Perspectives of nanotechnology in male fertility and sperm function

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A B S T R A C T

Recent advances in nanotechnology have tremendously expanded its possible applications in biomedicine. Although, the effects of nanoparticles (NPs) at cellular and tissue levels have not been fully understood, some of these biological effects might be employed in assisted reproduction to improve male fertility particularly by enhancing sperm cell quality either in vivo or in vitro. This review summarises the available literature regarding the potential applications of nanomaterials in farm animal reproduction, with a specific focus on the male gamete and on different strategies to improve breeding performances, transgenesis and targeted delivery of substances to a sperm cell. Antioxidant, antimicrobial properties and special surface binding ligand functionality and their applications for sperm processing and cryopreservation have been reviewed. In addition, nanotoxicity and detrimental effects of NPs on sperm cells are also discussed due to the increasing concerns regarding the environmental impact of the expanding use of nanotechnologies on reproduction.

1. Introduction

Nanoparticles (NPs) are particles that are synthesized with extremely small size, at the nanometer scale, with flexible fabrication and high surface-area ratio. Nanoparticles can be made from a variety of materials including metals, polysaccharides, and proteins. Recent advances in nanotechnology have tremendously expanded its possible applications in several scientific branches including medicine. This is mainly attributed to the engineering of nanoparticles with various physical and chemical properties that make them more stable, soluble and more biologically effective compared to their corresponding unengineered homologues. In addition, NPs have been increasingly employed in the field of drug delivery for generating therapeutic formulations either for lipophilic or unstable hydrophilic substances. Systemic delivery of poorly water-soluble (hydrophobic) drugs remains in fact a major problem in clinical pharmacology. In the WHO (World Health Organization) Model List of Essential Medicines, about 25% of the drugs are considered poorly water-soluble\textsuperscript{(1)} for which the application of nanotechnology could prove highly valuable.

Nanoparticles or nanoparticle-loaded drug formulations are currently being developed to increase drug efficiency through e.g.; 1) protecting against digestion and degradation in the gastrointestinal tract and thus maximizing intestinal absorption and increased oral bioavailability\textsuperscript{(2)}; 2) prolongation of the half-life of drugs in circulation; 3) bypassing blood-tissue barriers and delivery to specific target tissues, or even at cellular level; 4) rapid onset and prolonged therapeutic action; and 5) reduced effective dose and side effects or toxicity. Several reviews of literature provide comprehensive overviews on the expanding applications of nanotechnology in the field of drug delivery\textsuperscript{(3–5)}.

However, a better knowledge of the effects of nano-compounds at cellular and tissue levels is necessary, and little information is available about their activity in the reproductive system. The capacity of nanocarriers to cross the hematotesticular barrier\textsuperscript{(6)} have been demonstrated raising concerns about their distribution and biocompatibility at systemic level and suggesting that in vivo effects may be the result of systemic alterations but also a consequence of direct effects at the testicular level.

This review will focus on the reported in vitro and in vivo effects of different types of nanoparticles, either beneficial or detrimental, on male fertility and sperm cell function in farm animals, keeping in mind that these effects strictly depend on the chemical nature of the compound (size, surface charge, coating etc.) but also on the biological system involved (animal species, tissue, cell variability). A special
attention was given to the anti-oxidant properties of some nano-com-
ounds and to the possibility to build nano-cargos, promising tools for
improving transgenesis and targeted delivery in assisted reproductive
techniques.

2. Effects of NPs as antioxidants on sperm functions

Recent advances in nanoparticle technologies resulted in the de-
velopment of several NP formulations with potent antioxidant, anti-
flammatory, and antimicrobial properties [7–9]. This obviously opens
great potentials in the area of improvement of reproductive functions
either in vitro or in vivo [10].

Applications of NPs based on their antioxidant properties can be
particularly valuable for sperm functions and male fertility. Sperm
cooling and cryopreservation are known to increase oxidative stress
in spermatozoa leading to a significant reduction in their fertilization
capacity [11]. We have recently tested the efficiency of cerium oxide
(CeO₂) NPs, which are able to store oxygen and act as ROS (reactive
oxygen species) scavengers, to protect ram sperm cell viability during
cooling [12]. Supplementation of CeO₂ in semen extender improved
motility parameters after 48 h and up to 96 h of incubation and in-
creased sperm velocity of sperm cells. This was associated with pro-
tecting the integrity of plasma membranes and DNA of spermatozoa
[12]. These effects could not be linked with any reduction in in-
tracellular ROS as detected by H2DCFDA staining [12], however, the
sensitivity of this cytoplasmic stain may not be sufficient to detect
minor differences in sperm cells due to the cellular structure of the
sperm head which is mostly occupied by a nucleus. These beneficial
effects on sperm preservation by cooling can be very useful to increase
productivity of small sheep farms where routine artificial insemination
is done using liquid cooled semen prepared in house.

Nano-selenium (SeNPs) has also been used in several studies as a
scavenger of ROS to protect against oxidative damage in sperm cells.
Addition of Nano-Se to semen extender improved the post-thawing
quality and oxidative variables of rooster semen [13]. In addition, oral
supplementation of SeNPs also protected the quality of spermatozoa
(motility, DNA integrity) and spermatogenesis against oxidative damage
induced by Cisplatin, an anticancer agent with male reproductive
toxicant properties [14]. When supplemented in the diet of bucks,
SeNPs proved to be more effective in increasing antioxidant enzyme
activity (GSH-Px, SOD, CAT) and sperm quality when compared with
other selenium forms (sodium selenite or selenized yeast) [15,16].

Nano-zinc is also among the metal NPs that were efficiently used to
improve semen quality. In young rams, supplementation of the diet
with 50 mg/kg or 100 mg/kg ZnNPs in diet improved epididymal semen
quality, seminal plasma anti-oxidase activities and the expression of
bronze-zinc superoxide dismutase (Cu-Zn SOD) [17]. Similarly, sup-
plementation of bull semen extender with zinc nano-complex decreased
the levels of malondialdehyde (MDA) and improved mitochondrial
functionality [18]. Moreover, Zn nano-complex improved the functionality
of sperm plasma membranes in a dose dependent manner without any
deleterious effect on motility parameters [19]. In streptozotocin-in-
duced diabetic rats, ZnO NPs have been shown to enhance the activity
of antioxidant enzymes in the testicular tissue, to increase sperm count
and improve sperm characteristics by protecting against oxidative stress
[20].

3. Nanotechnologies for sperm purification and sorting in farm
animals

Modern breeding systems require, nowadays, the diffusion of su-
perior genotypes and thus the selection of high quality gametes and
embryos. In male reproduction, many factors such as routine proce-
dures of handling and storage, might affect negatively the quality of
spermatozoa, and several strategies have been designed to overcome
these limitations in order to achieve high in vivo and in vitro fertility
rates [21,22]. Among others, sperm purification and sorting represent a
valid tool for the selection of sub-populations of the best spermatozoa
(in terms of motility and morphology) within an ejaculate and the
discard of defective cells, contaminations and debris. These techniques
also allow to recover spermatozoa from sub-optimal ejaculates from
animals of high genetic values. In literature, several techniques have
been developed to achieve this purpose and have been tested in farm
animals: among others, swim up assay [23–25]; density gradient cen-
trifugation [23,25–27], filtration through columns [28–32] and single
layer centrifugation (SLC) [33–35]. Although these procedures greatly
improve motility and functional parameters especially in post-thaw
semen, on the other side they lead to a reduction in the concentration of
the insemination doses due to their variable recovery rates, they are
time consuming and add costs and labour to the standard procedures of
semen handling and storage [36].

A novel approach for semen selection is represented by the devel-
opment of techniques that target specific negative biomarkers of sperm
quality, such as for example, defects in the acrosome and cytoplasm
membrane or the presence of ubiquitin on sperm surfaces [as reviewed
by [37]]. Magnetic beads coated with probes that specifically bind to
these biomarkers (peanut and pisum sativum agglutinin (PNA/PSA)
lectins, annexin V, anti-ubiquitin antibodies) have been successfully
used both in humans [38] and in bulls [39] for removing defective
spermatozoa by exposure to magnetic field (MACS, magnetic activated
cells sorting).

In this context, nanoparticles, with their peculiar characteristics of
tunable size, surface charge and binding properties, have recently
contributed to optimise MACS protocols. In particular, Fe₃O₄ NPs,
known for their magnetization, bio-compatibility and bio-functional-
isation properties [40], have been conjugated either with PNA/PSA
lectin, to selectively bind to glycans expressed on reacted acrosomes,
or with anti-ubiquitin antibodies. With the specific binding to defective
spermatozoa, they have been employed to nanopurify frozen-thawed
bull spermatozoa, resulting in an increase in conception rates following
artificial insemination [41]. Similarly, Fe₃O₄ NPs coated with PNA/PSA
lectins were used for nanopurification of boar semen, efficiently se-
lecting a subpopulation of highly motile and viable spermatozoa [42].
Interestingly, the authors of these studies reported that the procedure of
sperm nanopurification did not require extensive semen manipulation,
labour and additional costs, as it takes less than 1 h to prepare a puri-
fied semen sample without expensive equipment [36]. Removal of dead
spermatozoa is indeed of critical importance as it negatively affects the
motion characteristics and membrane integrity of live spermatozoa
[43,44]. These encouraging results suggest that nanotechnologies could
be easily included in the routine procedures of selection of high quality
sperm population to enhance fertility rates in farm animals.

Another promising application of nanotechnologies was suggested
by Farini et al. [45] for bovine sperm selection using the Cell-SELEX
(systematic evolution of ligands by exponential enrichment) technique.
In this method, avidin-coated superparamagnetic Fe₃O₄ NPs (SPION)
selectively bound to synthetic DNA aptamers specific for spermatozoa
with damaged membranes. The authors reported that the removal of
damaged spermatozoa with the aptamer/SPION system significantly
improved semen quality, in terms of viable cells, and did not affect in
vitro embryo development [45]. Those magnetic NPs have been pre-
viously shown not to affect sperm motility or acrosome reaction [46].

NPs have also been recently suggested as valid alternatives to
fluorochromes and flow cytometry for sperm sorting, and targeting
specific sperm DNA sequences. The use of NPs as DNA tags has been
investigated with encouraging results by Barchanski et al. [47], pro-
posing bio-conjugated AntiNPs as tools for genetic labelling in bull
sperm. Recently, in the bovine, Gamrad et al. reported successful la-
belling of specific sites of the Y chromosome by oligonucleotide func-
tionalised AntiNPs [48]. In future, the development of specific nanop-
robes will provide precious tools to sex sort sperm cells in routine
reproductive management of farm animals, replacing flow cytometry.
and its limitations [49–51].

4. Sperm transport of NPs functionalised substances

In the past years, nanotechnologies have been gaining increasing interest in the development of nanoplatforms for delivery of biological compounds to specific targets within tissues or cells. These applications would provide great advantages in the progress of assisted reproductive technologies in farm animals and the potential of these nanocarriers interacting with gametes is yet to be fully understood, opening new perspectives but also new controversies. The majority of the reports on transport of nanocargos and reproduction focused on the spermatozoa for the peculiar characteristics of this cell: motility, scarce intracellular uptake, great capacity to interact through its membranes with several compounds. These properties, combined with the great plasticity, targeted action, binding attitude and small size of NPs have been increasingly investigated in the past decades to achieve successful loading of sperm cells with nucleic acids or proteins. Makhluf et al. in a series of experiments described the spontaneous loading of polyvinyl alcohol (PVA) -Fe₃O₄ and PVA-Eu₂O₃NPs by bovine sperm cells, without detrimental effects on motility and acrosome integrity [46]. Following these observations the same research group designed PVA-Fe₃O₄ NPs conjugated with an antibody raised against protein kinase C. They further described up-take of the antibody/NPs cargo by sperm cells (mainly in the post-acrosomal and upper regions of the head) and functional activity of the antibody in binding its specific antigen. These results suggested a strong ability of nanocarriers to deliver functional cargos into spermatozoa representing a promising tool for protein identification in target tissues or cells [52]. Similarly, in boar spermatozoa, mesoporous silica NPs (MSNPs) loaded with either a fluorescent nucleic acid (lamin siRNA) or a fluorescent protein (mCherry) interacted with cell membranes without compromising sperm function, indicating these NPs as potential candidates of biological cargos for in-vagame delivery [10]. These encouraging results pave the way for important applications in the field of animal reproduction. Loading sperms with specific nucleic or protein markers may help understanding a number of mechanisms that regulate physiological processes such as fertilization and embryo development. NPs might also be conjugated with target nutrients or treatments for direct supplementation to sperm cells. Moreover, the use of nanocarriers conjugated with specific labels of sperm quality, might help the investigation of the processes of oxidative stress and membrane damage during semen storage at low temperatures (refrigeration or cryopreservation) that greatly impair sperm function, especially in those species in which handling and storing semen is sub-optimal (for example ovine, endangered species, etc) [11].

Mesoporous silica NPs (MSNPs), functionalised with aminopropyltriethoxysilane, polyethileneimine and optionally loaded with two common types of cargo (nucleic acid/protein) were designed to form strong associations with boar sperm without negatively affecting sperm functions such as sperm viability, acrosomal reaction and DNA fragmentation [10,53]. Together with the high affinity of these MSNPs to sperm cells [53], they are suggested to serve as promising nanomaterial to develop or increase the efficiency of existing diagnostics and therapeutic compounds related to sperm quality and male fertility.

5. Treatment of genital infections

In literature, several reports described the antibacterial, antiviral and antifungal properties of metal NPs or dendrimers [54–56]. These characteristics derived either by their actions of lysis on the microbial cell walls, inhibition of viral adhesiveness and penetration into cells, pro-oxidant action with consequent induction of apoptosis and death of the microbial cell [57] or by their drug delivery capacity, enhancing the target release of specific antimicrobial substances [58]. In the field of human reproduction, nanotechnology has been applied for potential treatment of a variety of genital infections raised by different pathogens: among others Clamydia trachomatis [59], Candida albicans [60], vaginal Herpes-virus [61]. On the other hand, a very limited number of reports on this topic has been published on farm animals. Recently, the antimicrobial effects of AgNPs have been tested against Gram negative Prevotella Melaninogenica and Gram + Arcanobacterium pyogenes, two of the multiple drug resistant and most frequently isolated pathogens responsible for bovine clinical endometritis. The results showed that NPs were able to induce metabolic disturbance increasing intracellular production of free radicals, and decrease in biofilm activity [62], representing a valid alternative to traditional antibiotic treatments. No trials to counteract male genital tract infections in farm animals using NPs have been reported. A great deal of information still need to be collected in order to develop therapies in vivo and to introduce nanotechnologies in clinical practice and in farming routine. However, this novel approach to contrast infectious diseases in farm animals will provide great health and economic benefits, leading to targeted therapies and contrasting drug resistance.

6. Other recent applications of NPs in semen preparations

An assay using heparin AuNPs (HAuNPs) has been developed as a biomarker for human fertility [63]. This assay utilize the polycationic properties of protamine, the most abundant nuclear protein in the sperm. The electrostatic interactions between polycationic protamine and polyanionic heparins result in visibly observable color change that can be detected by naked eye [63]. Development and optimization of similar “lab-free” tests can be used in the future as animal-side tests to assist in the selection of males for breeding in pets, rabbits and farm animals.

7. Controversies in the use of nanotechnology in reproduction: Environmental impact of NPs dispersion and reprotoxicity

Nanoparticles, particularly the metal oxide NPs, are increasingly used in many fields of everyday life e.g. in cosmetics, electronics and food packaging. Therefore, there are environmental concerns about the dispersion of these NPs and their biological effects on living organisms. Controversial reports about the reprotoxic effects of NPs has been addressed in several recent articles and particular attention has been given to the specific effects on male gametes.

Zinc oxide NPs were shown to exert a cytotoxic action on murine testicular germ cells in a dose dependent manner [64]. Xu et al. [65] found that SNP primarily affect the maturation process of spermatozoa in the epididymis that turned out to be more sensitive to NPs toxicity compared to both spermatogonia and spermatocytes. ZnO NPs lead to the reversible damage of epididymal sperm without affecting fertility. The NPs have detrimental effects on the quantity and quality of sperm in the epididymis by causing oxidative stress and by damaging the structure of mitochondria, resulting in energy metabolism dysfunction [65].

Silver NPs are a potential cytotoxic agent for sperm cells and exert adverse effects, possibly through the induction of oxidative stress. Furthermore, exposure of murine sperm cells to AgNPs reduces the success rate of in vitro fertilization, delays subsequent blastocyst formation, and downregulates gene expression responsible for embryonic development [66]. Lafuente et al. [67] showed that in rats, exposure to oral sub-chronic doses of 50–200 mg/kg/day PVP-AgNPs possesses slight negative influence on sperm morphology.

Hong et al. [68] reported that dose-dependent exposure to TiO₂NPs caused testicular toxicity, reduced sperm production, and induced sperm lesions in mouse testes, which were closely related to reductions of daily food and water intake, biochemical dysfunctions and oxidative stress.

Gold and AgNPs are also widely used metal NPs. Administration of AuNPs for 35 days in mice resulted in a significant reduction in sperm.
motility and normal morphology, as well as sperm chromatin remodeling, lower chromatin stability and increased DNA damage [69]. Similar effects could be detected in epididymal sperms in adult rats after exposure to AgNPs [67], even if the exposure occurs during the prepubertal stage [70]. These effects are also evident in bull sperms [71] and are likely to be due to direct interaction with the sperm cells; since in bulls, AuNPs were shown to impair sperm functions and reduce sperm fertilizing ability, solely by interaction with the sperm surface membrane, and without cellular penetration [72].

Similarly, administration of TiO2 NPs caused functional defects and DNA damage in male mice [73], and affected the fertility of male offspring after maternal exposure [74]. It was shown to exert cytotoxic and genotoxic damage in the testicular tissue in rats [75] and increased heat-shock protein 70 expression [76] suggesting high levels of cellular stress that may significantly affect subsequent embryo development if these spermatozoa are used for fertilization. Titanium oxide was also toxic for buffalo sperm in vitro [77].

Other metal NPs such as dimercapto succinic acid (DMSA)-coated maghemite and CeO2 NPs did not influence ram or bull sperm structure or function when added in vitro [78,79]. Although CeO2 NPs were shown to enhance ram semen preservation by chilling [12], it may also have detrimental effects if orally supplemented, since in mice, induction of oxidative stress and lower sperm characteristics were reported [80].

8. Other limitations for practical applications of NPs

Major limitations for practical applications of NPs in vivo include overcoming the biological barriers to drug delivery, avoiding non-specific distribution and targeting, as well as minimizing opsonization and clearance by the immune system after oral or intravenous administration [81]. Recently, several strategies have been developed to create multifunctional NPs with incorporation of active targeting moieties to enhance uptake in specific cells [82]. Functionalization with protein corona and PEGylation, modification of surface charge, and attachment of specific ligands for target tissue, among other strategies have been successfully developed to avoid the mentioned obstacles and enhance targeting [81]. This has not been widely used in treatment of male infertility, especially in farm animals. However, there are indications that this approach can be successfully used to enhance testicular functions; SOD-loaded biodegradable NPs targeted to in vitro cultured Sertoli cells using FSH-hormone peptide have been developed and reported to have higher protective effects against oxidative stress [83]. Understanding of the mechanisms by which these NPs are taken up by the target cells and their intracellular fate is of primary importance to optimize their intended functions [84]. Further research is still needed before such technology can be translated into effective products for in vivo use.

9. Conclusions

The above mentioned reports show evidence that different types of NPs have different effects on sperm cell functions either upon direct exposure under in vitro conditions or if administered in vivo. The antioxidant properties of some NPs are among the most promising applications to protect sperm cell functions during cryopreservation. Other surface ligand functionalizations were proven efficient in sperm cell purification and selection. Fewer reports considered utilizing the anti-microbial properties of NPs on sperm preparations or treatment of genital infections. Nevertheless, toxic effects of NPs on male reproductive performance are evident, which increase the environmental concerns about the dispersion of these NPs and their biological effects on the fertility in both humans and animals.

Competing interests

The authors have no conflict of interest to declare.

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