

Clark, G.F. and Schust, D.J., 2013. Manifestations of immune tolerance in the human female reproductive tract. *Front. Immunol.,* **4**: 26. https://doi.org/10.3389/fimmu.2013.00026

Davidson, L.M., Barkalina, N. and Coward, K., 2016. Development of nanoparticle-mediated delivery tools to investigate the role of molecular genetic mechanisms underlying male infertility. *Sci. Adv. Today*, **1**: 25210.

Ding, Y., Zhi, Q., Zuo, Q., Jin, K., Han, W. and Li, B., 2024. Transcriptome-based analysis of key signaling pathways affecting the formation of primordial germ cell in chickens. *J. Integr. Agric.*, **23**: 1644–1657. https://doi.org/10.1016/j.jia.2023.09.003

Donnellan, E.M., Perrier, J.P., Keogh, K., Štiavnická, M., Collins, C.M., Dunleavy, E.M., Sellem, E., Bernecic, N.C., Lonergan, P., Kenny, D.A. and Fair, S., 2022. Identification of differentially expressed mRNAs and miRNAs in spermatozoa of bulls of varying fertility. *Front. Vet. Sci.*, **9**: 993561. https://doi.org/10.3389/fvets.2022.993561

Fagerlind, M., Stålhammar, H., Olsson, B. and Klinga-Levan, K., 2015. Expression of miRNAs in bull spermatozoa correlates with fertility rates. *Reprod. Domest. Anim.*, **50**: 587–594. https://doi.org/10.1111/rda.12531

Gapp, K., Jawaid, A., Sarkies, P., Bohacek, J., Pelczar, P., Prados, J., Farinelli, L., Miska, E. and Mansuy, I.M., 2014. Implication of sperm RNAs in transgenerational inheritance of the effects of early trauma in mice. *Nat. Neurosci.*, **17**: 667–669. https://doi.org/10.1038/nn.3695

García-López, J., Alonso, L., Cárdenas, D.B., Artaza-Alvarez, H., de Dios Hourcade, J., Martínez, S., Brieño-Enríquez, M.A. and Del Mazo, J., 2015. Diversity and functional convergence of small noncoding RNAs in male germ cell differentiation and fertilization. *RNA*, **21**: 946–962. https://doi.org/10.1261/rna.048215.114

García-Ruiz, A., Cole, J.B., VanRaden, P.M., Wiggans, G.R., Ruiz-López, F.J. and Van Tassell, C.P., 2016. Changes in genetic selection differentials and generation intervals in US Holstein dairy cattle as a result of genomic selection. *Proc. Natl. Acad. Sci.*, **113**: E3995–E4004. https://doi.org/10.1073/pnas.1519061113

Gonçalves, F.M., Santos, V.L., Farina, G., Oliveira, C.O., Anciuti, M.A. and Rutz, F., 2015. Nutritional aspects and reproductive management of roosters in Brazil. *World's Poult. Sci. J.*, **71**: 515–522. https://doi.org/10.1017/S0043933915002238

Grandjean, V., Fourré, S., De Abreu, D.A.F., Derieppe, M.A., Remy, J.J. and Rassoulzadegan, M., 2015. RNA-mediated paternal heredity of diet-induced obesity and metabolic disorders. *Sci. Rep.*, **5**: 18193. https://doi.org/10.1038/srep18193

Guo, S., Cong, B., Zhu, L., Zhang, Y., Yang, Y., Qi, X., Wang, X., Xiao, L., Long, C., Xu, Y. and Sheng, X., 2024. Whole transcriptome sequencing of testis and epididymis reveals genes associated with sperm development in roosters. *BMC Genom.*, **25**: 1029. https://doi.org/10.1186/s12864-024-10836-8

He, J., Shi, N., Yao, H., Li, J., Wang, Y. and Zhang, J., 2025. Genome editing in the chicken: From PGC-mediated germline transmission to advanced applications. *Int. J. Mol. Sci.*, **26**: 9426. https://doi.org/10.3389/fgeed.2025.1558432

Hill, W.G., 2010. Understanding and using quantitative genetic variation. *Philos. Trans. R. Soc. B: Biol. Sci.*, **365**: 73–85. https://doi.org/10.1098/rstb.2009.0203

Knol, E.F., Nielsen, B. and Knap, P.W., 2016. Genomic selection in commercial pig breeding. *Anim. Front.*, **6**: 15–22. https://doi.org/10.2527/af.2016-0003

Kropp, J. and Khatib, H., 2015. Characterization of microRNA in bovine *in vitro* culture media associated with embryo quality and development. *J. Dairy Sci.*, **98**: 6552–6563. https://doi.org/10.3168/jds.2015-9510

Kubsad, D., Nilsson, E.E., King, S.E., Sadler-Riggleman, I., Beck, D. and Skinner, M.K., 2019. Assessment of glyphosate induced epigenetic transgenerational inheritance of pathologies and sperm epimutations:

generational toxicology. *Sci. Rep.*, **9**: 6372. https://doi.org/10.1038/s41598-019-42860-0

Li, X., Zhao, C., Han, B., Qu, L., Liu, C., Yang, N. and Lian, L., 2021. Gga-miR-181a modulates ANP32A expression and inhibits MDCC-MSB-1 cell. *In vitro Cell. Dev. Biol. Anim.*, **57**: 272–279. https://doi.org/10.1007/s11626-021-00550-0

Lian, L., Li, X., Zhao, C., Han, B., Qu, L., Song, J., Liu, C. and Yang, N., 2015. Chicken gga-miR-181a targets MYBL1 and shows an inhibitory effect on proliferation of Marek's disease virus-transformed lymphoid cell line. *Poult. Sci.*, **94**: 2616–2621. https://doi.org/10.3382/ps/pev289

Liu, S. and Sharma, U., 2023. Sperm RNA payload: Implications for intergenerational epigenetic inheritance. *Int. J. mol. Sci.*, **24**: 5889. https://doi.org/10.3390/ijms24065889

Lucy, M.C., 2019. Symposium review: Selection for fertility in the modern dairy cow. Current status and future direction for genetic selection. *J. Dairy Sci.*, **102**: 3706–3721. https://doi.org/10.3168/jds.2018-15544

Maddox-Hyttel, P., Dinnyes, A., Laurincik, J., Rath, D., Niemann, H., Rosenkranz, C. and Wilmut, I., 2001. Gene expression during pre- and peri-implantation embryonic development in pigs. *Reproduction (Cambridge Suppl.)*: pp. 175–189.

Martinez, C.A., Roca, J., Alvarez-Rodriguez, M. and Rodriguez-Martinez, H., 2022. miRNA-profiling in ejaculated and epididymal pig spermatozoa and their relation to fertility after artificial insemination. *Biology*, **11**: 236. https://doi.org/10.3390/biology11020236

Meuwissen, T.H., Hayes, B.J. and Goddard, M., 2001. Prediction of total genetic value using genome-wide dense marker maps. *Genetics*, **157**: 1819–1829. https://doi.org/10.1093/genetics/157.4.1819

Moelling, K., 2024. Epigenetics and transgenerational inheritance. *J. Physiol.*, **602**: 2537–2545. https://doi.org/10.1113/JP284424

Ostermeier, G.C., Miller, D., Huntriss, J.D., Diamond, M.P. and Krawetz, S.A., 2004. Delivering spermatozoan RNA to the oocyte. *Nature*, **429**: 154–154. https://doi.org/10.1038/429154a

Reilly, J.N., McLaughlin, E.A., Stanger, S.J., Anderson, A.L., Hutcheon, K., Church, K., Mihalas, B.P., Tyagi, S., Holt, J.E., Eamens, A.L. and Nixon, B., 2016. Characterisation of mouse epididymosomes reveals a complex profile of microRNAs and a potential mechanism for modification of the sperm epigenome. *Sci. Rep.*, **6**: 31794. https://doi.org/10.1038/srep31794

Rodgers, A.B., Morgan, C.P., Bronson, S.L., Revello, S. and Bale, T.L., 2013. Paternal stress exposure alters sperm microRNA content and reprograms offspring HPA stress axis regulation. *J. Neurosci.*, **33**: 9003–9012. https://doi.org/10.1523/JNEUROSCI.0914-13.2013

Schagdarsurengin, U. and Steger, K., 2016. Epigenetics in male reproduction: Effect of paternal diet on sperm quality and offspring health. *Nat. Rev. Urol.*, **13**: 584–595. https://doi.org/10.1038/nrurol.2016.157

Sengar, G.S., Deb, R., Singh, U., Raja, T.V., Kant, R., Sajjanar, B., Alex, R., Alyethodi, R.R., Kumar, A., Kumar, S. and Singh, R., 2018. Differential expression of microRNAs associated with thermal stress in Frieswal (*Bos taurus* × *Bos indicus*) crossbred dairy cattle. *Cell Stress Chaper.*, **23**: 155–170. https://doi.org/10.1007/s12192-017-0833-6

Shangguan, A., Zhou, H., Sun, W., Ding, R., Li, X., Liu, J., Zhou, Y., Chen, X., Ding, F., Yang, L. and Zhang, S., 2020. Cryopreservation induces alterations of miRNA and mRNA fragment profiles of bull sperm. *Front. Genet.*, **11**: 419. https://doi.org/10.3389/fgene.2020.00419

Sharma, U., 2019. Paternal contributions to offspring health: Role of sperm small RNAs in intergenerational transmission of epigenetic information. *Front. Cell Dev. Biol.*, **7**: 215. https://doi.org/10.3389/fcell.2019.00215

Sharma, U., Sun, F., Reichholf, B., Herzog, V., Ameres, S. and Rando, O.J., 2017. *Small RNAs are trafficked from the epididymis to developing mammalian sperm* [preprint]. https://doi.org/10.1101/194522

Shi, Z., Yu, M., Guo, T., Sui, Y., Tian, Z., Ni, X., Chen, X., Jiang, M., Jiang, J., Lu, Y. and Lin, M., 2024. MicroRNAs in spermatogenesis dysfunction and male infertility: Clinical phenotypes, mechanisms and potential diagnostic biomarkers. *Front. Endocrinol.*, **15**: 1293368. https://doi.org/10.3389/fendo.2024.1293368

Sindhu, P., Magotra, A., Sindhu, V. and Chaudhary, P., 2024. Unravelling the impact of epigenetic mechanisms on offspring growth, production, reproduction and disease susceptibility. *Zygote*, **32**: 190–206. https://doi.org/10.1017/S0967199424000224

Tomar, A., Gomez-Velazquez, M., Gerlini, R., Comas-Armangué, G., Makharadze, L., Kolbe, T., Boersma, A., Dahlhoff, M., Burgstaller, J.P., Lassi, M. and Darr, J., 2024. Epigenetic inheritance of diet-induced and sperm-borne mitochondrial RNAs. *Nature*, **630**: 720–727. https://doi.org/10.1038/

s41586-024-07472-3

Vaz, C., Burton, M., Kermack, A.J., Tan, P.F., Huan, J., Yoo, T.P., Donnelly, K., Wellstead, S.J., Wang, D., Fisk, H.L. and Houghton, F.D., 2025. Short-term diet intervention comprising olive oil, vitamin D, and omega-3 fatty acids alters the small non-coding RNA (sncRNA) landscape of human sperm. *Sci. Rep.*, **15**: 7790. https://doi.org/10.1038/s41598-024-83653-4

Vaz, C., Kermack, A.J., Burton, M., Tan, P.F., Huan, J., Yoo, T.P., Donnelly, K., Wellstead, S.J., Fisk, H.L., Houghton, F.D. and Lewis, S., 2021. Short-term diet intervention alters the small non-coding RNA (sncRNA) landscape of human sperm. *BioRxiv*: 2021–07. https://doi.org/10.1101/2021.07.08.451257

Yeste, M., Barrera, X., Coll, D. and Bonet, S., 2011. The effects on boar sperm quality of dietary supplementation with omega-3 polyunsaturated fatty acids differ among porcine breeds. *Theriogenology*, **76**: 184–196. https://doi.org/10.1016/j.theriogenology.2011.01.032