Reproductive Management of Farm Animals

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Reproductive efficiency of farm animals has central consequences on productivity and profitability of livestock farming systems. Optimal reproductive management is based on applying different strategies, including biological, hormonal, nutritional strategies, as well as reproductive disease control.

nano-delivery system | reproductive management | bio stimulation | nutrition | hormones | antibiotics | reproductive diseases | livestock

1. Introduction

In livestock farming systems, reproductive efficiency has central consequences on the productivity, profitability, and sustainability of farms. The reproductive performance of farm animals determines the efficiency of milk and/or meat production, either directly or through managing decisions, such as replacement and culling rates. Optimal reproductive management is based on applying precision strategies which also needs to consider costs, animals' welfare, environmental impacts, and human health. Most of the reproductive management practices are ready for their use in commercial livestock farms after selecting the strategy which meets goals of every farm [1][2]. Such strategies may include one or more bio stimulation tools (e.g., male effect), reproductive assisted techniques (mainly estrous synchronization and artificial insemination), nutritional management, and prevention/treatment of reproductive diseases [3][4][5].

Although these reproductive management strategies are widely and predominantly applied in different livestock production systems, their efficiency is challenged by several practical and ethical aspects. For example, hormonal-based reproductive therapies are the preferred method for reproductive management; however, their effectiveness is highly dependent on their pharmacokinetics and pharmacodynamics, which may be affected by biological factors [6][7]. The male effect is a sexual bio stimulation method that confers an opportunity to eliminate the intensive use of hormones in reproductive management and the production of hormone residues-free animal products; however, its outputs are challenged by the sexual activity of both males and females, male to female ratio, and age and the experience of the male [4][8][9][10]. Similarly, nutritional management practices for improving the reproductive performance of farm animals may be negatively impacted by the lack of nutrients bioavailability and insufficient delivery of required nutrients [11][12]. Lastly, the management of reproductive-related diseases is challenged by the concerns regarding the intensive use of antibiotics and the development of antimicrobial-resistant strains [13][14][15].

2. Management of Reproductive-Related Diseases

2.1. Importance and Challenges of Antibiotic Applications

Animal production, and specifically reproduction, is usually associated with the episode of reproductive diseases. Postpartum diseases, specifically endometritis caused by different bacterial species (mainly *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Prevotella melaninogenica*, and *Arcanobacterium pyogenes*), are accompanied by impaired reproductive performance reflected in reduced conception rate and increased risk of reproductive culling [16]. The hazard of pregnancy, days elapsed from calving until pregnancy, reached 0.60, 0.31, and 0.24 in cows with metritis, clinical end metritis, and subclinical end metritis, respectively, when compared to healthy herd mates [17]. Pregnancy-associated diseases, such as toxoplasmosis (*Toxoplasma gondii*) and neosporosis *(Neospora caninum)*, are protozoan diseases that lead to significant economic loss in farm ruminants. Worldwide, toxoplasmosis infection is a zoonosis that mainly causes reproductive failure in small ruminants, whereas neosporosis infection is a common zoonosis that causes abortion in cattle [18]. In dairy farms, bovine mastitis, mainly caused by *Staphylococcus aureus* causes significant economic losses due to severe declines in milk yield (about 380 tons of milk are lost every year in the world), dumped milk, reproductive disorders, and expenses paid to the replacement of infected animals, increased costs of pharmacologic costs, and replacing tainted milk [15][19]. Additionally, the contamination of raw milk with *Staphylococcus aureus* raises public health problems throughout the food chain.

Overall, these diseases have negative impacts on the animals' reproductive efficiency and welfare, public health, and the final profit of the production process. The clinical symptoms of most of these bacterial and/or zoonotic diseases are mediated directly by microbial products (endotoxin) and tissue damage, or indirectly by inflammatory (cytokines and eicosanoids) and/or oxidative stress (nitric oxide) mediators [18]. These changes have a negative impact on sperm function and quality (sperm motility and sperm phagocytosis), ovarian function (follicular steroidogenesis and growth, ovulation, and ova competence development to blastocyst), uterine competence (implantation failure), and embryonic development (retarded development into blastocyst) [15][17].

Currently, antibiotic-based therapy is the commonly recommended therapy for tackling different microbial/protozoan diseases, including reproductive-related diseases. The effectiveness of antibiotics-based therapies is controlled by the pharmacokinetics of the drug. The delivery of antibiotics into targeted infected sites depends on the rate of absorption and distribution of the drug, which can be limited by different biological factors as the stability of the antibiotics against degradation by gastrointestinal enzymes (oral administration), blood hydrolytic enzymes (parenteral administration), drug solubility, and thus cellular uptake and bioavailability. Furthermore, some diseases cause fibrous damage in infected tissues limiting the penetration of the antibiotics into infected sites when local treatment is applied, such as in the direct infusion of the drug into the uterus in endometritis cases or through teats in mastitis cases [15][20].

Despite the limiting biological and therapeutic efficiency of the current antibiotics-based therapies, emerging concerns regarding the development of antimicrobial-resistant species add other limitations on the applications of antibiotic-based therapies, specifically in food-producing farm animals. The fear of developing more wild pathogenic microbial species, creating infectious and cross-transited microbial species, transferring of antibiotic

residues into animal products (meat and milk), and the release of antibiotics into the environment are all aspects that should be taken into account [21]. Antimicrobial resistance leads not only to an encumbrance on public health but also extends to the risk of therapy failure and repeated infection, along with subsequent economic impacts. Actually, these factors make the treatment of reproductive-related diseases (mastitis [15], toxoplasmosis, and neosporosis [18]) by antibiotics a controversial strategy. Accordingly, novel, safe, and effective antibiotics-based therapeutic approaches are needed, particularly when treatments are directed to food-producing farm animals [18].

2.2. Nanotechnology Approaches

Many studies have shown the opportunity of using many engineered nanomaterials (e.g., liposomes, polymeric nanoparticles, solid lipid nanoparticles, nanogels, and inorganic nanoparticles), which are synthesized with specific physicochemical properties to overcome the therapeutic limitations of antibiotics-based therapies [19][22][23][24].

The use of nano-formula for antibiotics-based therapies may offer additional advantages over conventional antibiotics formula, such as (1) reducing the dose of the antibiotic, (2) allowing efficient delivery of the antibiotic to the infected sites, (3) shortening the therapeutic timing and side effects, and (4) preventing burst release and degradation of the antibiotic [19]. Nanomaterials may be protective against the rapid degradation of the antibiotic and may improve its delivery to the infected site, but, moreover, nanomaterials themselves could be engineered to show cytotoxic and destructive properties against microorganisms. Moreover, some nanoparticles have destructive effects on the bacterial cell membrane, enzymes, and functional and structural cell proteins mainly through evoking cellular oxidative pathways, in addition to their ability to inhibit the formation of bacterial biofilm, to induce changes in the gene expression, and to stimulate innate and adaptive immunity [22]. Additionally, nanoparticles could be engineered to hinder the bacterial adhesion, colonization, and biofilm development of bacteria [15]. Furthermore, nanomaterials have the ability to incorporate one or more drugs without any effect on the structure of the compound but increasing its pharmacological action [23].

Specifically, in swine, enrofloxacin antibiotic is used to treat several bacterial infections, such as *Pasteurella*, *Mycoplasma*, *Escherichia coli*, or *Salmonella*, with an intramuscularly recommended dose average between 2.5 to 5 mg enrofloxacin/kg Bw/day for 3 to 5 days. Paudel et al. [24] showed that enrofloxacin-loaded poly(lactic-co-glycolic acid) nanoparticles may be delivered orally in a suspension in drinking water, and the minimum inhibitory concentration against *Escherichia coli* was reduced by 23% compared to free enrofloxacin alone. Such finding, combined with increased bioavailability, maybe an interesting first step to reduce the dose of enrofloxacin and, therefore, its side effects (including the propagation of antibiotics resistance). El-Zawawy et al. [25] reported that incorporating triclosan into the lipid bilayer of liposomes allowed its usein lower doses, which in turn reduced its biochemical adverse effects. In another study, sodium dodecyl sulfate-coated atovaquone nanosuspensionsconsiderably increased the therapeutic efficiency against experimentally acquired and reactivated toxoplasmosis by improving the passage of gastrointestinal and blood–brain barriers [26]. Similarly, tilmicosin (a semi-synthetic macrolide antibiotic)-loaded hydrogenated castor oil with lower dosage showed better therapeutic efficacy than free tilmicosin for *Staphylococcus aureus* mastitis infectiondue to the enhanced bioavailability and sustained-release performance [27]. Recently, nano drugs have also been used as astrategy to solve the multi-drug

resistance and intracellular persistence of *Staphylococcus aureus*, which is associated with the subclinical and relapsing infection of bovine mastitis [19]. Yang et al. [13] showed the possibility of prolonging post-antibiotic effects and thus dosing intervals when amoxicillinnanoparticles are used for treating bovine mastitis. This would decrease the rate of antibiotic use and the costs of medication.

Recently, the combination of the advantages of nano-drug delivery technology and alternative medicine, which depends on the usage of natural products with antimicrobial activity, opens the way to innovative natural and safe antibiotic alternatives. Numerous studies have shown probiotic species, microbial extracts, and plant secondary metabolites (essential oils and polyphenols) as potential antimicrobial agents [28][29]. A nano-formulacontaining 0.4–10% of oregano oil was developed for treating dairy cow endometritis. The uterine infusion of this nano-formula for 2–5 days showed a remarkable curative effect, being able to diminish inflammation, sterilizing uterus, draining pus by contracting the uterus, and promoting the recovery of the uterine function (Patent: CN104288222A, china https://patents.google.com/patent/CN104288222A/en; accessed on 12 February 2021). In another study, poly(lactic-coglycolic) acid(PLGA)-epigallocatechin gallate-deoxycyclin nanoparticles have been successfully used as an assisted-endometritis therapy [30]. In context, chitosan-TPP nanoparticles have been used for treating mastitis [31].

Additionally, many metal nanoparticles, such as silver oxide (Ag_2O), gold (Au), zinc oxide (ZnO), titanium dioxide (TiO_2), and copper oxide (CuO), have shown effective antimicrobial activity against a broad spectrum of microorganisms [32]. These approaches may give an opportunity to completely substitute antibiotics-based treatments with more safe therapies. The emergence of biological biosynthesis procedures of nanometal (silver) using microorganisms (*Escherichia coli*, *Acinetobacter species*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*) and/or natural reducing agents (polyphenols, flavonoids, and phenolic biomolecules of Camellia, green tea, and black tea leaf extracts)has encouraged the use of nano metals as an alternative to antibiotics, meeting both therapeutical and environmental aspects [28][33]. In this context, apigenin (a polyphenolic compound) was successfully used to synthesis silver nanoparticles with a size of 10 nm.These nanoparticles showed antimicrobial activity against pathogenic bacteria *Prevotella melaninogenica* and *Arcanobacterium pyogenes* isolated from endometritis infected uterine discharges by inhibiting cell viability and biofilm formation in a dose-and time-dependent manner [16]. Similarly, Yuan et al. [34] confirmed the antibacterial activity of biologically synthesized silver nanoparticles against two multiple drug-resistant strains of *Pseudomona saeruginosa* and *Staphylococcus aureus* isolated from mastitis-infected goats milk samples.

Regarding the toxicity of such nanomedicines to animal tissues, Radzikowski et al. [35] confirmed the potential of commercially available silver nanoparticles, copper nanoparticles, and their combination in decreasing the viability of mastitis-borne pathogens without showing toxic effects on mammary gland tissues. Furthermore, Paudel et al. [24] confirmed the lower toxicity of enrofloxacin entrapped nanoparticles to mammalian cells relative to a free drug as the incorporation of the drug into the PLGA matrix minimized the production of reactive oxygen speciesevoked by the antibiotic.

A summary of studies on the nano drugs developed to treat reproductive-related diseases is shown in **Table 1**.

Table 1. Summary of studies on the nano drugs developed to treat reproductive-related diseases.

Type of Drug	Formula	Technique	Particle Characteristics	Drug Activity	Usage
Antibiotic [24]	Enrofloxacin-poly lactic-co-glycolic acid NPs	-	Size = 102 nm PdI = 0.095 Zp = −32 mV	Antimicrobial agent against *Staphylococcus aureus*, *Escherichia coli*	Endometritis and mastitis treatment
Antibiotic [27]	Tilmicosin-loaded hydrogenated castor oil NPs	Hot homogenization and ultrasonication	Size = 343 nm PdI = 0.33 Zp = 7.9 mV EE = 60.4%	Antimicrobial agent against *Staphylococcus aureus*	Mastitis treatment
Antibiotic [25]	Triclosan-loaded liposome NPs	Dehydration-rehydration	Size = 53.3 nm EE = 90%	Antimicrobial agent against *Toxoplasma gondii*	Toxoplasmosis treatment
Antibiotic [26]	Atovaquone-poloxamer 188 - sodium dodecyl sulfate	-	-	Antimicrobial agent against *Toxoplasma gondii*	Toxoplasmosis treatment
Nitric oxide (NO) [36]	NO-alginate-chitosan NO-chitosan-TPP	-	Size= 270–375 nm PdI=0.27–0.31 Zp = 16−17 mV	Antimicrobial agent against *Staphylococcus aureus*, *Escherichia coli*	Mastitis treatment
Metal [16]	Silver NPs	Biosynthesis by apigenin	Size = 10 nm	Antimicrobial agent against *Prevotella melaninogenica* and *Arcanobacterium pyogenes*	Antibiotic alternative for endometritis treatment
Metal [34]	Silver NPs	Biosynthesis by quercetin	Size = 20 nm Zp= 37.7mV	Antimicrobial agent against *Staphylococcus aureus* and *Pseudomonas aeruginosa*	Antibiotic alternative for mastitis treatment
Chitosan [31]	Chitosan-TPP Nps	Ionotropic gelation	Size = 19.1 nm PdI = 0.41 Zp = 49.9 mV Yield particle = 92.8%	Antimicrobial agent against *Pseudomona* sp.	Antibiotic alternative for mastitis treatment
Antibiotic + polyphenol [30]	Poly(lactic-co-glycolic) acid-epigallocatechin gallate-doxycycline Nps	Modified double emulsion solvent evaporation/extraction technique	Size = 176 to 211 nm PdI = 0.124 to 0.466 EE= 78.5 to 86.3%	Anti-inflammatory agent	Assisted-endometritis therapy

References

Type of Drug	Formula	Technique	Particle Characteristics	Drug Activity	Usage
	Singh et al., 2015				
Essential oil [1]	Oregano oil Nps	-	-	Antimicrobial agent against *Staphylococcus aureus, Escherichia coli, Streptococcus* spp.	Antibiotic alternative for endometritis treatment

Nps = nanoparticles, Zp = zeta potential, PdI = Polydispersityindex, and EE = encapsulation efficiency. [1] https://patentimages.storage.googleapis.com/patent/WO2021042822A1/en, accessed on 02 February 2022.

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