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MULTI-OMICS APPROACHES FOR UNDERSTANDING REPRODUCTIVE PERFORMANCE IN FARM ANIMALS: A COMPREHENSIVE REVIEW

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ABSTRACT
Reproductive efficiency is the extent to which helpful breeding opportunities for livestock are transformed into healthy heifers and productive stock. Common measurements, such as semen quality, conception rate, calving interval, provide end results which may offer very little information about underlying biology. Reproduction as an interacting system is examined through multi-omics, which uses information from the genomes, epigenomes, transcripts, proteins, metabolites and microbial communities. These approaches can identify heritable variation among contrasting cellular activity and environmental exposure with physiological performance. The greatest contribution of these multi-omics approaches is to understand why animals reared under apparently comparable conditions would have different reproductive performance based on reproductive examination. Truly useful molecular differences exist if they are reproducible, biologically interpretable, and are linked to clearly defined, actionable veterinary reproductive decisions. This narrative review focuses on key omics technologies used to decipher reproductive performance in cows, buffalo, sheep, goats, pigs, and poultry. It especially looks at gamete competence, ovarian activity, uterine receptivity, embryo development and survival, reproductive disorders and environmental resilience. It also incorporates experimental design, computational integration, biomarker validation and practical implementation into consideration. Across these applications, discovery must be distinguished from prediction, and prediction from demonstrated improvement following intervention. An increasing number of molecular signatures still exist largely as research projects, rather than in utility as diagnostic tests. Advances in multi-omics will rely on large, representative populations, multiple records. including external validation and affordable testing. Ultimately, multi-omics should strengthen veterinary judgement, breeding programmes and animal welfare through better biological understanding and more reliable management decisions.

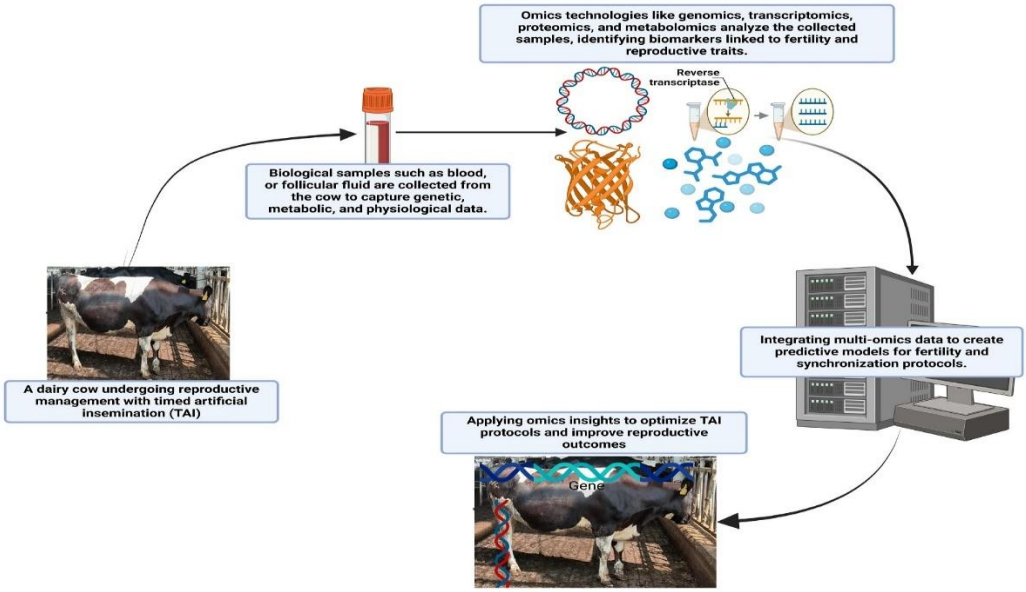
KEYWORDS: Multi-omics; Reproductive efficiency; Farm animals; Genomics; Reproductive biomarkers; Fertility prediction; Precision livestock farming

1. INTRODUCTION
Reproductive failure is usually caused by more than one isolated defect. Essential to the success of breeding is the interaction of endocrine signalling, the availability of functional gametes, correct mating or insemination, the functional reproductive tract and continued embryo development. Nutrition, disease, temperature, social conditions and management can influence many of these simultaneously (Bruinje *et al*., 2025). Consequently, two animals that are labelled infertile may have different biological limits. One may fail to ovulate, another may produce developmentally compromised oocytes, and another may lose embryos after fertilisation. Molecular investigations are most useful when these are teased apart through detailed phenotyping. Assuming the same condition exists for each failed pregnancy conceals mechanisms and it raises the possibility that any biomarker identified will not aid in distinguishing between herds and production systems (Castro *et al*., 2026).
Traditional reproductive assessment is still irreplaceable because it connects laboratory findings with actual animal performance. Molecular profiles are not enough to resolve problems. Breeding histories, ultrasonography, hormonal, semen evaluation and clinical examinations can make problems easier to spot than molecular profiles. Omics technologies extend this foundation by measuring many biological components simultaneously, potentially

detecting disturbances before obvious clinical signs emerge (Costes *et al*., 2024). Their relevance, however, lies in what is already out there for use by veterinarians and farmers. There is little point to a complex molecular test that simply repeats a cheap established test. On the other hand, there could be great benefits in normal looking animals when a test is validated and evidence is used to guide the breeding process. It is important to ask not what new decision is on offer but what decision that becomes possible, reliable or realistic (Ayantoye *et al*., 2025).

The term multi-omics should imply meaningful integration rather than the simple collection of several datasets. Genomics is distributed sequence variation, but other layers are regulatory activity, molecular products or interactions with microorganisms. They are measurable at different timescales and in different biological compartments. A variant may be constant throughout life, whereas a metabolite concentration does waver within hours of a meal (Abdelaziz *et al*., 2024). In order to merge this information, one must make how these layers interrelate to the biology of reproduction underpinning an explicit hypothesis. Our review is mechanistic and translational in nature rather than an evidence synthesis. It synthesises the confirmed biological facts, representative research presentations and what preconditions necessary for decisions for promising findings to be used in routine reproductive management of livestock (Wen *et al*., 2026).

Figure 1. Integration of Multi-Omics Technologies for Optimizing Timed Artificial Insemination and Reproductive Performance in Dairy Cattle



Source- Ayantoye *et al*., 2025

2. Biological Foundations of Reproductive Performance

The reproductive axis links environmental and metabolic information with gonadal activity through communication among the brain, pituitary and gonads. The Gonadotropin release, which is influenced by follicular development, ovulation, steroid synthesis and spermatogenesis, is feedback regulated by gonadal hormones (Hassanein *et al*., 2024). Omics studies may be used to investigate components of this coordination but it is known that the samples obtained are not necessarily reflective of what is occurring in the brain or gonad (Abdelaziz *et al*., 2024). Blood measurements often reflect systemic effects rather than the initiating stimulus. Precise endocrine anatomy combined with tissue information and accurate times of sampling clarifies these relationships. Puberty, cyclicity and seasonal breeding should also be distinguished, because similar molecular pathways may participate in different developmental or physiological transitions without serving identical functions across all species or management conditions (Bao *et al*., 2025).

Gamete development is a uniquely informative model for integrated molecular analysis. Developmental competence of oocytes is provided by the interactions with surrounding follicular cells, the accumulating maternal molecules, and the coordinated nuclear and cytoplasmic maturation. Production of sperm is associated with transitions of successive germ cells, extensive chromatin remodelling and later maturation in the epididymis (Herbicht *et al*., 2026; Kiefer *et al*., 2021). Defects at various points can lead to similar end results, such as lower embryo development or low sperm motility. Multiple omics layers help distinguish altered gene regulation from impaired energy metabolism, membrane disruption or abnormal protein processing. Though successful fertilisation is just one endpoint; studies to assess the larger impact of variation at the molecular level on gamete must also account for the effects of changes in genome on embryo competence, viable pregnancy and health of the offspring (Kassim *et al*., 2026; Frezarim *et al*., 2025).

Once fertilization occurs, success of reproduction is dependent on the coordinated dialogue between the developing embryo and maternal tissues. The early developmental stages occur in the oviduct, while the uterus provides a favourable environment that is nutritional, immunological and structural (Saint-Dizier *et al*., 2019). Greatest risk of direct extrapolation is due to differences in pregnancy recognition mechanisms across species

(Peterson *et al*., 2025). In ruminants, conceptus signals to maintain corpus luteum; a different signalling system is involved in pigs and poultry develop embryos outside of the maternal body after laying. multi-omics can point to secretions, cellular pathways and tissue responses that are likely stage dependent. Nonetheless, differences between pregnant and nonpregnant individuals may instead be a result of the presence of an embryo, not predisposing conditions for fertility. Sampling before and after breeding is therefore essential for resolving direction (Ott *et al*., 2025).

3. Genomics and Reproductive Potential

3.1 Genome-Wide Association Studies and Genetic Determinants of Fertility

Genomics examines DNA variations influencing reproductive traits such as conception, puberty, semen quality and litter size. Genome-wide association studies locate genetic regions that are related to the markers of interest in, while functional systems, fine mapping and transcriptomic microarray integration provide insights into causal mechanisms. However, reproductive traits are usually complex and affected by many genes and environmental factors, which must be validated across breeds and populations (de Carvalho *et al*., 2025). While some variants have a large impact on fertility and prolificacy, there is a degree of dependence on the genetic background and the mother (Zhao *et al*., 2026). As a result, genomic selection must consider factors including reproductive efficiency, calf survival, dam performance, and overall animal health while limiting undesirable correlated responses (Han *et al*., 2025).

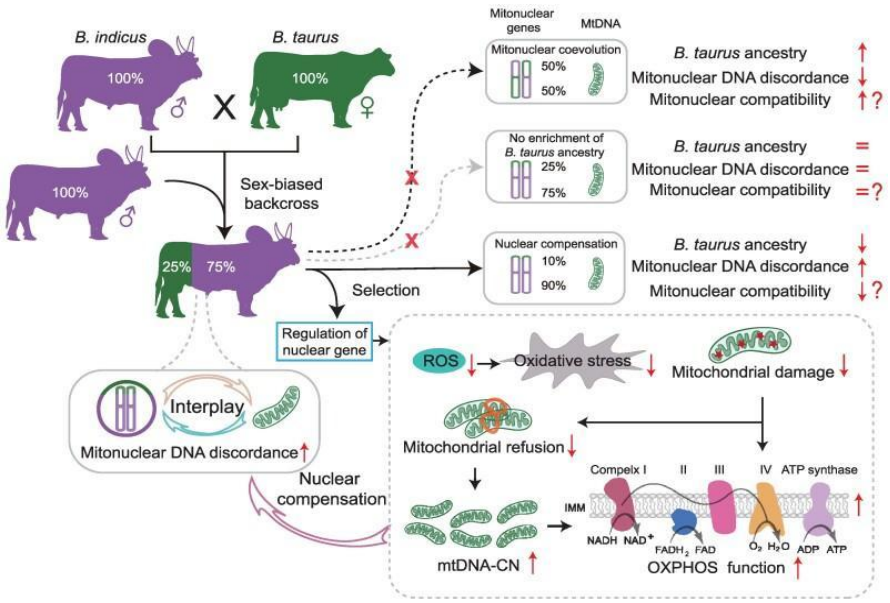
3.2 Genomic Selection and Prediction of Reproductive Performance

Genomic selection uses information across many markers to predict breeding value without knowing every genetic mechanism involved. For fertility its performance is a function of the quality and size of reference populations, good reproductive records and good statistical models. Multi-omics could improve interpretation or prioritisation of markers, but does not guarantee better prediction when adding molecular layers (Wen *et al*., 2026). Expensive assays can add noise due to small sample sizes or less than optimal matching between the assay and the target trait. Comparisons should also include a solid genomic baseline and assess prediction in animals that are truly independent of model development. Ultimately it should lead to sustained genetic gain under commercial circumstances, not merely a more involved model with an impressive performance exemplified only in its training population (Tabet *et al*., 2025).

3.3 Structural Variants, Mitochondrial Variation and Genetic Diversity

Structural variants, mitochondrial variation and breed diversity take the genomic investigation beyond the commonly tested markers. Changes in gene dosage or reproductive development may be altered by deletions, duplications and rearrangements, whereas mitochondrial function is at the core of gamete energy balance (Zhang and Xu, 2025). Poorly suited reference assemblies are prone to miss relevant variants when genetically diverse animals are aligned to an unsuitable reference. This is particularly relevant for minority breeds and little-understood agricultural species where reproductive characteristics could differ from better-known populations about reference (Panigrahi *et al*., 2026). However, sequence novelty in itself does not signify biological relevance. Putative variants have to be subject to reliable genotyping, appropriate population biases and functional confirmation. Conservation of diversity should be done in conjunction with selection to prevent short-term reproductive gains at the price of resiliency or probability of inherited disease arising from excessive relatedness (Meszaros *et al*., 2026).

Figure 2. Figure: Mitonuclear Genome Interactions, Nuclear Compensation and Mitochondrial Function in *Bos indicus* × *Bos taurus* Crossbred Cattle



Source- Shi *et al*., 2025

4. Epigenomics and Developmental Regulation

4.1 Epigenetic Mechanisms in Reproductive Development

Epigenomics is the study of regulatory features that influence genome activity without altering its base sequence. Which genes are expressed within which cells are largely dictated by DNA methylation, histone modifications and chromatin accessibility. These help define which genes are expressed within particular cells. These features change during germ cell development, fertilisation and early embryogenesis marking widespread regulatory reorganisation (Ivanova and Kelsey, 2026). Their relevance to reproduction is due in part to their ability to coordinate developmental programmes at the proper time. When combined with transcriptional measurements, epigenomic assays can identify what changed in the regulatory state that may contribute to fertility differences. However, an epigenetic difference does not automatically result from an environmental stimulus or independent inheritance. All of the factors of genetic variation, cell composition, age, developmental stage, can alter the pattern published and should be considered in analysis and experimental design (Khatib and Graybeal, 2026).

4.2 Sperm DNA Methylation and Fertility Biomarkers

Sperm methylation studies illustrate both the promise and limitations of epigenomic biomarkers. Bull fertility-related differences in methylation signatures have been reported, and some studies have used insemination records to test their predictive value. It appears that some information may exist in sperm aside from conventional morphology and motility (Bourassi *et al*., 2026). However, this predictive association does not establish that a methylation change is the cause of infertility or will be durable in the next generation. The results may also be influenced by sample processing, sperm purity, bull age and genetics. Reliable application would necessitate repeatability across ejaculates, validation in independent studs and evidence that the assay provided more information than current semen evaluation and genetic screening. Predictive models should be complemented by, rather than replace, mechanistic studies (Asset *et al*., 2026).

4.3 Environmental Influences and Epigenetic Reprogramming

Maternal nutrition, thermal conditions Assisted in reproductive technologies coincide with changes in the epigenetic profiles of oocytes or embryos. These findings matter as early developmental regulation can affect later physiology. But exposure-effect all must be separated from baseline developmental variation and selection among surviving embryos (Lang *et al*., 2024). A permanent effect means the effect needs to be reproduced beyond the early cleavage or pregnancy establishment period. Transgenerational inheritance claims require extreme designs to separate transmission from direct exposure of developing germ cells to the next generation. In practice, epigenomics allows us to study developmental competence and further enhance reproductive environments. It cannot be used as a ground to claim all management stress permanently changes one aspect of heritability or that all future progeny will necessarily be unhealthy (Diaz *et al*., 2025).

4.4 Chromatin Accessibility and Integrated Epigenomic Analysis

Chromatin accessibility profiling marks the possibility of regulatory proteins on the condition of methylation events. Combined analysis of accessibility, expression, and genetic variants enables researchers to identify regulatory elements influencing follicular development or spermatogenesis. As with all other research, the predictive value is from context to context, as an accessible region may not always drive the regulation of a nearby gene (Nasir *et al*., 2026). Histone profiling and experimental perturbation can further strengthen the evidence though reproductive tissues return few samples. The combination of these should be performed at cellular resolution, especially where several germ and support cell populations are intermingled. The long-term aim is to identify regulatory events that explain reproductive differences, while retaining a clear distinction between computationally inferred connections and experimentally demonstrated control (Cheng *et al*., 2025).

5. Transcriptomics and Cellular Activity

Transcriptomics assesses RNA level in a sample and infer which biological programmes are activated in part of the tissue or in cell population. Its applications vary from follicular development, to functioning of the corpus luteum, uterine receptivity, spermatogenesis and embryogenesis. RNA sequencing detects differences in the expression of genes, in their structure and of a small number of detected types of noncoding RNA (Kasimanickam *et al*., 2024). However, RNA abundance does not directly measure protein activity, and increased expression may represent compensation rather than improved function. Time of sampling is of particular interest in reproductive tissues due to the rapid changes that they undergo, and where comparing fertile and subfertile animals, relevant stages match or and differences are explicitly modelled. Otherwise, a fertility signature could just reflect that animals were sampled at different points in a reproductive cycle or developmental process (Beng *et al*., 2025).

Cellular and Spatial Resolution

Bulk transcriptomic analysis averages signals across all cells within a sample. This can be informative for tissue level responses, but changes in cellular composition may resemble changes in gene regulation. An inflamed endometrium, for example, may appear to have more immune transcripts without equivalent changes within resident cells due to more infiltrating immune cells than a healthy sample (Wolfram-Schauerte *et al*., 2025). Single cell and single nucleus approaches help to separate these possibilities by resolving multiple populations and states. In this regard, their usage has been highlighted by atlases of the livestock gonia tracing germ cells and supporting cells development. Nevertheless, tissue dissociation, recovery and analytical decisions determine which populations make the map, so cellular maps are still measurements with constraints not a definitive account of the cellular composition (Lyons *et al*., 2025).

Spatial transcriptomics provides anatomical context via positioning of RNA signals relative to tissue sections. Reproduction relies on spatially structured microenvironments such as ovarian follicles, seminiferous tubules and maternal interfaces, where neighbouring cells communicate. The actual location of expression may tie molecular activity with these structures and may explain why apparently similar cells behave differently (Vlieghe *et al*., 2026). Linking spatial info to cellular atlases may improve interpretation, although resolution and sensitivity differ between methods. Intercellular communication proposed by prediction does not represent direct observation of molecular exchange, and additional experiments are required to confirm that the relevant receptors, ligands and functional responses involve. Hence, comparative spatial approaches are particularly advantageous for formulating anatomically relevant conjectures that would be missed by conventional tissue averages in reproductive studies of farm animals (McCutcheon *et al*., 2025).

Regulatory and Circulating RNAs

Noncoding RNAs add another level its regulation of reproduction and situations involving biomarkers. MicroRNAs affect either the stability or expression of target transcripts, as other classes of RNA may be involved in chromatin regulation, RNA processing or cellular organisation. Many are therefore alluring as indirect probes for lower-invasive samples including in reproductive fluids and extracellular vesicles (Roca *et al*., 2022). However, to establish an RNA in joint circulation does not demonstrate its tissue of origin and its biological function. In silico target predictions also offer many possible interactions which are often unvalidated. Studies need to differentiate regulatory signals from signals that are merely reflective of tissue damage or changing cell populations. Appropriate extraction controls, normalisation strategies and repeatable measurement techniques for these signatures are also needed before these can influence reproductive decisions beyond research settings (Abeysinghe *et al*., 2025).

6. Proteomics and Functional Regulation

Proteomics analyses all the proteins necessary for cellular functions including catalysis, transport, signalling and organisation. It therefore provides insights that cannot be reliably derived from RNA abundance alone. Although there are no changes relative to the transcripts, reproductive function can be altered by protein translation, degradation, localisation and chemical modification (Parameswari *et al*., 2026). An extensive protein profile of sperm, seminal plasma, follicular fluid, uterine secretions and embryonic material can be generated with mass spectrometry. However, highly abundant proteins can hide less abundant regulators, and protein extraction can affect the detected profile. It is important to have deliberately designed sample preparation and quality controls. The most relevant studies are those that associate specific changes in protein abundance with specific processes and functional measurements, rather than assuming that every difference in abundance represents an improved or impaired reproductive capacity (Guedes de Azevedo Viana *et al*., 2025).

Sperm and seminal plasma proteomes together give multiple viewpoints on male reproduction. Sperm are needed for movement, interaction with the membrane, capacitation and fertilisation, whereas seminal plasma is rich in molecules that may derive from diverse reproductive tissues. Their effect depends on concentration and processing conditions as well as interaction with the female tract (Leites *et al*., 2026). Integrative examinations of bull sperm transcripts, proteins and metabolites have uncovered metabolic pathways related to different fertility rates. Although providing plausible biological evidence, these studies do not identify an overall panel of fertility markers. Repeated ejaculates, subsequent outcomes with breeding attempts, and proper calibration for the female and work of her husband remain necessary. An effective biomarker would predict reproductive performance on top of what knowledge of normal semen analysis already provides, for every lot of semen basis, and under different handling conditions (Talluri *et al*., 2022).

Female reproductive proteomics can investigate how the follicular and uterine environments support development. The follicular fluid contains proteins associated with transport, inflammation, extracellular organisation and metabolic regulation, coming from the local tissues and circulation. Uterine secretions also alter with hormonal state occurring conceptive status. They may be utilized as surrogates for blood due to the lack of access in some circumstances (Jamwal *et al*., 2023). However, the local protein yield can also be altered by fluid dilution, blood contamination and differences in sampling procedures. Because media add background signals and volumes are small, embryo associated proteins are the hardest to identify. Studies must address these technical issues before interpreting a detected protein as a marker of oocyte competence or embryo viability (Damayanti *et al*., 2026).

Protein modifications provide another important dimension because reproductive events frequently depend on rapid activation rather than new protein synthesis. Phosphorylation promotes signalling during sperm capacitation and oocyte maturation, whereas oxidation and other added groups may modulate protein activity. Consequently, measuring only the total amount of protein may potentially overlook biologically decisive changes (Thomalla and Wolfner, 2025). Broad but unspecific proteomic discovery can be complemented by carefully scheduled analyses of target modifications, though enrichment methods and sample handling add to the problem. Antibody specificity, calibration and sample stability must be verified independently. Ultimately, translation correlates the predictive power of a very small protein panel with performance as such is suited to standard field conditions, as opposed to metering every single laboratory discovery feature (Kumaresan *et al*., 2024).

7. Metabolomics and Lipidomics

Metabolomics analyses small molecules that reflect biochemical activity, nutrient availability and communication between tissues and microorganisms. Metabolites, positioned near to biological expression, have the capacity to serve as indicators of disruptions in reproduction due to energy balance, oxidative stress and changes in substrate

utilization. Mass spectrometry and nuclear magnetic resonance each complement the other in metabolomic coverage and analytical capabilities (Torres-Calzada *et al*., 2025). Untargeted analysis looks at overall patterns, while targeted techniques measure concentrations of specific substances, but systems may not measure all metabolites from the start. Results can be affected by diet, feeding time, hydration, drug administration, and sample processing. Investigators can interpret these reproductive data accurately with standardized collection, controls that are in place, and physiological measurements, but not automatically assuming altered levels of all molecules instead reflect fertility or reproductive tissue function (Girka *et al*., 2026).

The transition around calving depicts how metabolism and reproduction intersect. Resumption of ovarian activity and subsequent conception may be affected by changes in energy need, reserves, inflammation, and disease susceptibility. Metabolomic profiles may be able to separate animals which are coping with effects from those experiencing chronic upset. However, a metabolite correlated with delayed breeding may be more directly a sign of lower feed intake, or other disease (Sarno *et al*., 2026; Gao *et al*., 2023). Longitudinal measurements are more indicative than individual samples because they capture directions and recovery pathways. These profiles need to be combined with body conformation, clinical records and endocrine data to interpret the results. The goal should be to identify actionable physiological barriers, rather than attaching molecular labels that are superfluous to careful observation and the traditional herd health assessments (Mendina *et al*., 2025).

Follicular fluid and oocyte metabolomics seek to profile the biochemical microenvironment, where developmental competence is first defined. Maturation, antioxidant defence and early developmental preparation depend on energy substrates as well as amino acids and lipid related molecules. Integrated studies in bovine and porcine oocytes have investigated metabolic profiling dynamics that align with transcriptional programmes throughout maturation (Abdulrahman Alrabiah *et al*., 2023; Sheng *et al*., 2026). Key pathways for experimental testing are suggested by this work, but abundance changes cannot be equated to metabolic flux. An increase in the amount of a compound can be due to its production or its utilization. Biosynthetic, functional experiments with limits on culture conditions and if possible labelled substrate studies are required to distinguish these passionate. Finally, this must ultimately be assessed against fertilisation, outcome of embryo development and pregnancy not merely through molecular normalisation (Lipinska *et al*., 2025).

Sheng, Z., Gao, Y., Chong, Y., Lu, Y., Shi, J., Liu, H., ... and Wu, J. (2026). Metabolomics and Lipidomics in the Study of Reproductive Performance in Livestock. *Animals: an Open Access Journal from MDPI*, *16*(4), 588.

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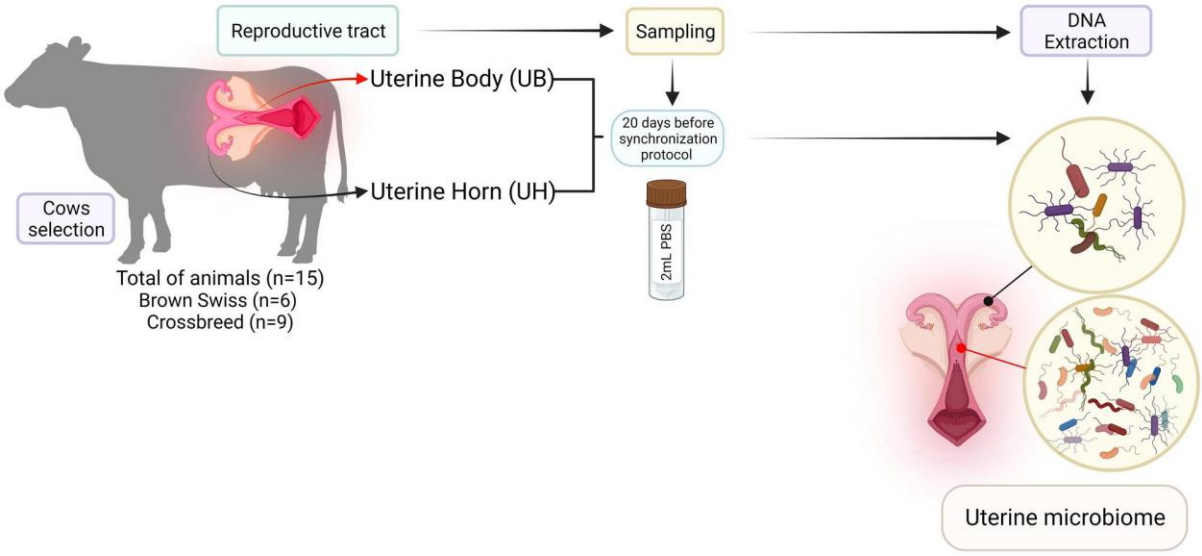
Lipidomics focuses on the diversity of lipids involved in membranes, energy storage and signalling. This is particularly relevant to sperm survival, oocyte competence and responses to cooling or cryopreservation. Lipid composition plays a key role in determining the behaviour of the membrane, while lipid oxidation could compromise the integrity of cells. Conservation methods effective for one type of semen or embryo may not directly translate to another due to interspecies variations (Evans *et al*., 2021). These differences can be narrowed down, tested and verified through lipidomic studies. It is however a challenge, as lipidomic signatures alone do not declare a better reproductive performance. Future experiments should measure membrane integrity, functional competence and relevant breeding outcomes, looking to separate improvements and stress unrelated adaptations or cell loss (Pedrosa *et al*., 2026).

8. Microbiomics and Reproductive Interactions

Microbiomics is the study of microbial communities and their likely contribution to reproductive physiology. The support microorganisms of the reproductive tract interact with local immunity, morphology of epithelium, and the milieu in which the does a game of hit-and-miss with the gametes. Community composition is addressed by sequencing approaches, whereas metagenomics indicates what functions are potentially possible for that community. This is why a lot of microbiomics studies here rely on detecting microbial DNA after performing cultivation steps (Fierer *et al*., 2025). Low microbial biomass samples are especially sensitive to contamination from collection devices and laboratory reagents. Appropriate negative controls, quantitative measurements and clinical context are essential. One should therefore treat a microbial pattern associated with infertility as a candidate trait needing further study and not as a diagnosis of reproductive tract infection (Gupta *et al*., 2024).

Digestion microbial communities may affect reproduction, mostly via the metabolism of nutrients, inflammatory signalling and the circulating metabolites. This is especially relevant among ruminants, animals whose physiology is tightly reliant on microbial fermentation. Integrated studies in sheep have linked microbial and metabolic profiles with semen characteristics or reproductive outcomes, providing hypotheses about communication between digestive and reproductive systems (Kilama *et al*., 2025). However, diet, age, housing and body condition can affect both microbiota and fertility, which can create associations without direct microbial causation (Poole, 2026). Interventional studies are needed to test whether change in the community is proportional to reproductive benefit. Probiotic and or dietary strategies should thus be tested on animal performance and health and should not just be the marketing tool of selection based on changing bacterial abundance or improving a computationally inferred microbial pathway (Sha *et al*., 2024).

Figure 3. Potential pathways through which reproductive and gastrointestinal microbiota may influence bovine reproductive physiology and fertility



Source- Murga Valderrama *et al*., 2023

Microbial findings must also respect species specific reproductive ecology. A community considered favourable in one host or anatomical site may not indicate health elsewhere. Human concepts of vaginal microbial dominance, for example, should not be transferred uncritically to livestock. Hormonal stage, mating, insemination, antimicrobial exposure and sampling location can all change the observed community (Adnane and Chapwanya, 2022; Skoufos *et al*., 2025). Combining microbial measurements with host transcripts, immune markers and metabolites provides a more informative picture than taxonomic lists alone. Even then, intervention requires evidence about mechanism, safety and durability. The practical goal is to identify microbial contributions that can be managed beneficially while avoiding unnecessary antimicrobial use, disruption of normal communities or overinterpretation of organisms detected at very low abundance (Jonas *et al*., 2025).

9. Integrating Molecular Evidence

Integration begins with an explicit biological question and a sampling strategy that aligns the molecular layers. Ideally, this means from the same animals, within the same time, with a consistent reproductive outcome linked. Some tissues may be mandatory, but their biological relationship needs to be declared (Van Den Bossche *et al*., 2026). Merging blood metabolites derived from one population with ovarian transcripts from another enables us to formulate proposes, but does not verify coordinated regulation within individual animals. With statistics integration might occur at the level of raw features, shared factors, networks or predictions, selection is dependent on data availability of quality and purpose of the study. Unlocking fewer well-matched layers may be far more informative than many more unlinked datasets collected in the absence of an explicit reproductive question (Wen *et al*., 2026).

Pathways network analyses turn huge feature lists into a possible biological relationship. They are one way of identifying the changes, be it steroid synthesis, mitochondrial function, immune signalling or extracellular organization that occur together. This aids in correlating physiological findings with their molecular underpinnings, but databases are uneven across species and tissues (Gable *et al*., 2022). Highly studied genes more likely appear connected, while genes poorly annotated in livestock could be missed. Enrichment is also background set dependent and subject to statistical assumptions. It is important that researchers explain these settings as never use a pathway diagram as evidence of a mechanism. Consensus on multiple levels of data is akin to convergent evidence, but a hypothesis is best supported by independent functional evidence and a temporal relationship compatible with the process under investigation (Slobodyanyuk *et al*., 2024).

Computational Prediction

Machine learning can be used to integrate molecular and conventional variation to predict reproductive outcomes, but results are especially prone to optimism when working with small data sets. Many of the thousands of features enjoyed by relatively few animals is bound to capture noise. Feature selection, normalisation and model tuning should be performed on training partitions with the validation data clean (Sasse *et al*., 2025). Care must be taken to not randomly split repeated samples from the same animal between training and testing, otherwise that leaks individual details. Validation should also consider herd, season and breed splitting. Accuracy is not enough performance needs discrimination, calibration and decision relevance. A model must improve on practical baselines and remain reliable when applied to genuinely new animals under realistic commercial conditions (Hoorn *et al*., 2026).

Establishing Causality

A causal interpretation requires more than correlation over molecular layers. A transcript, protein and metabolite may change together because they share an upstream cause, rather than forming a direct regulatory chain. Longitudinal designs place emphasis on temporal ordering and controlled interventions and functional

perturbations can evaluate more specific mechanisms (Svinin *et al*., 2026). Often genetic instruments lend credence to causal reasoning, but their assumptions can be more or less flexible. Culture cells, organoids and embryo systems provide powerful experimental control, though they can't cover every feature of whole animal physiology. Robust conclusions resulting when different methods point to the same explanation. The difference is important because a good predictor does not have to be causal, but an intervention target must change the outcome of interest rather than simply be associated with reproductive success or failure (Dimitrov *et al*., 2026).

10. Species-Specific Applications

Reproductive omics research on cattle is heavily influenced by the massive number of relevant studies due to the powerful presence of artificial insemination systems, pedigree data and production records. Important targets include bull fertility, postpartum recovery, embryo loss and responses to assisted reproduction. While keeping many physiological aspects in common, other aspects of reproduction, environmental response, and available genomic resources differ for buffaloes. It is hence mandatory to directly validate biomarkers established in the cattle host species (Pausch and Mapel, 2023). Tropical production systems further amplify this heat load, feed availability and management, which may be underrepresented in temperate studies. Although some advantage also may be gleaned within indigenous and crossbred yield studies as ambient climatic factors would fit to a closer representation of agricultural mechanisms. Practicability is contingent on whether a molecular relationship holds under the conditions in which farmers actually breed their animals (Chitkara *et al*., 2025).

The use of sheep and goats facilitate studies in seasonal breeding, puberty, prolificacy and maternal investment while pigs are the ideal species to study the coordination of ovulation, uterine capacity and litter development. These strategies of reproduction require different phenotypes and sampling designs. It offers an incomplete picture when solely counting embryos or offspring, particularly in prolific species, without investigating survival and the condition of the mother (Kleemann *et al*., 2026). Porcine ovarian transcriptomic and metabolomic studies have tracked modifications through development and pregnancy, demonstrating how the molecular context of the body shifts in relation to an individual's physiological stage. However, litter size associations must account for parity, breed, management and timing. Comparative work works best when it finds common as well as species-specific limitations to pathways but not with particular assumptions that a given molecular change relates to a reproductive role in all organisms (Pan *et al*. 2024).

Poultry reproduction generates phenomena that cannot be directly inferred from mammalian models. Reproductive output is affected by follicular hierarchy, egg formation, sperm storage within the female tract and incubation conditions. Transcriptomic and metabolomic evidence of molecular environments related to longer sperm survival have come from research of sperm storage areas (Assersohn *et al*., 2024). These findings could potentially direct breeding and semen management, but egg fertility and hatchability must be treated as separate metrics as they signify different events. An unfertilised egg and an embryo that dies during incubation are two different failures. Studies should separate maternal allocations to egg composition from paternal fertility and hatchery effects. For a more comprehensive explanation of reproductive performance, molecular data should be combined with data relating to breeder age, mating history, egg characteristics and incubation records (Agbehadzi *et al*., 2024).

11. Practical Reproductive Applications

Assisted reproductive technology provides a tangible avenue to apply molecular insights into improved procedures. Artificial insemination, semen cryopreservation, oocyte maturation, embryo production and embryo transfer expose reproductive cells to conditions that can be varied and measured. Multi-omics are potential expected to identify injury pathways, to compare culture conditions or to analyse donor or recipient heterogeneity (Rickard and De Graaf, 2026). However, it does not seem that a supposedly healthy laboratory profile will result in a higher chance of getting pregnant. Assessment should progress through cellular function in vitro and transferable embryos to initiated pregnancies and the development of healthy offspring. While the non-invasive assessment of spent culture media is compelling, low analyte levels and background contamination add complexity to data interpretation. An effect any selection approach claims should provide benefit without compromising embryos or adding avoidable cost and time (Veldhuizen and Rabaglino, 2026).

Reproductive disorders form another important application particularly when standard tests show nonspecific abnormalities. Endometrial inflammation, persistent ovarian dysfunction and unexplained subfertility may result from overlapping immune, endocrine and metabolic disturbances. Integrated profiles may allow the identification of biological subgroups or animals requiring closer clinical attention (Juli *et al*., 2024). However, molecular tests should complement to an appropriate diagnostic workup rather than replace an examination, investigations of the pathogen cause or evaluation of management failures. An inflammatory signature alone does not identify the responsible organism, or the right treatment. Molecular classification should be assessed to determine whether or not it changes management and improves clinical outcomes. This approach protects against turning exploratory clusters into premature disease categories or using expensive tests without clear implications for animal care and reproductive recovery (Song *et al*., 2026).

Heat stress demonstrates the effect of environmental exposure on reproduction across multiple molecular layers. This high level of thermal load could also be detrimental to endocrine factors, the oxidative balance, cellular metabolism and gamete development. To assess the oocyte transcriptome, epigenetics and follicular metabolites in different thermal environments, bovine studies have investigated these topics. Though these lead to insights into the most sensitive processes, further scrutiny is always required since there is, also, a decrease in food intake,

and/or other associated factors (Held-Hoelker *et al*, 2025). Controlled experiments and field studies answer sets of questions that are complimentary to one another. The practical objective is to determine which animals, tissues and reproductive stages respond best to cooling, nutritional support, or altered breeding schedules. Molecular enhancement be evaluated conjoined with animal comfort, conception and maintenance of pregnancies, instead of being used as an isolated demonstration of successful adaptation (Celeghini *et al*., 2024).

12. Study Quality and Translation

Reliable phenotyping is the backbone of every important multivariate study. Fertility should be defined using the outcomes and the time of observation, not after a single failure of service via a non-restrictive labelling definition. Male evaluations should include enough data for mating or insemination records and then adjust accordingly to female characteristics, technician effects and management. Female studies need to look at submission for breeding, conception, early loss and late pregnancy loss cases (Kertz *et al*., 2023). Non-random animals are more likely to result from attrition of cows with missing records and excluded animals, which can lead to performance bias. Keep the inclusion criteria, follow-up and relevant competing events in mind when making any statements on this. Without these safeguards, precise molecular measurements can be linked to wrong outcomes, explaining complex relationships that actually arise from reported practices or management decisions (Stephen *et al*., 2023). Laboratory and sampling procedures can vary as much as the effects being examined in the biological study. Fasting status, time of collection, anticoagulants, temperature of storage and repeated freezing affect molecular integrity and count. Find a method to ensure samples are analysed together, with suitable quality controls, reference materials, and blanks across analytical batches. Consistent recordkeeping of reproductive stage and recent treatments is necessary (Trindade *et al*., 2026). Biological replication and technical replication are not the same: measuring one sample more accurately does not involve studying more animals. To account for repeated samples and the structure of animals per farm, statistical models have to take into account repeated observations and the nesting of animals within farms. Predefined analysis plans and non-selective reporting aid in transparent interpretation while accessible metadata facilitate independent verification and can enable meaningful comparison of studies across laboratories and production systems (Woodland *et al*., 2025).

Biomarker Validation

Biomarker development necessitates a range of validation steps that many discovery studies may not accomplish. Analytical validation assesses whether an assay measures its target with accuracy and reproducibility. Biological validation confirms if the association is maintained across independent populations and relevant conditions. Clinical or management validation asks whether using the result improves a meaningful decision (Ou *et al*., 2021). Thresholds should be set to apply to the target population rather than be retrospectively selected to optimise performance. False positives and false negatives can have different costs, including over-treatment, barren periods in breeding, or the unjustified culling of the herd. Consequently, a panel must be evaluated on its value within realistic disease or subfertility prevalence. Longitudinal stability, turnaround time and compatibility with routine sampling also impact the transition of a promising marker into practice (Juli *et al*., 2024).

Economic Feasibility

The economic and operational feasibility of molecular tools determines whether they can even be considered beyond specialist labs. Farmers need the results fast enough to affect breeding decisions and obtain returns commensurate with the costs and logistics involved in sampling, transport, analysis and interpretation. Fewer but high yield panels for specific testing will be preferred over repeated whole profile testing except in very specific circumstances (Berry and Spangler, 2023). Laboratory infrastructure, veterinary support, and robust animal identification are equally important. Smallholder systems may need shared services and simple sample preservation methods, but these simplifications must not compromise the validity of the methodology. Economic evaluations should integrate the costs of adverse decisions and the value of gain beyond conception alone. A test which may be profitable for a high value breeding centre may not have to be for every farm, species or production goal (Wadood *et al*., 2025).

13. Future Priorities and Responsible Implementation

Future progress should prioritise coordinated longitudinal resources rather than isolated collections of molecular snapshots. Following animals from development through breeding, pregnancy and subsequent reproductive cycles can reveal stable predispositions and temporary disturbances. Shared protocols would allow populations to be combined while retaining information about breed, environment and management. Reference atlases should include underrepresented species and locally adapted animals, with appropriate annotation of reproductive tissues and stages (Tan *et al*., 2026). Such resources can strengthen both mechanistic research and prediction. However, larger datasets are valuable only when measurement quality and outcome definitions remain reliable. Investment in accurate records, specimen banks and collaborative veterinary networks may yield greater practical benefits than adding another analytical layer to a poorly characterised small study (Bao *et al*., 2025).

Experimental priorities should focus on testing actionable mechanisms emerging from integrated analyses. Candidate pathways can be examined using controlled nutrition, thermal management, culture modifications or carefully justified molecular perturbations. Organoids and other tissue models may help reduce animal use while allowing closer study of local responses, but findings require confirmation in relevant biological settings (Afzal, 2026). Interventions should specify the intended reproductive benefit and assess possible trade-offs involving health, development and welfare. For prediction tools, the next step is prospective evaluation in which decisions are made using a predefined model. For mechanistic discoveries, the next step is evidence that modifying the

proposed target changes the reproductive process. These pathways require different experiments and should not be conflated (Kwon *et al*., 2025).

A responsible implementation also needs to take into account issues of data ownership, communication and access to the benefits. Commercial value can be derived from genomic and reproductive records, so agreements on consent, sharing and reuse need to be very clear. Farmers and veterinarians need understandable explanations of uncertainty rather than unexplained numerical rankings (Williamson and Hartley, 2025). Molecular results should not drive the eradication of animals that otherwise have good welfare and conditions. Diversity should be safeguarded and tiny fertility gains not made at the expense of resilience in breeding programmes. Access to training helps professionals to understand when tests are appropriate and when traditional evaluation resembles the process. Public and cooperative research can support useful applications in less profitable species and production systems that might otherwise receive limited commercial investment (Barton *et al*., 2025).

14. CONCLUSION

Multi-omics has transformed the understanding of reproductive performance in farm animals by integrating genomic, epigenomic, transcriptomic, proteomic, metabolomic and microbial information to explain complex biological interactions. These methods provide the insights into gamete competence, fertility, late embryonic development, and reproductive resilience. However, the real-world value of multi-omics depends on proper experimental design, precise phenotyping, functional validation and reproducibility across independent populations. Future research must focus on turning molecular findings into economical diagnostic tools, better breeding strategies, and effective reproductive management practices. Collaboration between veterinarians, geneticists, reproductive biologists, researchers, and farmers is required for a successful implementation of multi-omics strategies. In summary, multi-omics should complement traditional reproductive traits instead of replacing them for improving fertility, healthy production of offspring, animal welfare, cost-effective livestock production and genetic diversity and adaptation to changing environmental and production conditions.

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