



ISSN: 2456-2912

NAAS Rating (2026): 4.61

VET 2025; SP-11(7): 16-23

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www.veterinarypaper.com

Received: 21-04-2026

Accepted: 25-05-2026

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Application of nanotechnology in animal reproduction

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DOI: <https://www.doi.org/10.22271/veterinary.2026.v11.i7Sa.3599>

Abstract

Nanotechnology, involves the manipulation of materials at the nanoscale (1-100 nm) and offers unique physicochemical properties that significantly enhance their functionality in various biological applications. In animal reproduction, nanotechnology enables precise drug and hormone delivery, enhances nutrient bioavailability, and provides innovative tools for diagnostics and disease management. Applications include nanoparticle-based pheromone delivery systems to mimic the male effect, nanoformulated antibiotics for treating reproductive infections, and sustained release systems for hormones such as GnRH and PGF_{2a} which reduce dosage and improve efficacy. In nutrition, nanotechnology improves absorption of key nutrients, optimizing reproductive health. Advanced semen purification and cryopreservation techniques using magnetic and antioxidant nanoparticles preserve sperm quality and increase fertility outcomes. Additionally, nano-enabled sperm sexing and non-invasive fetal sex determination technologies are emerging as practical solutions for reproductive management. Collectively, nanotechnology represents a transformative advancement in livestock reproduction, offering improved productivity, sustainability, and precision in animal health interventions.

Keywords: Nanoparticles, biostimulation, drug delivery, hormones

1. Introduction

The concept of "nanotechnology" was first introduced by Nobel Laureate Richard P. Feynman in his seminal 1959 lecture titled "*There's Plenty of Room at the Bottom*" (Ijaz *et al.*, 2020). Nanotechnology involves the manipulation and study of materials at the nanoscale, typically ranging from 1 to 100 nanometers (10^{-9} to 10^{-7} meters) in at least one dimension (Jiang *et al.*, 2008; Albanese *et al.*, 2012). At this scale, materials exhibit distinct physicochemical properties compared to their bulk counterparts, primarily due to an increased surface area to volume ratio. This enlarged surface area leads to a higher proportion of atoms located on the surface of the particle, significantly influencing its chemical reactivity and physical behavior. For instance, while bulk copper is known for its ductility and is widely used in applications such as wiring and piping, its nanoscale counterpart exhibits reduced malleability. This is attributed to the predominance of surface atoms in nanoparticles, which resist deformation, unlike the interior atoms that facilitate bending in bulk copper (Sharma and Sharma, 2012). Nanoparticles can function either as independent entities or as carriers for active agents, which may be adsorbed onto their surfaces or encapsulated within. Their enhanced surface reactivity and small size make them highly versatile for a wide range of applications, including biomedical diagnostics, drug delivery, and gene therapy (Albanese *et al.*, 2012; Swain *et al.*, 2015). From a biological perspective, nanoparticles often demonstrate improved solubility, increased bioactivity, and structural stability. These attributes make them less susceptible to degradation through oxidative processes or other environmental stressors.

In the field of animal sciences, particularly livestock production, nanotechnology has shown promising potential. Nano-formulated minerals have been reported to inhibit pathogenic microorganisms in animal feed, modulate rumen fermentation processes and improve reproductive performance in livestock species such as cattle and sheep (Laurent *et al.*, 2008)

[39, 40, 2, 71, 2, 75, 47].

The deployment of nanoscale drug delivery systems allows for more targeted and efficient therapeutic interventions, including the administration of antibiotics and hormones, thereby reducing both dosage requirements and treatment costs. This approach also contributes to minimizing the risk of antimicrobial resistance.

Nanotechnology further offers novel opportunities in livestock reproductive management. For example, smart delivery platforms can enhance the bioavailability of reproductive hormones and therapeutic agents, thus improving fertility outcomes (Feugang, 2017; Feugang *et al.*, 2019). Nanoparticles, whether naturally occurring or synthetically engineered, can be fabricated in various geometries (e.g., spherical, cubic) and may consist of a diverse range of materials including metals, salts, lipids, proteins, and polymers (Sun *et al.*, 2014). These diverse material properties and morphologies expand the functional possibilities for nanotechnology in enhancing animal health, productivity, and overall farm sustainability [27, 29, 74].

Applications of nanotechnology in the Animal Reproduction

Biostimulation and Pheromones

Reproductive efficiency in farm animals is a cornerstone of livestock productivity. Several tools have been developed to enhance reproduction, including biostimulation methods (e.g., male effect), reproductive assisted techniques (such as estrous synchronization and artificial insemination), nutritional strategies, and disease prevention or treatment (Caraviello *et al.*, 2006; Hashem *et al.*, 2021).

Among these, pheromones play a unique biological role. Pheromones are volatile chemical signals secreted by specific organs and scent glands in animals. In mammals, including livestock, pheromones are crucial for intra-species communication, mediating a wide range of behavioral and physiological responses. One of the most significant pheromone mediated reproductive phenomena in livestock is the “Male Effect” (Murata *et al.*, 2014).

The male effect refers to the stimulatory influence of a sexually active male on the reproductive activity of females. Pheromones present in the wool, wax, and urine of males can initiate puberty, resume ovarian activity in seasonally anestrus females, and reduce postpartum anestrus. This effect has been well-documented in sheep, goats, and swine (Ferreira-Silva *et al.*, 2017; Meza-Herrera *et al.*, 2017). Its practical utility in farm management includes advancing the breeding season by 4-6 weeks, achieving synchronization in estrus, and shortening the interval between parturitions, particularly when used 35-40 days postpartum (DeSantiago-Miramontes *et al.*, 2008; Martin *et al.*, 2004).

In swine, the presence of boars significantly reduces the postpartum interval in lactating sows and advances puberty in gilts by approximately 30 days. Intriguingly, even the odor left by a boar in a pen can elicit these effects, underscoring the potency of olfactory cues (Signoret and Lindsay, 1982). Recent innovations such as BOARBETTER, a synthetic 3-molecule boar pheromone spray, have been shown to improve sow reproductive performance (McGlone *et al.*, 2019).

Despite the demonstrated efficacy of the male effect, its practical implementation at scale faces logistical challenges, such as the seasonal sexual inactivity of males and the need for managing male-female contact. A promising solution is the use of aerosol nano-drug delivery systems. These systems can transport bioactive molecules, including pheromones, through olfactory pathways or systemic circulation, bypassing

biological barriers such as the blood-brain barrier (Cerbu *et al.*, 2021; Dugasa and Williams, 2016).

By encapsulating pheromonal compounds-identified from wool wax, saliva, urine, or feces-into nanoparticles suitable for nasal spray formulations, it becomes feasible to mimic the male effect year-round. This approach could minimize reliance on live males and eliminate the need for priming sexually inactive males before use (Kekan *et al.*, 2017; Archunan, 2020).

The application of nanotechnology opens avenues for developing pheromone-based protocols not only for the male effect but also for broader reproductive events such as estrus detection, puberty induction, postpartum anestrus management, pregnancy diagnosis, and even modulating maternal behavior.

Potential innovations include commercial bio-stimulating pheromones and pheromone based bioassay kits. For instance, urinary pheromones like 4-methyl phenol (p- Cresol), oleic acid (9-octadecenoic acid), and luteinizing hormone (LH) have been proposed as candidates for the development of nanoparticle-based estrus detection kits in buffaloes (Archunan, 2020) [9, 37, 57, 26, 55, 15, 51, 73, 52, 12, 20, 43, 6].

Treatment and Diagnosis of Infectious Reproductive Disorders in Livestock

Bacterial and zoonotic infections of the reproductive tract pose significant challenges in livestock management due to their impact on fertility and productivity. The clinical manifestations of these infections are often driven either directly by microbial products such as endotoxins or indirectly through the host's immune response involving cytokines, eicosanoids, and oxidative mediators like nitric oxide (Sánchez-Sánchez *et al.*, 2018). The emergence of antibiotic resistance, limited drug bioavailability, and tissue-specific delivery barriers further complicate therapeutic outcomes. In this context, nanotechnology-based interventions are gaining traction as promising alternatives.

A range of engineered nanomaterials-including liposomes, polymeric nanoparticles, solid lipid nanoparticles, nanogels, and inorganic nanoparticles-have been explored for their ability to overcome the limitations of conventional antibiotic therapies (Zhou *et al.*, 2018; Wang *et al.*, 2017; Paudel *et al.*, 2019). These systems offer several advantages [69, 82, 78, 63]:

- Enhanced targeting and retention at the site of infection
- Protection of the drug from enzymatic degradation
- Controlled and sustained drug release
- Reduced therapeutic dosage and side effects
- Shorter treatment durations

In addition to serving as carriers, some nanomaterials possess intrinsic antimicrobial activity. Their mechanisms include disruption of bacterial membranes, interference with enzyme function, and damage to essential proteins, largely through oxidative stress pathways. Moreover, they can suppress bacterial biofilm formation, modulate gene expression, and even stimulate host immune responses (Wang *et al.*, 2017; Algharib *et al.*, 2020).

Metal-based nanoparticles such as silver oxide (Ag₂O), gold (Au), zinc oxide (ZnO), titanium dioxide (TiO₂), and copper oxide (CuO) have demonstrated broad-spectrum antibacterial efficacy. These particles can hinder bacterial colonization and biofilm development by directly disrupting cell integrity or through oxidative mechanisms (Ferreira-Silva *et al.*, 2017).

A practical example of nanotechnology in action is the encapsulation of enrofloxacin, a widely used antibiotic in

swine. Traditionally administered intramuscularly at 2.5-5 mg/kg body weight for 3-5 days, enrofloxacin faces limitations such as low oral bioavailability and potential side effects. Paudel *et al.* (2019) reported that oral administration of enrofloxacin loaded poly (lactic-co-glycolic acid) (PLGA) nanoparticles via drinking water reduced the minimum inhibitory concentration (MIC) against *Escherichia coli* by 23%, compared to the free drug. The nano-formulation also exhibited enhanced bioavailability, indicating a potential for lower dosage requirements and reduced risk of resistance development.

In addition to therapy, nanotechnology offers diagnostic benefits. Nanosensors incorporating highly sensitive biomolecular probes have been developed for the detection of reproductive tract infections, hormonal imbalances, and even for estrus detection. These nanosensors provide real-time, accurate, and non-invasive diagnostic capabilities, greatly enhancing reproductive monitoring and management in livestock (Saragusty and Arav, 2011).

Nanotechnology has emerged as a transformative tool in combating reproductive tract infections in livestock. By facilitating targeted drug delivery, reducing antibiotic doses, and enhancing antimicrobial efficacy, nanomaterials offer a sustainable and effective solution to many challenges posed by conventional therapies. Additionally, advances in nanosensor technology promise earlier and more precise diagnostics, supporting better reproductive health management. Continued research and translational efforts are essential to fully harness the potential of these technologies in field conditions [78, 4, 26, 63, 70].

Sustain Delivery of Hormone/Nutrition

Reproductive efficiency is a fundamental determinant of productivity in farm animals. Traditional hormonal treatments and nutritional interventions have improved animal fertility; however, they come with limitations such as low bioavailability, immunogenicity, and environmental risks. Recent advances in nanotechnology-based delivery systems offer promising solutions by enhancing hormone stability, reducing doses, and improving nutrient utilization.

Some hormones commonly used in reproductive management, such as gonadotropin releasing hormone (GnRH) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), have low molecular weights and short half-lives, which compromise their sustained delivery and biological efficacy. In contrast, glycoprotein hormones like follicle-stimulating hormone (FSH), luteinizing hormone (LH), human chorionic gonadotropin (hCG), and equine chorionic gonadotropin (eCG) are more stable but may elicit immune responses, leading to antibody formation and reduced responsiveness upon repeated administration (DeCastro *et al.*, 2009; Forcada *et al.*, 2011).

Nanostructured drug delivery systems, particularly those utilizing biodegradable and biocompatible polymers, are being employed to overcome the pharmacokinetic limitations of conventional hormones (Lombardo *et al.*, 2019). These systems protect hormones from enzymatic degradation, prevent rapid clearance, and extend their half-life.

For example, Hashem and Sallam (2020) demonstrated that GnRH nanoparticles conjugated with chitosan-sodium tripolyphosphate (TPP) enabled a 75% dose reduction in goats without compromising fertility or prolificacy. Similarly, Casares-Crespo *et al.* (2018) incorporated nanoparticles into rabbit semen extenders, achieving a 50% reduction in hormone use while maintaining reproductive outcomes.

Beyond dose reduction, NHDS enable alternative administration routes (e.g., intranasal delivery), enhancing animal welfare and minimizing hormone exposure risks for workers and technicians (Hashem *et al.*, 2015; Tomoda *et al.*, 2012).

Conventional hormonal delivery systems, such as progesterone-impregnated intravaginal devices, rely on non-biodegradable silicon polymers and require high hormone concentrations to be effective. These devices pose environmental hazards due to hormone residues and risk of human exposure (Rathbone and Burke, 2013; DeGraaff and Grimard, 2018). In contrast, biodegradable nanofiber-based systems, such as poly (lactic acid) progesterone nanofibers, provide controlled hormone release while eliminating ecological and health concerns (Oliveira *et al.*, 2013).

Nutrition plays a critical role in modulating reproductive function across key physiological stages-fetal development, puberty, and reproductive maturity (CordovaIzquierdo, 2016). Specific short and long-term nutritional strategies have been employed to optimize breeding efficiency. For example, short-term supplementation (7-10 days pre-mating) or long-term feeding (about 60 days before mating) of energy-dense diets (e.g., protected fats, glycerol) has shown to enhance reproductive parameters in small ruminants (Hashem and El-Zarkouny, 2014; Meza-Herrera *et al.*, 2017; Cannas *et al.*, 2019).

Moreover, certain nutrients exhibit 'functional effects' on reproductive physiology. Amino acids, for example, are essential for the development of the oviductal and uterine histotroph, and for the composition of amniotic and allantoic fluids, which support embryo development (Abdelnour *et al.*, 2021). In dairy cows, linseed oil supplementation, rich in linolenic acid (C18:3), has been associated with increased plasma progesterone levels and improved first-AI conception rates (Castro *et al.*, 2019).

On the other hand, nutritional imbalances can lead to poor body condition, delayed puberty, longer open days, and infertility due to hormonal dysfunction and ovarian inactivity. The integration of nanotechnology into livestock nutrition holds promise for enhancing the effectiveness of feed additives and supplements. Nano-formulated nutrients exhibit improved solubility, bioavailability, and cellular uptake due to their reduced particle size and increased surface area (Gawad and Fellner, 2019). This allows for sustained and site-specific nutrient release, optimizing their biological function and reducing wastage [30, 48, 35, 10, 36, 77, 67, 16, 62, 13, 33, 55, 8, 1, 11, 32].

Nanotechnology represents a transformative approach in reproductive and nutritional management of livestock. From enhancing the efficacy and safety of hormonal treatments to improving nutrient absorption and utilization, nano-delivery systems promise to elevate animal productivity while ensuring environmental sustainability and occupational safety. As research progresses, further integration of nanotechnology into livestock management protocols could redefine current reproductive and nutritional paradigms.

Semen Purification

Semen purification plays a critical role in enhancing reproductive efficiency by isolating high-quality sperm subpopulations-those with superior motility and morphology while simultaneously eliminating defective cells, cellular debris, and potential contaminants from the ejaculate. Conventional semen purification methods employed in farm animals include density gradient centrifugation (Sieme *et al.*, 2003), the swim-up technique (Arias *et al.*, 2017), column

filtration (Galarza *et al.*, 2018), and single-layer centrifugation (Nongbua *et al.*, 2017). While these approaches are widely used, they often involve significant manual handling, are time-intensive, and require moderate technical infrastructure.

Recent advancements have introduced nanotechnology-based purification strategies as promising alternatives. Specifically, magnetic nanoparticles (Fe_3O_4) functionalized with biological ligands-such as peanut agglutinin (PNA), *Pisum sativum* agglutinin (PSA), annexin V, and anti-ubiquitin antibodies-have shown efficacy in selectively binding and removing defective spermatozoa in bovine semen samples (Faazah *et al.*, 2014). These biofunctionalized nanoparticles facilitate the enrichment of viable sperm by targeting apoptotic or ubiquitinated cells, thus enhancing semen quality.

Such Nano-magnetic purification methods have demonstrated several advantages over traditional techniques. They minimize excessive semen manipulation, reduce labour intensity, and shorten processing times (typically under one hour), while also eliminating the need for costly laboratory equipment (Feugang, 2017; Meles *et al.*, 2022). Consequently, the integration of nanoparticle-based strategies offers a streamlined, efficient, and cost-effective solution for obtaining high-quality spermatozoa, particularly in resource-limited settings.

Semen Preservation (cryopreservation)

The integration of nanotechnology into semen extender formulations has emerged as a promising strategy to enhance the preservation of sperm quality during storage and cryopreservation. Among various nanomaterials, cerium oxide nanoparticles (CeO_2 NPs), recognized for their oxygen buffering capacity, have demonstrated significant potential in protecting spermatozoa. Supplementation of semen extenders with CeO_2 NPs during storage at 4°C for up to 96 hours has been shown to enhance sperm motility, particularly after 48 hours, and maintain plasma membrane and DNA integrity (Falchi *et al.*, 2018a).

Selenium nanoparticles (Se NPs), known for their reactive oxygen species (ROS) scavenging ability, have also been employed to mitigate oxidative damage in sperm cells. Their inclusion in semen preservation protocols has resulted in improved post-thaw sperm motility, morphology, and decreased apoptotic and necrotic cell populations, ultimately contributing to higher in vivo fertility rates (Khalil *et al.*, 2019). Similarly, nano-lecithin-based extenders, specifically those containing 2% lecithin, have been found superior to conventional egg yolk- or lecithin-based media in preserving goat spermatozoa, evidenced by increased motility, viability, and functional membrane integrity, along with reduced apoptosis (Nadri *et al.*, 2019).

Advancements in magnetic nanoparticle technology have enabled the targeted purification of sperm populations. For instance, Fe_3O_4 nanoparticles functionalized with lectins or antibodies have been effectively used to isolate viable spermatozoa from both fresh and extended porcine semen (Durfey *et al.*, 2019), as well as from cryopreserved bull semen (Odhiambo *et al.*, 2014). Other studies have utilized avidin-conjugated Fe_3O_4 nanoparticles along with DNA aptamers to sort both unsorted and sex-sorted bull sperm (Farini *et al.*, 2016).

In addition to metal oxide nanoparticles, "nanowater" has emerged as a novel extender medium. When used for ram semen, nanowater improved fertilizing capacity, with significant increases in conception and lambing rates

(Murawski *et al.*, 2015). Moreover, the antioxidant properties of various nanomaterials-such as cerium oxide, selenium, vitamin E nanoemulsions, and noble metal nanoparticles (gold, platinum)-have been associated with improved sperm motility, membrane integrity, and overall semen quality during chilled or frozen storage (Falchi *et al.*, 2018b; Jurado-Campos *et al.*, 2023; Hashem and Gonzales-Bulnes, 2020; Khalique *et al.*, 2024).

Cryopreservation of semen often results in functional impairments, including DNA fragmentation, premature capacitation, and disruption of acrosomal and plasma membrane integrity (Yoon *et al.*, 2015; Yeste, 2016). To mitigate these damages, liposome vesicles have been investigated for their ability to fuse with sperm membranes and deliver protective agents intracellularly. Liposomes incorporated into egg yolk-free extenders (e.g., OptiXcell®) have proven effective in minimizing cryodamage across several species, including bulls, buffaloes (Ansari *et al.*, 2016), and camels (Al Bulushi *et al.*, 2019; Swelum *et al.*, 2019).

Furthermore, unilamellar liposomes and mesoporous nanoparticles serve as effective carriers for controlled antioxidant release during semen storage. Liposomes loaded with compounds such as quercetin and lycopene have offered superior cryoprotection in spermatozoa of various livestock species, including bovines (Röpke *et al.*, 2011; Kumar *et al.*, 2015), porcines (He *et al.*, 2001), ovines (Luna-Orozco *et al.*, 2019; Mafolo *et al.*, 2020; Mortazavi *et al.*, 2020), and equines (Pillet *et al.*, 2012; Medina-León *et al.*, 2019).

Organic nanoparticles, particularly liposomes and exosomes, offer further potential due to their biocompatibility and membrane-binding ability. These nanocarriers have been shown to reduce the population of non-viable spermatozoa and may facilitate the repair of damaged sperm membranes. While synthetic liposomes can be engineered to deliver specific molecules (Feugang, *et al.*, 2022), naturally derived exosomes from biofluids-including seminal plasma-contain diverse bioactive cargos such as proteins, miRNAs, lipids, and mRNAs (Piehl *et al.*, 2013; Yang *et al.*, 2017; Dilsiz, 2022; Dlamini *et al.*, 2023). These attributes make both liposomes and exosomes promising vehicles for enhancing sperm viability and fertility potential during and after cryopreservation [7, 31, 60, 22, 27, 54, 23, 44, 59, 21, 61, 25, 58, 24, 42, 34, 45, 81, 80, 5, 3, 76, 68, 46, 38, 49, 50, 56, 65, 53, 28, 64, 79, 17, 18].

Nanotechnology-Based Strategies for Sperm Sexing and Fetal Sex Determination

Recent proteomic investigations have identified distinct surface protein profiles in bull spermatozoa, revealing that 12 proteins are upregulated in X-chromosome-bearing sperm, while 3 are more abundant in Y-bearing sperm (Quelhas *et al.*, 2021). These molecular markers offer promising targets for selective sperm separation through immune affinity approaches. The application of magnetic nanoparticles (MNPs), functionalized with specific ligands or antibodies that recognize these differential protein expressions, presents a novel and efficient technique for immune selection of sex-specific sperm cells.

Furthermore, surface-engineered magnetic nanoparticles have been successfully employed to exploit the differential surface charge between X- and Y-chromosome-bearing spermatozoa. By modifying nanoparticle coatings to selectively bind Y-bearing sperm based on their electrostatic properties, researchers have achieved effective enrichment of X-bearing

sperm within semen samples (Domínguez *et al.*, 2018). For practical application, scalable production of such functionalized nanoparticles is crucial to support the broader adoption of nanotechnology-assisted sex-sorting methods in livestock reproduction.

In addition to sperm sexing, advancements in nanoscale biosensing have contributed to non-invasive fetal sex determination. Technologies such as biochips integrated with nanomaterials have shown potential for accurately identifying fetal sex at early stages of pregnancy by detecting sex-specific biomarkers in maternal biological fluids (Joanitti and Silva, 2014). These platforms combine high sensitivity and specificity with rapid analysis, offering a valuable tool for reproductive management in both veterinary and human medicine [66, 19, 41].

Conclusion

In conclusion, the integration of nanotechnology into gynecology and reproductive management in livestock presents a transformative shift toward precision, efficiency, and sustainability. From enhancing biostimulation via pheromone delivery and improving diagnosis and treatment of reproductive infections to enabling sustained hormone and nutrient release, nanotechnology offers novel solutions to long-standing challenges. Moreover, innovations in semen purification, preservation, and sex selection-along with non-invasive fetal sex determination-underscore its broad applicability across reproductive stages. As research continues to evolve, these nanotechnological advancements promise to revolutionize animal reproduction, optimize productivity, and contribute to more effective and environmentally responsible livestock management systems.

Conflict of Interest

Not available

Financial Support

Not available

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How to Cite This Article

Kumar V, Chaudhary SD, Verma AK, Patel A, Tripathi A, Dubey P, Shukla MK, Singh V. Application of nanotechnology in animal reproduction. *International Journal of Veterinary Sciences and Animal Husbandry*. 2026;SP-11(7):16-23.

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