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Abstract: Nanoparticles and nanotechnology may present opportunities to revolutionise the prevention, treatment, and diagnosis of a range of reproductive health conditions in women. These technologies are also used to improve outcomes of assisted reproductive technology. We highlight a range of these potential clinical uses of nanoparticles for polycystic ovary syndrome, endometriosis, uterine fibroids and sexually transmitted infections, considering in vitro and in vivo studies along with clinical trials. In addition, we discuss applications of nanoparticles in assisted reproductive technology, including sperm loading, gamete and embryo preservation, and preventing pre-term birth. Finally, we present some of the concerns associated with the medical use of nanoparticles, identifying routes for further exploration before nanoparticles can be applied to women's reproductive health in the clinic.

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What opportunities do nanoparticles present to revolutionise the prevention, treatment and diagnosis of reproductive health conditions in women, and improve outcomes of assisted reproductive technology?

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Keywords: Nanoparticles, women's reproductive health; assisted reproductive technology, endometriosis, polycystic ovarian syndrome, uterine fibroids, sexually transmitted infections

Main body of text:

Introduction

The application of nanotechnology to the field of medicine has the potential to improve the treatment and diagnosis of many medical conditions. Nanoparticles have many unique features that render them especially suitable for a variety of purposes, including controlled drug delivery, labelling, and direct functionality[1]. Nanotechnological approaches have already been implemented in a range of biomedical applications, with nanomaterials used in the clinic as drug delivery systems with clear benefits over a free drug[1], such as reduced toxicity and degradation while improving bioavailability and accumulation at the target site. The vast majority of research into nanoparticles has focused on their applications in the diagnosis and treatment of cancer[2], with good clinical success[3]; however, there is also significant potential for their application in other disciplines, from regenerative medicine[2] to the diagnosis and treatment of reproductive health conditions, and also in assisted reproductive techniques. Women's reproductive health conditions are challenging to diagnose and often lack effective treatment pathways. The growing field of nanomedicine may provide new opportunities for minimally invasive diagnostic approaches. In addition, nanoparticles may allow the enhanced storage and manipulation of gametes, as these specialized cells are difficult to penetrate. Consequently, the unique properties of nanoparticles pose an exciting opportunity to improve the outcomes of assisted reproductive technology.

This review explores the many roles that nanotechnology plays, and may potentially play, in the diagnosis and treatment of a selection of reproductive and sexual health concerns in women. We also investigate the possibility of using nanotechnology to facilitate assisted reproductive technology (ART) and the potential ways in which nanoparticles could provide novel solutions for infertility.

1. Reproductive Health

A variety of nanotechnology approaches have been applied to a range of reproductive health issues in women, shown in Tables 1 and 2.

1.1 Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) is a common reproductive endocrine disorder affecting an estimated 8% of women, and is the suspected cause of up to 40% of cases of female infertility[4]. PCOS is also considered a leading cause of reproductive cancers, including endometrial carcinoma, and is also likely to cause anovulation[5]. There are several challenges associated with PCOS in the clinic, including delayed diagnosis, diagnostic challenges, and a lack of clear treatment pathways[6].

Nanoparticles have the potential to aid in both the diagnosis and treatment of PCOS[7]; their small size (10 to 1000nm) and ability to carry metal and semiconductor materials, such as gold, silver and ferric oxide, can facilitate contrast for imaging, or by directly delivering these bioactive particles to cells and tissues[8]. Lipid carriers have also been produced on the nanoscale, which have several advantages: the lipids chosen are well tolerated by the human body, they are highly stable, and they can carry both hydrophilic and lipophilic compounds to target tissues, while also minimising the dose required due to targeted delivery[9]. In addition, come nanocarriers are produced naturally by cells in vivo. Exosomes are a category of extracellular vesicles produced by cells with a diameter of approximately 100 nm [10]. Cell-derived exosomes can carry a range of biomolecules as cargo, including lipids, nucleic acids, amino acids and cell metabolites. Developing nanoparticles for use in clinic will allow the fine-tuning of key parameters such as immunogenicity, release rate, solubility and half-life in the bloodstream, thus optimising delivery options for each cargo and target (Figure 1).

1.1.1 Diagnosis

The current diagnostic process for PCOS requires the presence of two of the three diagnostic criteria, oligo/anovulation, androgen excess, and ultrasound assessment of ovarian morphology, along with exclusionary tests for hyperprolactinemia, thyroid disease, and congenital adrenal hyperplasia, among other conditions[6]. Exosomes may represent suitable biomarkers that can aid in the diagnosis of PCOS. Recent studies have found that the small non-coding RNAs carried in follicular fluid exosomes is markedly different between PCOS patients and non-PCOS groups, which should be explored further as a possible biomarker for diagnostic techniques [11, 12]. The detection of exosomes requires high precision; for example, surface-enhanced Raman spectroscopy has recently gained attention for its possible application in exosome-based diagnostics of cancers [13].

1.1.2 Treatment

Nanotechnology offers a range of possible treatments for PCOS. This condition is frequently linked to obesity in women due to the cross-talk between PCOS, insulin resistance, and obesity. In particular, insulin signalling is likely to be impaired via the PI3K/Akt pathway. Selenium nanoparticles (SeNPs) have been investigated as a potential treatment for endocrine and reproductive dysfunction in a rat model of letrozole-induced PCOS[14]. SeNPs improved glycaemic control, eliciting insulin-like effects by activating Akt and other kinases in the insulin signalling cascade, thus increasing insulin sensitivity. In addition, the SeNPs also exhibited significant anti-inflammatory activity in the ovaries, and had a greater effect when combined with metformin[15]. This early study highlighted the possibility of using SeNPs as a direct treatment for PCOS. Additionally, in 2023, SeNPs were shown to modulate the expression levels of the androgen receptor, which are elevated in PCOS, thus reducing expression to almost the levels seen in the control group[16].

Another study showed that silver nanoparticles reduced the levels of inflammatory cytokines in rats with PCOS, with the levels of TNF-α, IL-6 and IL-18, cytokines associated with PCOS[17, 18], all being significantly lower in rats treated with *Cinnamomum zeylanicum* derived silver nanoparticles than the control[19].

There is also hope that extracellular vesicles derived from mesenchymal stem cells may prove to be a viable treatment for PCOS [20]. Mesenchymal stem cells are being investigated in a number of regenerative medicine applications due to their ability to inhibit inflammation, including PCOS in which stem cells help to relieve ovarian dysfunction [21]. While research is still limited, there are early results from in vitro human cell studies and mouse models of PCOS which used exosomes to upregulate miR-323-3p expression in cumulus cells,

reducing apoptosis an aiding cell proliferation [22]. Additionally, exosomes derived from human umbilical cord mesenchymal stem cells have been shown to inhibit inflammation in ovarian granulosa cells [23]. Studies testing mesenchymal stem cell-derived exosomes on rodent models of PCOS found that not only did this treatment reverse PCOS-related metabolic changes, but also restored ovarian function and fertility [24, 25]. Exosome-based treatments are advantageous over whole cell treatments as they reduce the risk of rejection by the immune system, and also reduce the cost and increase accessibility to treatment. These studies have far to go before exosomes can be used in clinic, but may eventually provide another line of treatment for PCOS.

Nanoparticles may also be employed to enhance the bioavailability of existing PCOS treatments. Curcumin is known to be an effective anti-inflammatory. Self-assembled curcumin-encapsulated nanoparticles with modified chitosan were successful in increasing the uptake of curcumin when compared with curcumin as a free drug, and also led to the recovery of the oestrous cycle in rats with PCOS[26]. This is an exciting prospect for potential drug-based management of this disease. A study of an alternative nanoparticle-based curcumin delivery method, using curcumin-loaded super-paramagnetic iron oxide (Fe_3O_4), demonstrated significant benefits in a mouse model of PCOS by acting as an anti-oxidant and by reducing the expression of several apoptosis-inducing factors[27]. Nanovesicle transethosomes have also been used to improve the delivery of progesterone, which has low bioavailability due to poor solubility and hepatic metabolism. Transethosomes are flexible lipid vesicles that can enhance permeation, particularly through the vaginal mucosa, making them suitable to act as a delivery vehicle for progesterone. In human trials, this vaginal gel formulation increased endometrial thickness along with pregnancy rate in anovulatory PCOS patients[28]. There is also scope for the production of synthetic exosomes that could carry these active pharmaceutical ingredients with fewer side effects [29]. Overall, enhanced bioavailability may provide several different treatment options to restore a typical menstrual cycle to patients with PCOS.

1.2 Endometriosis

Endometriosis is another common women's reproductive health condition plagued by diagnostic and therapeutic challenges. Endometriosis is estimated to affect 11% of all women[30] and is an inflammatory chronic pain condition in which uterine tissue grows outside of the uterus. It is characterised by symptoms of abnormal periods, infertility, and pain. At present, major surgery, usually an exploratory laparotomy, is required to confirm endometriosis at the histological level; however, this causes a significant delay in diagnosis such that the average wait from the onset of symptoms to diagnosis is more than 7 years[31].

1.2.1 Diagnosis

Nanoparticles could provide several alternative diagnostic pathways, including some that do not require surgery[32]. The potential use of extracellular vesicles in both the diagnosis and treatment of endometriosis has been reviewed extensively [33]; this strategy shows great potential despite research currently being limited to animal models. As with PCOS, an RNA signature has been detected in the exosomes of patients with endometriosis with unique microRNAs and long non-coding RNA which could theoretically be used as biomarkers to aid in diagnosis, thus requiring a biopsy rather than exploratory surgery for a diagnosis [34]. Nanoparticles have been successful in enhancing the magnetic resonance imaging (MRI) detection of endometriosis in a rat model of endometriosis. Although MRI can be used for the diagnosis of endometriosis, its sensitivity for deep pelvic endometriosis can be as low as 76%[35]. The intravenous delivery of ultrasmall superparamagnetic iron oxides (USPIOs) are efficiently taken up by macrophages, which are abundant in the peritoneal cavity of women with endometriosis. USPIOs can then act as an effective MRI contrast agent, identifying regions of ectopic uterine tissue that are indicative of endometriosis[36]. Magnetic iron oxide nanoparticles modified with hyaluronic acid have also been shown to clearly define lesion margins in a rat model of endometriosis[37]. These studies require further development before this strategy could be applied diagnostically in humans. Rats do not spontaneously develop endometriosis; consequently, surgically induced endometriotic lesions may not behave in the same manner as endometriotic tissue in humans[38]; however, these possibilities should certainly be investigated for their potential application in humans.

- Immunosensors are another option for the nanoparticle-based detection and diagnosis of endometriosis. Nanoparticle-based immunosensors can be used to identify biomarkers of endometriosis in the blood. Cancer

antigen 125 (CA 125) is the primary serum marker for late-stage endometriosis; a previous study synthesized a sensor made from a nanocomposite of conductive gold nanoparticles and reduced graphene oxide with an immobilised antibody for CA 125 attached. This immunosensor successfully detected CA 125 in blood samples acquired from patients with endometriosis [39]. Another immunosensor based on nanoparticle technology has also been developed, involving a bio-nanocomposite of a multi-walled carbon nanotube and magnetite nanoparticles in chitosan providing a surface to immobilise a monoclonal antibody for the sensing of carbohydrate antigen 19-9 (CA19-9), another candidate marker for endometriosis. This sensor showed high levels of sensitivity and may be suitable for early-stage diagnosis and the monitoring of disease progression [40].

Photoacoustic labelling, using gold nanoparticles as a contrast agent, has shown promise as a new non-invasive imaging method. This approach has been successfully used to image breast cancer cells without antibodies or complex nanoparticle surface modifications[41]. The use of gold nanoparticles as an exogenous contrast material, along with endogenous photoacoustic signals such as haemoglobin, allow the localization of endometriosis-like lesions within the peritoneal cavity[42]. This provides significant hope for non-invasive clinical imaging, which could be beneficial for a range of conditions. This strategy could streamline the diagnostic pathway for endometriosis, although there is a need for a significant amount of preclinical development before this can become a reality.

1.2.2 Treatment

There is no current treatment that can cure endometriosis; therefore, medications focus instead on managing the condition and its symptoms. Currently, pain management medication, hormone management through contraceptives, surgery to remove endometrial tissue and hysterectomy are all used to improve the quality-of-life of a patient[43]. However, endometriosis can reoccur frequently, thus requiring further surgery[44]. Furthermore, these methods may also compromise fertility. Fertility preservation is among the top ten research priorities for endometriosis in the UK and Ireland following a survey targeted to women with endometriosis[45], with almost half of infertile women with normal ovulation and normospermic partners being diagnosed with the condition according to a 2009 retrospective case series[46].

Nanoparticles may increase the efficacy of current endometriosis management techniques. P2X3, an ATP-gated ion channel, may be implicated in endometriosis pain. Chitosan oligosaccharide-g-stearic acid (CSOSA) polymer micelles-coated nanostructured lipid carriers have been developed as a delivery system for a selective P2X3 receptor antagonist, A-317491[47]; this treatment strategy induced a reduction of hyperalgesia in both endometriotic mice and rats.

Exosomes may pose another possible treatment strategy for endometriosis. Exosomes derived from menstrual blood mesenchymal stem cells in preliminary cell-based studies were shown to reduce apoptosis, inflammation, proliferation and angiogenesis markers, reducing lesion-forming behaviour [48]. Exosomes enriched with miR-214 from endometrial cells were also shown to reduce connective tissue growth factor in endometriosis xenograft mouse models, which is implicated in fibrogenesis in endometriosis (Figure 3) [49]. Both of these strategies may be able to reduce inflammation, potentially reversing some of the effects of this chronic reproductive health condition. Nucleic acids may also be delivered by synthetic nanoparticles in targeted therapies to modulate essential gene expression in endometriosis tissue. Chitosan oligosaccharide stearic acid micelles have been investigated as delivery vehicles for nucleic acids, particularly for siRNAs[50], due to their non-toxic and biodegradable properties and their ability to protect highly electronegative nucleic acid and facilitate cell entry. siRNAs have been investigated for the treatment of endometriosis by knocking down the expression of aquaporin 2 (AQP2) expression, which is known to support endometrial tissue migration, invasion and adhesion[51]. Chitosan oligosaccharide stearic acid micelles with polyethylenimine and hyaluronic acid and loaded with siRNAs targeted to AQP2 suppressed endometriotic lesion formation in a rat model of endometriosis while showing no adverse effects on the reproductive organs[52]. This approach has also been successful in gene therapy, introducing the gene encoding pigment epithelium derived factor (PEDF), a serine protease that is associated with the inhibition of angiogenesis and tumorigenesis; this led to a significant increase in apoptosis and a reduction in angiogenesis[53]. Poly(lactic-co-glycolic acid) (PLGA) nanoparticles have also been used as a delivery vehicle for miRNAs to promote apoptosis in endometriotic cells. miRNA-503 is implicated in the apoptosis of endometriotic cyst stromal cells and

prevents cell proliferation. In experiments involving human endometriotic tissue, miRNA-503-loaded PLGA nanoparticles caused an increase in apoptosis and a decrease in cell proliferation[54].

Cerium oxide nanoparticles have been shown to reduce endometriotic lesions that were induced in a mouse model by reducing oxidative stress and angiogenesis[55]. These nanoparticles act as free radical scavengers and can the levels of reduce reactive oxygen species (ROS) in vivo, thus inhibiting VEGF expression. Anti-inflammatory therapies using nanoparticles have also been investigated, such as acid-sensitive calcium carbonate nanoparticles associated with BML-11. This type of nanoparticle releases calcium and BML in an acidic environment; the resulting increase in local calcium concentration upregulated both apoptosis and efferocytosis in a mouse model of endometriosis, while also showing no severe side effects and strong biosecurity[56].

Rapidly dividing tumorigenic cells, including endometriotic cells, require the uptake of low-density lipoprotein (LDL). LDL receptor mRNA has been shown to be overexpressed in endometriotic lesions[57]. Lipid nanoparticles have been developed to exploit this event, targeting these cells and delivering a coupled chemotherapeutic agent[58]. These nanoparticles have been tested in a preliminary study and showed that this approach lacked systemic side effects and could provide a treatment option for endometriosis that avoids surgery.

Nanofibers have also been explored as a drug delivery system for the prolonged release of medication in the form of an implantable, cargo-carrying scaffold. This prolonged release is especially useful for conditions such as endometriosis that have a high chance of reoccurrence following surgical removal. The implantation of PCL-PEG nanofibers loaded with curcumin in the peritoneum of mice successfully reduced the size of endometriotic tissue and regions of inflammation when compared with a control group that received no treatment[59]. Furthermore, these nanofibers also continued to release the drug over a significant period of time, with 50% of the curcumin released after 30 days. Other formulations of nanofibers have been tested, but have not demonstrated such long-term release of their drug cargo[60].

Nanoparticles may also aid in physically locating and removing endometriotic tissue. Photothermal therapy is a novel strategy for disease treatment in which photothermal agents convert near-infrared light into heat to induce local cell death due to elevated temperature[61]. In a previous study, Moses et al. developed a nanoplatform that could both identify and ablate endometriotic tissues using near-infrared fluorescence and photothermal therapy[62]. This nanoplatform used a polymeric nanoparticle as a carrier for silicon naphthalocynanine (SiNc), a photothermal agent that accumulates in endometriotic tissue. When activated by near infrared light, the nanoparticles heat tissue to 53°C in vitro and 47°C in endometriotic grafts, thus causing cell death and removing the endometriotic tissue. This platform has significant potential for streamlining the process of lesion identification and surgical removal into a single procedure, although much progress is required in preclinical trials before this can become a reality in the clinic. Magnetic hyperthermia has also been investigated as an alternative therapy for endometriosis. Iron oxide-based magnetic particles with high heating efficiency were generated and encapsulated by poly(ethylene glycol)-block-poly(ε-caprolactone)-based nanocarriers to overcome the hydrophobicity of the magnetic particles alone. When activated, these nanoparticles heated endometriotic lesions to 51.6°C. There is also scope for the addition of specific ligands to target overexpressed receptors found on endometriotic cells, further targeting the nanoparticles to endometriotic tissue and avoiding healthy tissue[63].

Other uses of nanoparticles for the treatment of endometriosis are currently under investigation, including copaiba-oil resin encased in polyvinylpyrrolidone polymer, which successfully reduced inflammation and proliferation[64], neutrophil-mediated delivery of nanoparticles[65], and antibodies to modulate immune activity encased in PLGA[66]. These findings provide significant hope that we may discover a more effective treatment for endometriosis (Figure 2).

1.3 Uterine Fibroids

Uterine fibroids are the most common benign tumour of the female reproductive system. These are hormone-dependent tumours arising from the myometrium with an estimated cumulative incidence of 70 to 80%[67]. These tumours can cause pelvic pain, infertility, pregnancy complications, and menorrhagia, as well as further complications during childbirth[68]. Surgery and hormonal therapy can be successful but effects are only temporary; furthermore, these options are unsuitable for women who would like to conceive[69].

The pathology of uterine leiomyoma includes extracellular matrix remodelling and accumulation, thus providing a potential drug target for specific therapies[70]. 2-methoxyestradiol (2-ME), a promising anticancer agent that acts

as an angiogenesis inhibitor was previously loaded into nanoparticles and proved to be highly successful when tested in vitro against human leiomyoma cells[71]. 2-ME has previously been shown to be a potent antitumorigenic agent suitable for uterine leiomyoma cells with antiangiogenic, antiproliferative, proapoptotic and collagen synthesis inhibitor properties[72]; however, 2-ME has low bioavailability due its poor aqueous solubility, thus posing a challenge when administering the drug[73]. The polymeric liposomal nanoparticles in this study significantly improved bioavailability and induced cytotoxicity far more successfully than the free drug. PEGylated polymeric nanoparticles with 2-ME cargo led to a 51% inhibition in the growth of xenografted patient-derived human fibroid tumours in mice when compared with a control group[74], thus providing further evidence that this may represent a novel method for treating uterine leiomyoma.

Magnetic nanoparticles loaded with viral cargo have also demonstrated potential success for the non-surgical treatment of uterine fibroids. The possibility of nanotechnology-enabled gene therapy has been investigated in vitro studies by the application of magnetic nanoparticles complexed to an adenovirus[75]. These conjugates led to an increase in the efficiency of targeted suicidal gene transfer into tumour cells when compared against non-conjugated adenovirus. The magnetic properties of these nanoparticles permit molecules to be concentrated at their required sites by using external magnetic fields, thus promoting cellular uptake. However, reducing the effective dose of adenoviral vectors is critical if this method of gene therapy can be translated to the clinic.

Peptide-based nanoparticles provide an alternative route to gene therapy that is less likely to stimulate a detrimental immune response. iRGD, a tumour-penetrating peptide, ligand-modified nanoparticles with polycondensed arginine-histidine-rich oligomers were shown to be successful in herpes simplex virus thymidine kinase (HSV-TK) gene delivery to uterine leiomyoma cells, followed by ganciclovir treatment, significantly reducing the proliferation of leiomyoma cells [76]. A similar suicide gene therapy approach has been achieved with magnetic cationic nanoparticles targeting the delivery of HSV-TK, increasing delivery efficiency while reducing the time taken for successful transfection in primary human uterine leiomyoma cells[77].

Further in vitro studies showed that simvastatin induces calcium-dependent apoptosis in human uterine leiomyoma cells, while also inhibiting proliferation by inhibiting ERK phosphorylation in the growth factor signalling pathway[78]. Simvastatin has also proven successful in reducing tumour growth in patient-derived xenograft mouse models[79]. However, statins are often associated with poor bioavailability as they are sparingly soluble in water and have low tissue permeability[80]. Simvastatin-loaded liposome nanoparticles were therefore developed in an attempt to increase the bioavailability of these nanoparticles[81]. Although unsuccessful in this goal, alternative nanoparticle formulations optimised for uterine fibroid delivery could yield further treatment options for patients beyond surgery.

Nanoparticles may also facilitate typical treatment courses for uterine fibroids. For example, superparamagnetic iron oxide nanoparticle-embedded chitosan microspheres have been investigated as a novel agent for uterine fibroid embolization. These microspheres demonstrated improved segmental arterial occlusion than the typically used polyvinyl alcohol particles in uterine arterial embolization of rabbits while also being traceable by MRI scanning[82].

2. Sexual Health

Sexually transmitted infections (STIs) are a significant issue worldwide. The UK reported record numbers of gonorrhoea cases in 2022 and the highest number of cases of syphilis since 1948[83]. Streamlining the process of STI detection through a sensitive and user-friendly point-of-care diagnostic method is one of the best methods of control, as these infections are often asymptomatic with the risk of developing severe complications when left untreated.

2.1 Diagnosis

A possible sensitive, low-cost method for the treatment of Neisseria gonorrhoeae was developed by Liu et al[84] in 2017 using loop-mediated isothermal amplification of an N. gonorrhoeae pseudogene. This technique was then refined by the addition of a gold nanoparticle-based lateral flow biosensor. This biosensor provides a visual readout following the LAMP reaction and requires more basic equipment than the current gold standard PCR testing methods[85]. This method also proved suitable for the identification of Chlamydia trachomatis. These methods have the potential to develop home-testing kits which could increase accessibility to testing facilities, thus facilitating the rapid diagnosis of STIs before they are transmitted to others.

2.2 Prevention

Despite the use of barrier methods to prevent the transmission of STIs, male condoms provide variable levels of protection against transmission, ranging from >90% estimated efficacy against HIV and Hepatitis B to 10-50% against HSV-2[86]. Increasing the efficacy of STI prevention is important to the reproductive health of all people, regardless of gender, and improving the range of contraception methods available is especially beneficial for the sexual freedom of women. Polyurethane condoms coated with silver nanoparticles exhibit additional antimicrobial properties and were shown to successfully inactivate HSV-1, HSV-2 and HIV-1, but did not exhibit a toxic effect on three different cell lines (HeLa cells, 293 T cells, and C8166 T cells)[87]. This could provide an additional line of defence against STIs. Natural rubber latex, when mixed with 30nm silver nanoparticles, permitted the gradual release of these nanoparticles[88]. This provides another possible route for the development of nanoparticle-loaded condoms that could act as both a contraceptive as well as a chemical and physical barrier to STI transmission[89].

Nanoparticles continue to be investigated by researchers by virtue of their antimicrobial properties[90] and may have other applications for the reduction of STIs or in improving contraceptives. Mucus-penetrating nanoparticles coated with PEG diffuse through cervicovaginal mucus at speeds near diffusion through water, and can be loaded with acyclovir monophosphate and applied vaginally to improve protection against HSV-2 in a mouse model [91]; these mucus-penetrating particles could also be applied to the local delivery of other drugs for action in the cervicovaginal tract. Electrospun nanofibers may enhance the delivery of HIV pre-exposure prophylaxis drugs (tenofovir disoproxil fumarate and emtricitabine) in women, using vaginal delivery rather than orally. Both drugs were effectively released from the nanofibers in trials in a mouse mouse model, showing a higher concentration of drugs at the target site than mice treated with oral drug preparations [92]. Polymeric PLGA has been used as a pH-dependent capsule for tenofovir, which only releases its cargo when exposed to the increase of pH caused by the presence of semen[93]. Similarly, vaginal films have been developed to allow pH-dependent drug release [94] and with the addition of plasticizers (PEG and oleic acid), these films have been shown to reduce systemic exposure while maintaining effective mucosal levels of tenofovir in mice [95]. Polymeric nanoparticles, in the form of gels, have been investigated to prevent the vaginal transmission of HIV while also acting as a contraceptive. These nanoparticles, made of poly(methacrylic acid-co-acrylates), are pH responsive and dissolve to release their cargo of both an antiviral (Atazanavir sulphate) and a spermicide (fluoxetine hydrochloride) when exposed to the higher pH of semen (7.2), but will not release these drugs within the normal pH range of the vagina (pH 3.8-5)[96]. This potential dual-effect formulation showed no toxicity or irritancy in trials in experimental mice. Intrauterine devices often accumulate biofilms and may contaminate the endometrial cavity with bacteria when inserted[97]; this can lead to pelvic inflammatory disease. Many pathogenic bacteria have been identified on intrauterine devices following removal[98]. Coating intrauterine devices with antimicrobial nanoparticles, such as silver or zinc oxide[99], could help to reduce biofilm formation on intrauterine devices and thereby reduce associated inflammation.

2.3 Vaccination

Vaccination formulations may be improved by the integration of nanotechnology, thus providing an alternative form of protection from STIs. Estimates show that 67% of the global population could have herpes simplex type 1 (HSV-1) infections, with a further 13% estimated to have HSV-2 infections[100]. Women are infected by HSV-2 twice as often as men, as sexual transmission is more efficient from men to women; therefore, the development of a suitable vaccine would offer additional protection. A trivalent mRNA vaccine has been developed that can be delivered to cells when encapsulated in a lipid nanoparticle[101]. The encased mRNA encodes three glycoproteins found in HSV: gD2, which is required for receptor engagement; gC, which can block activation of the complement system, and gE2, which interacts with gI2 to block antibody-dependent cellular toxicity. This vaccine was effective in trials in both mice and guinea pigs, inducing both T-follicular helper cell and memory B cell responses.

Exosomes have provided a possible platform for the production of therapeutic vaccines to treat HPV. HPV infections are alarmingly high, with an estimated 13 million new disease-associated HPV infections in the US in 2018 [102]. Three different vaccine strategies were explored by Rezaei et al, including one strategy in which exosomes were loaded with recombinant heat-shock protein 27 fused to HPV16 E7. This vaccine platform successfully induced cytokine and antibody responses in mouse models, and also reduced tumour size when

administered as a prophylactic [103]. The exosome platform for vaccination has great potential for further applications and should be a growing area of exploration, as exosomes can be further modified to aid immunogenicity.

Nanotechnology has also provided potential nanovaccine formulations to combat gonorrhoea. Helicobacter pylori ferritin (Hpf) has been investigated as a possible antigen-presenting platform to prime the immune system for an adaptive immune response[104]. Hpf self assembles into a cage of 24 identical subunits, between which N. gonorrhoeae MtrE loops can be added. This makes these antigenic peptides accessible to antibody binding when presented on the nanocage surface[105], thus rendering these functionalised nanocage structures an attractive candidate for further vaccine development. A similar approach has been taken in the development of a candidate HIV vaccine, in which different formulations of the envelope protein from HIV strains were attached to the N-termini of bacterial ferritin, thus presenting multiple possible antigens that help to prime the immune system to mount a response upon infection[106]. This study was successful in stimulating neutralising antibody responses in guinea pigs, and therefore provides a viable starting point for developing vaccines that can simultaneously protect against multiple strains of HIV. Other nanotechnology vaccines for HIV are also under development[107].

A recent study showed that nanoparticles are a suitable vehicle with which to introduce HIV-specific broadly neutralising antibodies to humans via vaccination[108]. This self-assembling nanoparticle holds several advantages over recombinant subunit vaccines, including improved in vivo trafficking, stronger immunogenicity, and the potential to present multimeric antigens. This vaccine induced vaccine-specific CD4 T cell production, with diverse phenotypes and functions. This provides another potential route for vaccination against HIV. An alternative approach to developing a gonorrhoea vaccine with nanotechnology has also been investigated which utilizes whole-cell inactivated N. gonorrhoeae in microparticles. Whole cell vaccines have the advantage of carrying multiple possible antigens against which the adaptive immune system can mount a response. This vaccine produced an antigen-specific antibody and T cell responses in an in vivo vaccination study in mice[109], thus providing another possible route to successful vaccination.

3. Rethinking IVF/ART

Infertility is a common event in many reproductive health conditions; therefore, assisted reproductive technology (ART) is also a key concern in this area. Endometriosis alone is estimated to account for half of all infertile women, with up to 25% of all women with endometriosis requiring ART to conceive[110].

Infertility is a growing concern across the population, with 1 in 6 couples experiencing infertility over their lifetime according to the World Health Organisation (WHO)[111]. Infertility is defined as the failure to conceive following one year of unprotected sexual intercourse[111]. Infertility can be caused by female factors, male factors, a combination of male and female factors, or can be idiopathic. The birth of the first in vitro fertilisation (IVF) baby in 1978[112] led to the rapid development of ART, a technology that can help to overcome infertility. At present, a complex suite of sophisticated laboratory techniques is available under the umbrella term of ART, with a capability that is outpacing both legal and ethical regulation. However, despite these developments, the success rates of ART remain at 25% live births per cycle, with the majority of ART cycles resulting in failure[113]. Nanoparticles may be able to facilitate some of these conventional ART techniques, while also providing us with efficient tools to investigate methods with which to improve success rates (Figure 4).

3.1 Sperm Loading

Nanoparticles may provide a simple and efficient means of loading biological factors into sperm to augment fertility. For example, Makhluf et al. showed that magnetic nanoparticles could be loaded into sperm spontaneously without affecting their motility or their ability to fertilise an egg [114]; subsequently, the same group demonstrated that modified magnetite nanoparticles could act as protein carriers[115]. Sperm are not easy to penetrate; therefore, gaining the ability to load these specialized cells with supplementary biological factors could provide a route with which to load wild type versions of defective proteins into sperm cells that are not fertilisation competent, or allow the addition of drug molecules to influence sperm activity .

Mesoporous silica nanoparticles (MSNPs) may offer another safe delivery tool for introduction of biological factors to sperm. Barkalina et al. showed that MSNPs loaded with nucleic acid or protein can successfully interact with boar sperm without compromising key parameters of sperm function such as viability, motility, acrosomal status and DNA fragmentation index (Figure 5)[116]. Furthermore, MSNPs can be functionalised with a specific cell-

penetrating peptide (C105Y) to increase their binding affinity towards gametes without negatively affecting sperm function, instead associating with the head and midpiece and acting as a protective factor for acrosome morphology and duration of motility[117]. These developments may help to overcome issues of low efficacy in internalisation of compounds into gametes, thereby improving techniques that depend on intra-gamete delivery.

3.2 Gamete and embryo preservation

Not all sperm survive cryopreservation; this is due to the oxidative stress caused by the freezing and thawing processes[118]. Cerium oxide nanoparticles, as trialled for their use as reactive oxygen species scavengers in endometriosis, may also help to improve the survival and fertilisation capabilities of frozen/thawed sperm cells[119]. In a previous study, CeO_2 nanoparticles were shown to improve motility and membrane integrity, but without inducing effect on ROS levels; the mechanisms underlying these beneficial effects have yet to be elucidated. Cryopreserving human sperm with exosomes from seminal fluid led to an increase in sperm viability, motility and morphology as well as a reduction in ROS levels and DNA damage, thus suggesting that these play a cryoprotective role [120].

The oxidative stress associated with cryopreservation can lead to reduced plasma membrane integrity, as evidenced by alterations in protein-lipid interactions and a reduction in the amount of cholesterol in cell membranes[121]. This could lead to premature acrosome reactions in thawed sperm, thus leading to fertilisation failure. Membrane lipid replacement with nano-micelles has been posed as a possible solution to this issue[122]. The incorporation of cholesterol-loaded cyclodextrin prior to cryopreservation has been shown to improve the quality of frozen/thawed sperm from a range of different species[123]. Furthermore, the incubation of human sperm with micelles of glycerophospholipid mixtures was shown to increase both motility and the resistance to oxidative stress [124]. The application of micelles that carried both cholesterol-loaded cyclodextrin and glycerophospholipids resulted in increased motility, mitochondrial activity and acrosome integrity in frozen/thawed human sperm when compared with thawed but untreated sperm[122]. Low-density lipoprotein nanoparticles produced by the probe ultrasonification of egg yolk plasma may also offer cryoprotection for frozen sperm samples, as demonstrated in semen from the canine model. These nanoparticles have a high reactivity surface and help to preserve membrane integrity during the biological stress associated with freezing and thawing[125].

In ART, oocytes are often vitrified in order to preserve fertility in women who are likely to experience a loss in fertility, such as those who require gonadotoxic treatments. Fe_3O_4 nanoparticles have demonstrated potential as vitrification aids, as evidenced by a significant increase in the nuclear maturity of oocytes that were incubated with these nanoparticles prior to vitrification [126]. The lowest concentration of Fe_3O_4 nanoparticles tested showed the highest viability; this may be due to their ability to generate destructive free radicals. Exosomes in the follicular fluid do not affect the viability of thawed oocytes but have also been shown to increase the meiotic competence of domestic cat oocytes when added either to vitrification or thawing media; proteins that regulate tight junction and gap junction formation were detected in the exosomes of follicular fluid from cats, which may facilitate intercellular signalling post-vitrification [127].

Silica nanoparticles may facilitate the in vitro maturation of oocytes, as demonstrated in the domestic pig. The addition of highly dispersed silica nanoparticles was found to reduce apoptosis and increase embryo yield, thus suggesting that these nanoparticles would be a useful addition to culture media designed for the extracorporeal maturation of oocytes; this strategy could improve the quality of the collected eggs and their chances of successful fertilisation[128].

Nanoparticles could also help to improve the rates of cryopreservation and thawing of embryos produced by ART, which are typically considered as limiting factors in the improvement of embryo cryopreservation. Laser-assisted gold nanoparticle warming, in which gold nanorods are injected through the embryo to increase thermal conductivity, has been shown to improve the survival of zebrafish embryos following cryopreservation[129, 130]; this effect has been elusive for many years due to slow convective heating through the large mass of embryos. Thermally conductive graphene-based nanofluids may also maximise heating and cooling rates that are otherwise limited by the heat conductivity of the aqueous media of embryos. A trial that utilized mouse blastocysts showed that graphene oxide nanoparticle cryosolution was as effective as a typical sucrose solution in terms of hatching

and implantation rates, but also allowed embryos to maintain their spherical shape throughout dehydration[131]. This technique caused less thermal and physical stress on embryos and did not compromise their viability.

3.3 Pre-term birth

Nanoparticles may also aid women throughout pregnancy, increasing the likelihood of carrying a baby to term. The use of nanoparticles in pregnancy carries additional risks, as genetic or epigenetic changes to the foetus caused by nanoparticles in utero may be inherited, causing cross-generational detrimental effects.

Globally, an estimated 11.1% of all livebirths in 2010 were born preterm (prior to 37 weeks of gestation)[132] and this figure has remained constant for the last few decades. Preterm birth can be caused by a variety of medical, psychosocial and biological factors. Preterm birth is estimated to be a risk factor in over 50% of all neonatal deaths, and can lead to long-term complications in survivors. Despite this, only one drug has been approved for the prevention of preterm birth by the US Food and Drug Administration until its withdrawal in April 2023.

Nanoparticles are an attractive prospect for medications during pregnancy as they can travel through the cervico-vaginal mucous barrier and reach target tissues with lower systemic drug levels and further reduce placental barrier penetration[133]. Two different preclinical studies have shown that a self-nanoemulsifying drug delivery system (SNEDDS) can delay the onset of inflammation-induced preterm births; one study showed that the combination of a SNEDDS with sphingosine kinase inhibitor II drug cargo increased the number of mice pups rescued from preterm birth in lipopolysaccharide-induced pups[134], while the other tested a similar vaginal formulation with 17-alpha hydroxyprogesterone caproate as the cargo with a similar significant increase in the number of rescued pups[135]. Another nanosuspension, containing progesterone encapsulated in PEGylated nanoparticles, significantly prevented preterm birth and performed better than a gel equivalent, in a mouse model of progesterone withdrawal [136]. In addition, a vaginal nanoformulation carrying Trichostatin A, a histone deacetylase inhibitor, and progesterone, led to a reduction in inflammation-induced preterm birth by 50% in a mouse model[137]. These vaginal nanoformulations have the potential to reduce the number of preterm births and protect both the mother and new-born from the possible risks associated with preterm birth.

4. Concerns relating to the use of nanoparticles

Although nanoparticles provide us with a range of different methods to improve and enhance our ability to enhance the reproductive health of women and in our ability to provide ART, there are several concerns related to their application, ranging from safety to environmental issues. An estimated 20% of clinical trials involving nanoparticles fail due to safety concerns[138], clearly showing that this area requires further investigation.

The toxicity of nanoparticles must be carefully investigated before their widespread introduction in clinical scenarios, and this is an active area of current research[139]. A wide range of nanotoxicities have been reported, from gold nanorods aggravating immune-mediated hepatitis in mice[140] to abnormal offspring from maternal mice injected with reduced graphene oxide nanosheets[141]; gold nanorods have been investigated as an improved form of contrast agent for MRI [42] . Nanoparticles can cross the blood-brain barrier; while this is a benefit in some scenarios, this can also pose significant risk[142]. Nanoparticles can induce oxidative stress[143]; for example, silver nanoparticles are widely used as an antibacterial nanomaterial but can induce oxidative stress. Oxidative stress has been confirmed in the brain in rats following the inhalation of MnO_2 nanoparticles [144]. Oxidative stress in the brain has been implicated in the development of neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. However, silver nanoparticles have been investigated for a range of applications, including an oral PCOS treatment which may also induce systemic effects [19]. The potential application of nanoparticles in clinical applications to penetrate the nervous system must be carefully assessed prior to clinical translation.

Nanoparticles have also been implicated in reproductive and developmental nanotoxicity, with concerns over potential embryo-foetal toxicity due to their ability to cross biological barriers such as the blood-brain barrier, blood-testis barrier and the placenta[145]. Higher concentrations of titanium nanoparticles in the maternal blood are associated with a greater risk of congenital heart defects[146]. In the testes, both carbon and silver nanoparticles are known to negatively affect both Leydig and Sertoli cells by inducing oxidative stress and reducing mitochondrial membrane potential; these cell types are crucial for successful spermatogenesis[147, 148], which may warrant further investigation before the potential use of silver nanoparticle-coated condoms to prevent STI transmission [88, 89]. There is also evidence that the accumulation of nanoparticles may lead to abnormalities in

sperm production[149]. For example, titanium dioxide and zinc oxide nanoparticles have been linked to genotoxicity in human sperm cells by causing an increase in ROS [150]. Zinc oxide nanoparticles have also been shown to enhance apoptosis in Leydig cells, thus leading to reduced testosterone levels and impaired spermatogenesis[151]. Titanium dioxide nanoparticles have also been shown to cause a significant reduction in the number of eggs produced by female zebrafish following 13 weeks of exposure[152]. Furthermore, zinc oxide nanoparticles reduce fertility by inducing cytotoxicity, and by promoting oxidative stress, autophagy and apoptosis in developing zebrafish oocytes[153]. These types of nanoparticle have been primarily explored for use in ART; therefore, long-term effects on the foetus must be carefully investigated. Nanoparticles may even pose a barrier to ART; even very low levels of cerium oxide in a culture medium during IVF resulted in a reduction in fertilisation rate in mouse models[154].

Although extracellular vesicles do not face the same challenges as synthetic nanoparticles in terms of tolerance and toxicity, there are challenges in developing the required technology for their clinical use. Exosomes are complex and there is much more variability introduced through biogenesis than synthetic manufacture, posing significant challenges for reproducibility and reliability [155]. In addition, methods to purify exosomes and the demands of cell culture limit our ability to scale up production for clinical application. There are also concerns that exosomes may be rapidly degraded in the body; however, a previous study showed that antigen-presenting cellular exosomes express CD55 and CD59, which provide protection from lysis by the complement system [156]. Further research needs to focus on the modification of exosomes to optimise bioavailability and reduce variability before they can be applied reliably.

Future Perspective

Advancements in nanotechnology provide a range of new possibilities for the diagnosis, treatment and prevention of many conditions, including diseases of the female reproductive system, while also offering potential new avenues for ART. Many of these possibilities are directly associated with the ability of both lipid- and metal-derived nanoparticles to increase delivery efficacy and accuracy, along with their potential to reduce drug-taking non-compliance through sustained release delivery systems. Other new possibilities are related to their intrinsic properties as biologically active particles. With so many potential benefits for patients, it is vital that researchers increase their focus on preclinical testing in order to translate these findings safely into the clinic.

However, there are still several factors that require further consideration before nanoparticles can become accessible in a clinical context for reproductive health. Questions remain over the cytotoxicity of nanoparticles, and also over the potential effects of their bioaccumulation, which is a particular issue for synthetic and metal nanoparticles. Further research into the safety and application is paramount, especially given that the potential benefits of nanoparticles in a clinical setting cannot be understated. Further research into the safety and application is paramount, especially given that the potential benefits of nanoparticles in a clinical setting cannot be understated. Methods that can localise nanoparticles to reduce systemic risks may be of key importance to the field moving forwards. The use of extracellular vesicles as delivery vehicles are an exciting prospect for further research as drug vehicles, as they are already found and tolerated in the body, while further toxicity and tolerance testing on metal-based nanoparticles is required before their translation into clinical studies and onto the clinic. Nanoparticle research provides a valuable and exciting new direction for research into women's reproductive health conditions and assisted reproductive technology which will bring new approaches to improve outcomes.

Executive Summary (Review, Perspective, Special Report & Priority Paper Evaluation articles): bulleted summary points that illustrate the main conclusions made throughout the article. Less than 400 words.

- Polycystic ovary syndrome
  - Metal nanoparticles can exhibit direct effects on PCOS, reducing insulin resistance and inflammation.
  - Lipid-based nanoparticles significantly increase bioavailability of existing PCOS treatment drugs.
- Endometriosis

- Nanoparticles provide a possible non-surgical route for endometriosis diagnosis, while also potentially linking lesion identification and treatment through coupling imaging agents to nanoparticles with high heating efficiency to induce local hyperthermia.
- Endometriosis-related inflammation, oxidative stress and angiogenesis have all been reduced in animal endometriosis models by the addition of nanoparticles.

- Uterine fibroids
  - Nanoparticles both aid in traditional treatment methods (as an embolic material) and in the delivery of interfering peptides, nucleic acids and drugs that can reduce fibroid size.
- Sexually transmitted infections
  - Nanoparticles may allow fast and rapid diagnosis of sexually transmitted infections such as gonorrhoea and chlamydia through gold nanoparticle biosensors.
  - Nanoparticles can improve traditional contraceptive methods: silver nanoparticles cab ve used to coat condoms, exhibiting antimicrobial effects, as well as reducing biofilm formation of intrauterine devices.
  - Nanoparticles may be an effective way to formulate vaccinations against sexually transmitted infections due to their in vivo trafficking, ability to present multimeric antigens, and enhanced immunogenic responses.
- Sperm loading
  - Magnetic nanoparticles and mesoporous silica nanoparticles have been successfully used to interact with sperm and also to carry biological factors such as nucleic acids and protein.
  - This may allow missing or defective factors to be replaced during assisted reproductive technology to aid fertility.
- Gamete/embryo preservation
  - Oxidative stress induced by cryopreservation of gametes can be reduced by the use of nanoparticles either as part of the freezing medium or via membrane-lipid replacement.
  - Nanoparticles increase heating efficiency of cryopreserved gametes and embryos in a safe and effective manner.

Figure/Table legends

Table 1 Selected applications of nanoparticles in women's and reproductive health

Table 2 Active pharmaceutical ingredients and administration routes for experimental nanoparticle-based therapeutics in women's reproductive health conditions

Figure 1 Selected properties of nanoparticles showing their versatility

Figure 2 The uses of nanoparticles in endometriosis and polycystic ovary syndrome diagnosis and treatment, adapted from Mohammad Javad Javid-Naderi, Ali Mahmoud, Prashant Kesharwani, Tannaz Jamialahmadi, Amirhossein Sahebkar, Recent advances of nanotechnology in the treatment and diagnosis of polycystic ovary syndrome. Copyright (2023) [7] (Created with BioRender.com)

Figure 3 Microscopy images showing the effect of miR-214 enriched exosomes on endometriosis mouse models. **A)** Immunostaining of collagen αI and connective tissue growth factor in endometrial-like lesions **B)** Effect on collagen production shown by Sirius red staining, with black arrows indicating collagen. Scalebar = 50µm. Adapted from *Mol Hum Reprod* 24(7) Wu D, Lu P, Mi X, Miao J. Exosomal miR-214 from endometrial stromal cells inhibits endometriosis fibrosis. Copyright (2018).[49]

Figure 4 Uses of nanoparticles in sperm loading and gamete and embryo preservation

Figure 5 Microscopy images showing mesoporous silica nanoparticles (MSNPs) associating with boar sperm. **A)** MSNPs loaded with lamin A/C siRNA **B)** MSNPs loaded with mCherry. Scalebar = 5µm. Adapted from *Nanomedicine*. 10(4) Barkalina N, Jones C, Kashir J, et al. Effects of mesoporous silica nanoparticles upon the function of mammalian sperm in vitro. Copyright (2014) [116]

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- **Reference annotations**: authors should highlight 6–8 references that are of particular significance to the subject under discussion as **"* of interest"** or **"** of considerable interest"**, and provide a brief (1–2 line) synopsis.