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REVIEW

The cutting-edge progress of novel biomedicines in ovulatory dysfunction therapy

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Abstract Ovulatory dysfunction (OD) is one of the main causes of infertility in women of childbearing age, which not only affects their reproductive ability, but also physical and mental health. Traditional treatment strategies have limited efficacies, and the emergence of biomedicines provides a promising alternative solution *via* the strategies of combining engineered design with modern advanced technology. This review explores the pathophysiological characteristics and related induction mechanisms of OD, and evaluates the current cutting-edge advances in its treatments. It emphasizes the potentials of biomedicines strategies such as hydrogels, nanoparticles and extracellular vesicles in improving therapeutic precision and efficacy. By mimicking natural physiological processes, and achieving controlled drug release, these advanced drug carriers are expected to address the challenges in ovarian microenvironment reprogramming, tissue repair, and metabolic and immune regulation. Despite the promising progress, there are still challenges in terms of biomedical complexity, differences between animal models and human physiology, and the demand for intelligent drug carriers in the therapy of OD. Future researches are mainly dedicated to developing precise personalized biomedicines in OD therapy through interdisciplinary collaboration, promoting the development of reproductive regenerative medicine.

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1. Introduction

Globally, 8% to 12% of reproductive-age couples experience infertility, with ovulatory dysfunction (OD) being the most common cause, accounting for about 25% of cases[1]. OD is an abnormality in ovulation among non-pregnant women of reproductive age. It is most commonly caused by polycystic ovary syndrome (PCOS), a complex endocrine-metabolic disorder meeting ≥2 Rotterdam criteria, characterized by oligo/anovulation, hyperandrogenism, or polycystic ovaries (≥20 follicles/ovary), but can also result from the conditions like hyperprolactinemia, hypothalamic-pituitary dysfunction, diabetes, thyroid disorders, depression, certain medications, obesity, excessive exercise, or significant weight changes[2]. The causes are complex, and involve multiple physiological and pathological factors. The latest International Federation of Gynecology and Obstetrics (FIGO) classification system divides it into four types: Type I (hypothalamic), Type II (pituitary), Type III (ovarian), and Type IV (PCOS)[3].

In addition to impairing conception, OD can also lead to menstrual disorders and hormonal imbalances. Metabolic abnormalities are also prone to inducing various chronic diseases, such as type 2 diabetes and metabolic syndrome, and can increase the risk of cardiovascular diseases and even gynecological cancers[4,5]. Apart from physical manifestations, OD is also associated with anxiety, depression, eating disorders[6], and sexual psychological dysfunction, severely affecting the physical and mental health of women of childbearing age[7]. Premature ovarian insufficiency (POI), a syndrome defined as OD before age 40 with ≥4 months of amenorrhea and follicle-stimulating hormone (FSH) (>25 IU/L), and premature ovarian failure (POF), a syndrome defined as OD before age 40 with the absence of spontaneous menstrual recovery and menopausal-range FSH (>40 IU/L), affect approximately 3.7% of women. POI and POF are associated with the risks of premature menopause, including cardiovascular disease and osteoporosis[8], with an extremely narrow therapeutic window for intervention. This indicates that OD has surpassed the scope of reproductive system diseases, and has become a key issue in systemic metabolic disorders.

Traditional treatments for OD include lifestyle interventions, pharmacological treatments (including ovulation-inducing drugs, insulin sensitizers, and anti-androgen medications, etc.)[1], surgical treatments (ovarian drilling)[9], and assisted reproductive technologies[1]. Evidences indicated that 5%−10% weight loss could restore ovulation in 70% of obese PCOS patients, but their long-term compliance was still below 30%, with limited effects on normal weight patients[10]. For ovulation induction, clomiphene achieves 60%−85% ovulation rates, but the pregnancy rates is only 20%−40%, and the risk of multiple pregnancies and adverse perinatal outcomes is increased[11]. Letrozole offers lower multiple pregnancy risks than clomiphene, but there are teratogenic concerns that limit its use[12]. Gonadotropin therapy yields higher ovulation rates, but it possesses 20%−30% multiple pregnancy risks and 5%−10% ovarian hyperstimulation syndrome incidence[13]. Especially, for treatment-resistant cases, surgical options like ovarian drilling might be helpful, but there was a risk of pelvic adhesions and accelerated decline in ovarian reserve[14]. The pregnancy ratio of *in vitro* fertilization and embryo transfer (IVF-ET) could reach 40%−50%, but the cost and psychological pressure caused by repeated cycles are high[15]. Although these traditional methods have shown some efficacy, they have failed to treat OD at its root, and ignored its mechanisms, including ovarian microenvironment dysregulation, mitochondrial dysfunction, adipokine signaling, and metabolic-reproductive axis imbalance. Therefore, developing safe, effective, and economical treatment methods is an important requirement in the field of reproductive medicine.

Biomedicines refer to biomedical products with engineering bioactive materials or structures designed for medical applications, including metal nanoparticles (NP), hydrogels, extracellular vesicles (EVs), and similar molecular/nanosystems. These systems are specially designed for interaction with biological systems, and are used for targeted drug delivery or regenerative therapies[16]. Currently, the rapid development of biomedicines has brought great convenience to clinical medicine. For example, biomedicines possessed unique physicochemical properties, such as size effects, surface effects, and quantum effects, which gave them significant advantages in drug delivery[17,18], gene therapy[19], and tissue engineering[20]. Targeted modification in biomedicines can also help deliver drugs to the targeted sites, avoiding non-specific distribution[21]. Additionally, multifunctional biomedicines could reduce immune reactions, prevent *in vivo* degradation, promote drug absorption, and improve bioavailability[22]. Moreover, emerging therapeutic approaches, such as gene therapy, offer potentials for treating OD caused by genetic mutations[23]. However, the inherent complexity of the structure and function in ovary poses some challenges to biomedicines. Generally, systemic administration often results in insufficient local drug concentrations in the ovaries, leading to off-target effects, such as the gastrointestinal side effects of metformin[24]. Nevertheless, the current development of novel biomedicines shows great promise in overcoming these challenges. In this context, various biomedicines, including chitosan, hydrogels, poly(lactic-*co*-glycolic acid) (PLGA), NPs, and EVs, have been explored in preclinical studies for the treatment of OD[23].

In this review, it aims to provide a comprehensive overview of the pathophysiology of OD, and critically evaluate current treatment limitations. In Fig. 1A and B, it presents the causes and therapeutic strategies of OD. We highlight the potentials in biomedicines-based therapeutic strategies, such as advanced delivery systems, to address these challenges. Specifically, we examine how hydrogels and NPs can enhance therapeutic precision and efficacy by mimicking natural physiological processes, and enabling controlled drug release to the ovaries. We also discuss the role of biomimetic vehicles, like cell derived vesicles, in overcoming barriers to achieve targeted therapy. This review seeks to bridge the gap between preclinical research and clinical translation, offering new perspectives for advancing OD therapy through cutting-edge biomedicines and delivery technologies.

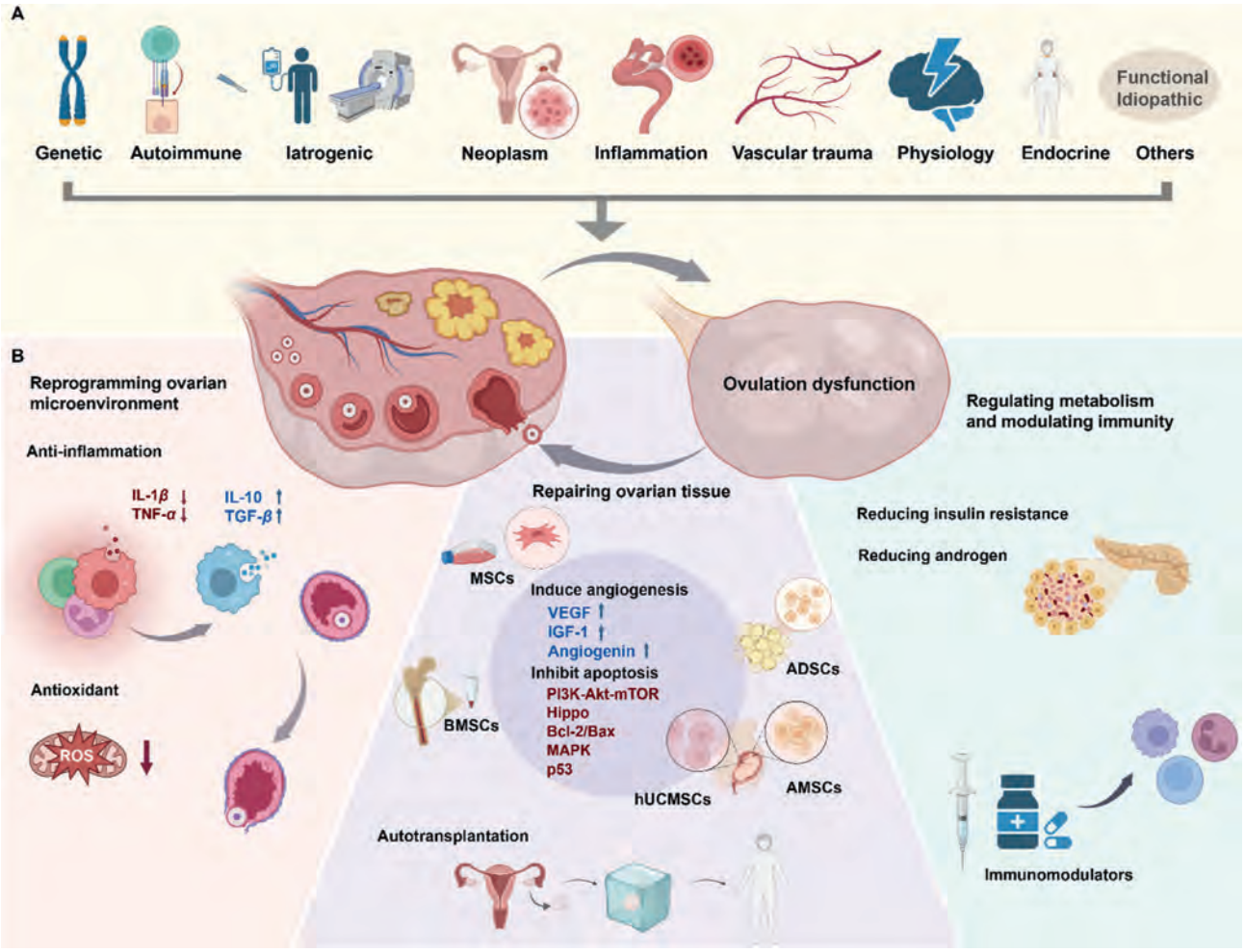

Figure 1 (A) The pathogenic causes of OD. (B) The recent therapeutic strategies for OD.

2. Traditional therapeutic strategies

The ovarian microenvironment is composed of follicular fluid, granulosa cells, stromal cells, and various bioactive factors such as growth factors and cytokines, all of which directly affect oocyte maturation and the ovulation process[25]. During physiological ovulation, the ovarian microenvironment undergoes significant dynamic remodeling, mainly triggered by a surge in luteinizing hormone (LH), involving precise regulation of multi-dimensional molecular events[26]. After binding to the LH/choriogonadotropin receptor on the surface of granulosa cells, LH activates downstream transcription factors, and induces a transient overexpression of epidermal growth factor (EGF)-like factors[27]. These factors promote cumulus expansion and secretion of matrix metalloproteinase (MMP) through EGF receptor signaling, thereby weakening follicle wall structure[27]. Simultaneously, a dramatic shift occurs in the steroid hormone synthesis pathway, where granulosa cells rapidly switch from an estrogen-dominated mode to a progesterone synthesis mode, and regulate inflammation and angiogenesis through progesterone receptors[28]. The ovulation period is also accompanied by a peak in prostaglandin synthesis, leading to the increased vascular permeability and uterine contraction, while the dynamic balance between protease inhibitors and MMPs ensures orderly degradation of extracellular matrix (ECM)[28]. In addition, chemokines recruit immune cells (macrophages and neutrophils) to the follicle wall, and further amplify the inflammatory response through the secretion of factors like interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), creating a dynamic interplay between pro-inflammatory and anti-inflammatory microenvironments[26]. These steps are interconnected, and any problems in any step may lead to OD. In traditional treatment therapies, the mechanisms targeting ovulatory disorders can be categorized into the following aspects: reprogramming ovarian microenvironment, repairing ovarian tissue, and regulating metabolism and immunity.

2.1. *Reprogramming ovarian microenvironment*

Remolding ovarian microenvironment is a fundamental strategy for improving OD, with the core goal of optimizing the local conditions for follicular development. Researches have shown that excessive oxidative stress and inflammation were important factors that disrupted the ovarian microenvironment. Antioxidants have been shown to effectively alleviate the pathological state by scavenging reactive oxygen species (ROS) and lowering inflammatory factors (*e.g.*, IL-1β and TNF-α) expression[29]. Clomiphene could correct sex hormone imbalances in PCOS patients, optimize the follicular development environment, and alleviate excessive generation of ROS caused by hormonal dysregulation[30]. It has been proven to significantly counteract elevated ROS levels in

ovarian tissue while enhancing the activity of antioxidant enzymes including superoxide dismutase (SOD) and catalase (CAT), thereby alleviating oxidative stress derived damage[31]. Vitamin C and vitamin E could reduce oxidative damage by scavenging free radicals[32,33], while coenzyme Q10 enhanced follicle energy metabolism by improving mitochondrial function[34]. A randomized controlled trial in patients with low ovarian reserve found that coenzyme Q10 supplementation (200 mg/time, 3 times/day for 60 days) significantly improved sinus follicle number, and oocyte quality[35]. In addition, vitamin D supplementation has been shown to regulate follicular development in women with PCOS. Clinical studies have shown that serum vitamin D levels were positively correlated with ovulation rate. Vitamin D supplementation could improve ovulation function in PCOS patients, which may be related to regulating anti-Mullerian hormone (AMH) levels, and inhibiting inflammatory factors[36]. These studies demonstrated that improving the ovarian microenvironment through targeting oxidative stress and nutritional interventions provided a basic pathway for OD treatment.

Although basic strategies to regulate the ovarian microenvironment have shown some efficacy in improving OD, there are still some limitations. Clinical evidence showed that clomiphene had drug resistance, decreased endometrial receptivity, and increased risk of multiple pregnancies[11]. Although it can temporarily ameliorate oxidative stress, discontinuation of the medication may cause ROS to rebound due to hormonal fluctuations, highlighting the necessity of combined treatment with antioxidants to achieve sustained therapeutic effects[30]. These limitations emphasize the importance of optimizing adjuvant antioxidant therapy, yet these methods face challenges in clinical implementation. First, although the administration of antioxidants such as vitamin C, vitamin E, and coenzyme Q10 could alleviate oxidative stress, the effect may vary according to individual differences. For patients with severely reduced ovarian reserve or older age, the therapeutic effect of antioxidants may be limited. In addition, the dosage and duration of antioxidants have not been fully standardized, and excessive supplementation may bring potential side effects, such as excessive vitamin E, which may lead to bleeding tendencies[37]. Secondly, although vitamin D supplementation was beneficial for PCOS patients, its efficacy depends on the basal vitamin D levels of patients. Vitamin D supplementation alone might not be sufficient to fully restore ovarian function in patients with vitamin D receptor gene polymorphisms or severe deficiency[38]. Finally, the adjuvant nature of these interventions is further compounded by poor drug delivery systems (DDSs), which limit their ability to effectively target ovarian tissue, and achieve sustained drug release, necessitating the advanced delivery platforms for ovarian targeting.

2.2. *Repairing ovarian tissue*

The restoration of ovarian function relies on tissue regeneration technologies, aiming to reestablish the normal structure and physiological functionality of the ovary. Stem cell therapy and ovarian tissue transplantation represent two validated strategies for POI patients or those suffering from iatrogenic ovarian damage, such as that induced by radiotherapy or chemotherapy[39].

Mesenchymal stem cells (MSCs), particularly those derived from the human umbilical cord, have shown significant promise due to their multipotent differentiation capacity and paracrine properties. Preclinical studies have demonstrated that the transplantation of human umbilical cord-derived MSCs (hUCMSCs) enhanced follicular survival and granulosa cell proliferation through promoting the levels of free amino acids, and activating the phosphatidylinositol 3-kinase (PI3K) pathway, thereby improving lipid metabolism, ultimately leading to the restoration of ovarian endocrine function[40]. Previous studies have explored the mechanisms by which MSCs exert their therapeutic effects on OD across different animal models. These studies have shown that MSCs could secrete a variety of growth factors, such as vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), angiogenin, insulin-like growth factor-1 (IGF-1), and hepatocyte growth factor (HGF). These factors are crucial for promoting the expression of B-cell lymphoma-2 (Bcl-2), reducing apoptosis in ovarian stromal or granulosa cells, enhancing neovascularization, and mitigating ovarian aging processes[41,42]. In clinical practice, ovarian tissue transplantation has been successfully used in cancer survivors. A long-term follow-up study revealed that auto-transplantation of cryopreserved ovarian tissue restored ovulation in 95% of patients, with spontaneous pregnancy and assisted reproductive technology (ART) pregnancy rates reaching 28.2% and 36.7%, respectively[43]. As a supplement to these regenerative approaches, drug interventions can enhance ovarian repair, and reduce fibrosis. Gonadotropin can stimulate granulosa cells to upregulate VEGF expression[44], promoting angiogenesis, while metformin reduces ovarian fibrosis by suppressing collagen deposition[45]. These adjuvants can work synergistically with regenerative therapies to optimize ovarian recovery.

Even though stem cell therapy and ovarian tissue transplantation have shown promise in restoring ovarian function, their clinical applications still face challenges. The safety and long-term efficacy of stem cell therapy are still unclear, and further research is needed on differentiation mechanisms, post-transplant survival, and immune rejection[46]. In addition, the therapeutic potential of transplanted stem cells is still limited by suboptimal delivery strategies at target sites[46]. Ethical issues, and the lack of standardized protocols also limit its application. Although ovarian tissue transplantation is successful in some cases, it is mainly suitable for cancer survivors or those patient with iatrogenic injuries, with variable recovery times, and risks of tissue damage or infection. The high cost and technological complexity further restrict the widespread adoption, especially in resource limited regions. The current adjuvant drug therapies are limited by inefficient DDSs. Gonadotropins often cause ovarian hyperstimulation syndrome due to uncontrolled activation of granulosa cell[13], while metformin's anti-fibrotic effects are restricted by poor ovarian bioavailability[45]. These challenges push the need for advanced delivery strategies, such as gonadotropin releasing hydrogels for sustained stimulation or NPs encapsulated metformin for targeted fibrosis reduction, to improve therapeutic efficacy while minimizing side effects.

2.3. *Regulating metabolism and immunity*

Metabolic and immune dysregulation are significant causes of OD, and systemic regulation of metabolic and immune functions indirectly restores ovarian function by improving the overall systemic condition[47,48]. For patients with PCOS, conventional ovulation induction agents include clomiphene, FSH, gonadotropin, and letrozole[49]. Additionally, anti-androgenic agents, such as spironolactone[50], competitively bound to the androgen receptor (AR), thereby neutralizing their inhibitory effects on follicular development. However, patients with PCOS often exhibit insulin resistance or hyperandrogenemia, which can reduce their

responsiveness to ovulation induction agents. Metformin, as an insulin sensitizer, could inhibit hepatic gluconeogenesis, and reduced insulin levels by activating the adenosine 5′-monophosphate (AMP)-activated protein kinase (AMPK) pathway, thereby improving local androgen synthesis in the ovary[51,52]. Moreover, metformin exerted its therapeutic effects by inhibiting NF-κB signaling pathway. This mechanism leads to reduced secretion of pro-inflammatory cytokines such as TNF-α while also mitigating insulin resistance and hyperandrogenemia, thereby alleviating systemic inflammation[53]. Glucagon-like peptide-1 (GLP-1), a key metabolic regulator, which stimulated insulin secretion, and suppressed appetite, and has been widely utilized in the treatment of type 2 diabetes and obesity. Recent studies have revealed that GLP-1, acting as a novel multi-agonist, held significant potentials for improving metabolic complications in PCOS[54]. It regulated appetite, energy balance, insulin secretion, and lipid metabolism through the modulation of multiple hypothalamic signaling pathways, thereby enhancing overall metabolic status[54].

For POF patients, the supplementation of estrogen and progesterone is the basic treatment method to restore the women's ovarian function, an important strategy to ensure women's quality of life, maintain physical health, and reduce bone loss[55]. In addition, immune abnormalities, such as autoantibodies attacking ovarian tissue, may contribute to POF[56]. In the autoimmune POF mice models, glucocorticoids could restore ovarian function by suppressing immune related inflammatory responses, and the protective effect of the 100 mg/kg dose was more obvious than that of 25 mg/kg dose[57]. These systemic interventions not only target local ovarian area, but also provide a comprehensive therapeutic framework for the complex etiologies OD by regulating metabolism and immune networks.

The limitations of improving ovulatory function through metabolic and immune regulation cannot be ignored. The efficacy of metformin varies depending on the phenotype, and its efficacy is reduced in non-obese PCOS patients. And its long term use may cause gastrointestinal side effects, and reduce compliance[58]. Although immune modulators like glucocorticoids, are effective for treating autoimmune POI, long term use can lead to osteoporosis, metabolic disorders, and infection risks. Studies have shown that they might damage follicles and exhibited ovarian toxicity under non pathological conditions[59]. In addition, there is a lack of standardized diagnostic criteria for autoimmune POI, which poses a risk of misdiagnosis. Long term use of these drugs, the efficacy depends on baseline metabolism or immune status, failing to achieve stable outcomes.

3. Biomedicines in OD therapy

Although traditional methods have achieved some success in improving ovulatory function, their core issues still focus on the lack of targeting, inability to reverse pathological microenvironments, and inherent invasion risks. Systemic administration often results in insufficient local drug concentrations in targeted organs, and may lead to off target effects. Surgical interventions can accelerate ovarian reserve depletion, while long term use of ovulation inducing drugs increases the risk of POI. In addition, existing approaches are difficult to eliminate the accumulation of ROS within the ovary, or remodel immune cell phenotypes (such as pro-inflammatory M1 macrophages), making it difficult to fundamentally block pathological cascades. The introduction of biomedicines offers a novel approach to overcome these limitations.

Therapeutic approaches involving biomedicines mainly rely on their physicochemical properties, such as surface charge, to enable drug to be delivered across barriers, thereby improving the targeting ability of biomedicines[23,60]. The types and additives of biomedicines can greatly influence the drug release kinetics[61]. Biomedicines designed for drug delivery can achieve controlled release, targeted delivery, and stimuli responsive release, which are critical for treating OD. These systems improve the precision of drug delivery to ovarian tissues, minimize systemic side effects, and optimize therapeutic outcomes. In Fig. 2, it summarizes the categories of biomedicines used to treat OD, classified by their DDSs and delivery mechanisms. Biomedicines can be divided into four categories: biomedicines with carrier-free, traditional drug carriers, hydrogel drug carriers, and biobased drug carriers. The focus of this review is on the research of OD therapy to restore impaired ovarian function. Table 1 summarizes the specific examples of OD treated with biomedicines, highlighting the innovative use of DDSs to enhance therapeutic efficacy[62-104]. To clarify the underlying mechanisms, the representative schematic diagram (Fig. 3) depicts the interactions between biomedicines and ovarian cellular targets, highlighting the involvement of typical pathways such as PI3K/AKT and TGF-β.

3.1. *Biomedicines with carrier-free*

The carrier-free strategy refers to therapeutic agents that achieve their expected biological effects without relying on exogenous delivery carriers, while these agents themselves may still contain intrinsic delivery features, such as covalent targeting ligands and self-assembling motifs[105,106]. Biomedicines with carrier-free either exert intrinsic effects through their native structure or chemical properties, or undergo chemical modifications to achieve self-sufficient delivery capabilities without the need for a separate carrier system. Biomedicines with carrier-free utilize the intrinsic properties, such as antioxidant, anti-inflammatory, and hormonal

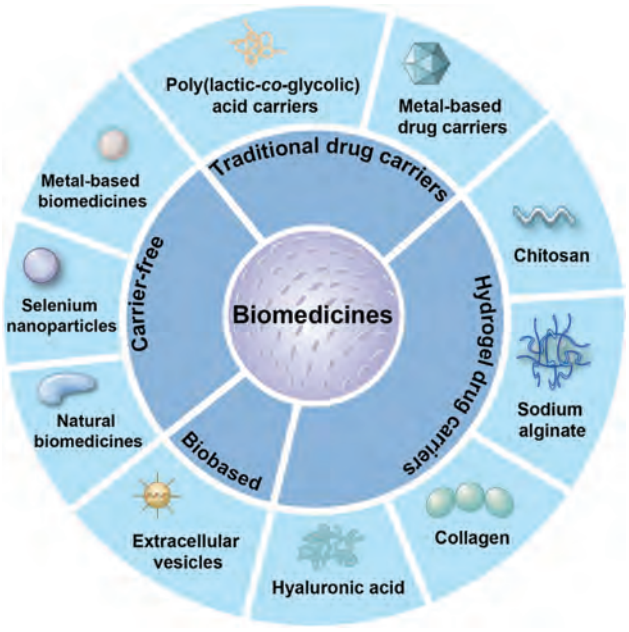

Figure 2 The classification of biomedicines in OD therapy.

Table 1 List of biomedicines in OD therapy.

Classification of biomedicines		Biomedicine	Disease	Therapy strategy	Related mechanism	Ref.
Biomedicines with carrier-free	Metal-based biomedicines	CeO_2 NPs	Obesity-induced OD	Antioxidant, improving metabolic disorders, protecting follicle equality, and regulating mitochondrial function	Alleviating endoplasmic reticulum stress *via* regulating related genes (*Atf4, Chop, Grp78, Xbp1s*)	62
		Ag NPs	PCOS	Antioxidant, anti-inflammation, regulating hormone levels, and inhibiting apoptosis	Inhibiting ferroptosis *via* reducing caspase-3 activity and the expression of Panx1 and *Tlr4*	63
		Ag NPs	PCOS	Anti-inflammation	–	64
		MgO NPs	PCOS	Antioxidant, regulating hormone levels, and improving ovarian tissue pathology	Up-regulating *Cyp19a1* gene expression	65
	Selenium NPs	Se NPs	PCOS	Antioxidant, anti-inflammation, and regulating metabolic	Regulate KEAP-1/NRF2/HO-1 pathway	66
		Se NPs	PCOS	Reducing oxidative stress, improving ovarian function, and regulating hormone levels	–	67
		Se NPs	PCOS	Antioxidant and anti-inflammation, improving metabolic function,	Reducing androgen receptor expression and down-regulating the expression of steroidogenic related genes (*Star*, *Cyp11a1*, *Cyp17a1* and *Hsd17b3*)	68
		Se NPs	PCOS	Anti-inflammation, reducing oxidative stress, improving ovarian pathological changes, promoting proliferation, and regulating hormone levels, and repairing mitochondrial function	Up-regulating the expression of *PI3K* and *Akt* genes	69
	Natural biomedicines	Collagen extracted from sturgeon swim bladder	POF	Antioxidant, regulating hormone levels, inhibiting apoptosis	Activating PI3K/AKT and BCL-2/BAX pathways, inhibiting MAPK pathway	70
		LMWC	Ovarian senescence	Promoting follicle development	Enhancing the phagocytic function of macrophages, up-regulating the expression of CD68, CD204, CD36	71
		SA	PCOS	Regulating blood lipids and reducing androgens	–	72
Biomedicines with traditional drug carriers	PLGA carriers	PLGA-$Mg(OH)_2$ sponge scaffold loaded with hESCMPCs	POI	Regulating the ovarian microenvironment and reducing apoptosis	Secreting cytokines and growth factors, and regulating PI3K/AKT pathway	73
		miR-146 encapsulated in PLGA (miR-146@PLGA)	POF	Inhibiting oxidative stress damage and cell senescence	Silencing the expression of *p38-Mapk14* gene	74
		miR-503 encapsulated in PLGA (miR-503@PLGA)	Ovarian endometriosis	Increasing the apoptosis and inhibiting proliferation of endometriosis cells	–	75
		Metformin	PCOS	Antioxidant and anti-inflammation	Activating AMPK pathway	76

		encapsulated in PLGA (metformin@PLGA)				
	Metal-based drug carriers	CeO_2@RSV	PCOS	Antioxidant, anti-inflammation, and regulating macrophage polarization	Activating JAK/STAT pathway	77
		Curcumin loaded SPIONs	PCOS	Antioxidant, anti-apoptosis, and regulating hormone levels	Decreasing the expression of BAX and caspase-3, and increasing the expression of BCL-2	78
Hydrogel drug carriers	Chitosan-based drug carriers	Chitosan loaded with FSE	PCOS	Antioxidant and anti-inflammation	–	79
		Chitosan-tripolyphosphate loaded with two plant extracts	PCOS	Regulating hormone levels and improving ovarian histopathological changes	–	80
		Chitosan loaded with fennel seed extract	PCOS	Antioxidant and regulating lipid metabolism	–	81
		Arg-CS-NAcHi/Cur NPs	PCOS	Antioxidant, anti-inflammation, and antiandrogen	Inhibiting NF-κB pathway and up-regulating the expression of SOD and CAT	82
	SA-based drug carriers	SA-BG composite hydrogel encapsulated hAECs	POF	Repairing granulosa cell function and restoring follicular development	Up-regulating VEGF, IGFBP, TIMP-1, and coagulation factor III secretion	83
		SA-based progesterone	PCOS	Increasing progesterone levels	–	84
	Collagen-based drug carriers	Collagen scaffolds loaded with hADSCs	POI	Restoring the oestrous cycle, increasing estradiol levels, and promoting follicular development	Promoting VEGF, TGF-β, FGF2 and HGF secretion	85
		Collagen scaffold loaded with hUCMSCs	POF	Promoting granulosa cell proliferation and angiogenesis, improving hormone levels, and reducing ovarian atrophy	–	86
		Collagen scaffold loaded with hUCMSCs	POF	Activating primordial follicles and promoting follicular development	Phosphorylating FOXO3a and FOXO1 and activating PI3K/AKT pathway	87
	HA-based drug carriers	HA scaffold loaded hUCMSCs	POI	Protecting follicle survival and ovarian function	Activating PI3K/AKT pathway by paracrine mechanism	88
		HAMA microvector encapsulating MSCs-Exos	POF	Promoting angiogenesis, reducing fibrosis, and regulating inflammation and immune responses	Increasing CD34 and VEGF expressions	89
Biobased drug carriers		hADSC-Exos	POI	Increasing follicles and restoring hormone levels	Modulating TGF-β/SMAD pathway	90
		MSCs-EVs	PCOS	Anti-inflammation, inhibiting androgen synthesis, improving metabolic disorder	Down-regulating the expression of CYP17A11, *CYP11A1*, and DENND1a	91
		hUCMSCs-EVs	POF	Promoting proliferation and inhibiting apoptosis	Activating PI3K/AKT pathway	92
		hUCMSCs-EVs	POF	Anti-inflammation, inhibiting apoptosis, regulating immune cells	Regulating AKT/P38 pathway	93

(*continued on next page*)

Table 1 (*continued*)

Classification of biomedicines	Biomedicine	Disease	Therapy strategy	Related mechanism	Ref.
	BMSCs-Exos	PCOS	Promoting follicle development, reducing apoptosis, and regulating angiogenesis	Increasing the expression of CD31	94
	AF-Exos	POI	Reducing ovarian fibrosis, increasing healthy follicles, and regulating hormone levels	Regulating TGF-β/SMAD pathway	95
	CLU protein carried by hUCMSCs-EVs	POF	Inhibiting apoptosis, promoting the proliferation, and repairing ovarian function	Activating PI3K/AKT pathway, down-regulating cleaved-caspase-3, BAX, and up-regulating BCL-XL	96
	miRNAs target PTEN carried by iPSC-MSCs-EVs	POF	Inhibiting apoptosis and promoting proliferation	Inhibiting PTEN and activating ILK/PI3K/AKT pathway	97
	miR-144-5p carried by BMSCs-Exos	POF	Inhibiting apoptosis and reducing follicular atresia	Targeting PTEN and activating PI3K/AKT pathway	98
	miR-21 carried by AFMSCs -EVs	Premature OD	Inhibiting apoptosis	Regulating PTEN and caspase-3	99
	miR-323-3p carried by AMSCs-Exos	PCOS	Inhibiting apoptosis, promoting proliferation, and regulating steroidogenesis	Targeting PDCD4	100
	miR-21-5p carried by AMSCs-Exos	PCOS	Repairing ovarian function and reducing ovarian cysts	Inhibiting the expression of BTG2, activating IRS-1/AKT pathway	101
	miR-664-5p carried by BMSCs-Exos	POF	Inhibiting apoptosis	Targeting the 3′UTR of *p53* and inhibiting *p53* expression	102
	miR-200c, 122, and 99 carried by MSCs-EVs	Ovarian failure	Inhibiting apoptosis and promoting proliferation	Regulating PI3K/AKT/mTOR pathway	103
	miR-126 carried by hUCMSCs-EVs	POF	Promoting ovarian angiogenesis, inhibiting apoptosis, and improving ovarian function and structure	Targeting PIK3R2 and activating the PI3K/AKT/mTOR pathway	104

−, not applicable. ADSCs, adipose-derived stem cells; AF, amniotic fluid; *AKT*, potein kinase B; AMPK, AMP-activated protein kinase; *Atf4*, activating transcription factor 4; Arg-CS-NAcHis/Cur, curcumin (Cur) encapsulated arginine (Arg) and *N*-acetyl histidine (NAcHis) modified chitosan; BTG2, B-cell translocation gene 2; BCL-2, B-cell lymphoma 2 protein; BAX, BCL-2 associated X protein; BCL-XL, B-cell lymphoma-extra large; CAT, catalase; CeO_2, cerium dioxide; CLU, clusterin; *Chop*, C/EBP homologous protein; *Cyp19a1*, cytochrome P450 family 19 subfamily A member 1; *Cyp11a1*, cytochrome P450 family 11 subfamily A member 1; *Cyp17a1*, cytochrome P450 family 17 subfamily A member 1; DENND1a, DENN domain containing 1A; EVs, extracellular vesicles; Exos, exosomes; FGF2, fibroblast growth factor 2; FOXO3a, forkhead box O3a; FOXO1, forkhead box O1; FSE, flaxseed extract; *Grp78*, glucose-regulated protein 78; HAMA, hyaluronic acid methacryloyl; hAECs, human amniotic epithelial cells; hUCMSCs, human umbilical cord-derived mesenchymal stem cells; hESCMPCs, human embryonic stem cell-derived mesenchymal progenitor cells; HGF, hepatocyte growth factor; *Hsd17b3*, 17β-hydroxysteroid dehydrogenase 3; HO-1, heme oxygenase-1; iPSC, induced pluripotent stem cells; IRS-1, insulin receptor substrate-1; ILK, integrin-linked kinase; IGFBP, insulin-like growth factor binding protein; JAK, janus kinase; KEAP-1, Kelch-like ECH-associated protein 1; LMWC, low molecular weight chitosan; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; NPs, nanoparticles; *NRF2*, nuclear factor erythroid 2-related factor 2; OD, ovulatory dysfunction; PCOS, polycystic ovary syndrome; POF, premature ovarian failure; POI, premature ovarian insufficiency; PTEN, gene of phosphate and tension homology deleted on chromosome ten; *PI3K*, phosphatidylinositol 3-kinase; *p38-MAPK14*, p38 mitogen-activated protein kinase 14; Panx1, pannexin 1; PDCD4, programmed cell death protein 4; PIK3R2, phosphoinositide-3-kinase regulatory subunit 2; SA-BG, sodium alginate-bioglass; Se, selenium; SMAD, mothers against decapentaplegic homolog; SOD, superoxide dismutase; STAT, signal transducer and activator of transcription; STAR, steroidogenic acute regulatory protein; SPIONs, super-paramagnetic iron oxide nanoparticles; TIMP-1, tissue inhibitor of metalloproteinase-1; TGF-β, transforming growth factor-beta; *Tlr4*, toll-like receptor 4; VEGF, vascular endothelial growth factor; *Xbp1s*, X-Box binding protein 1 (spliced).

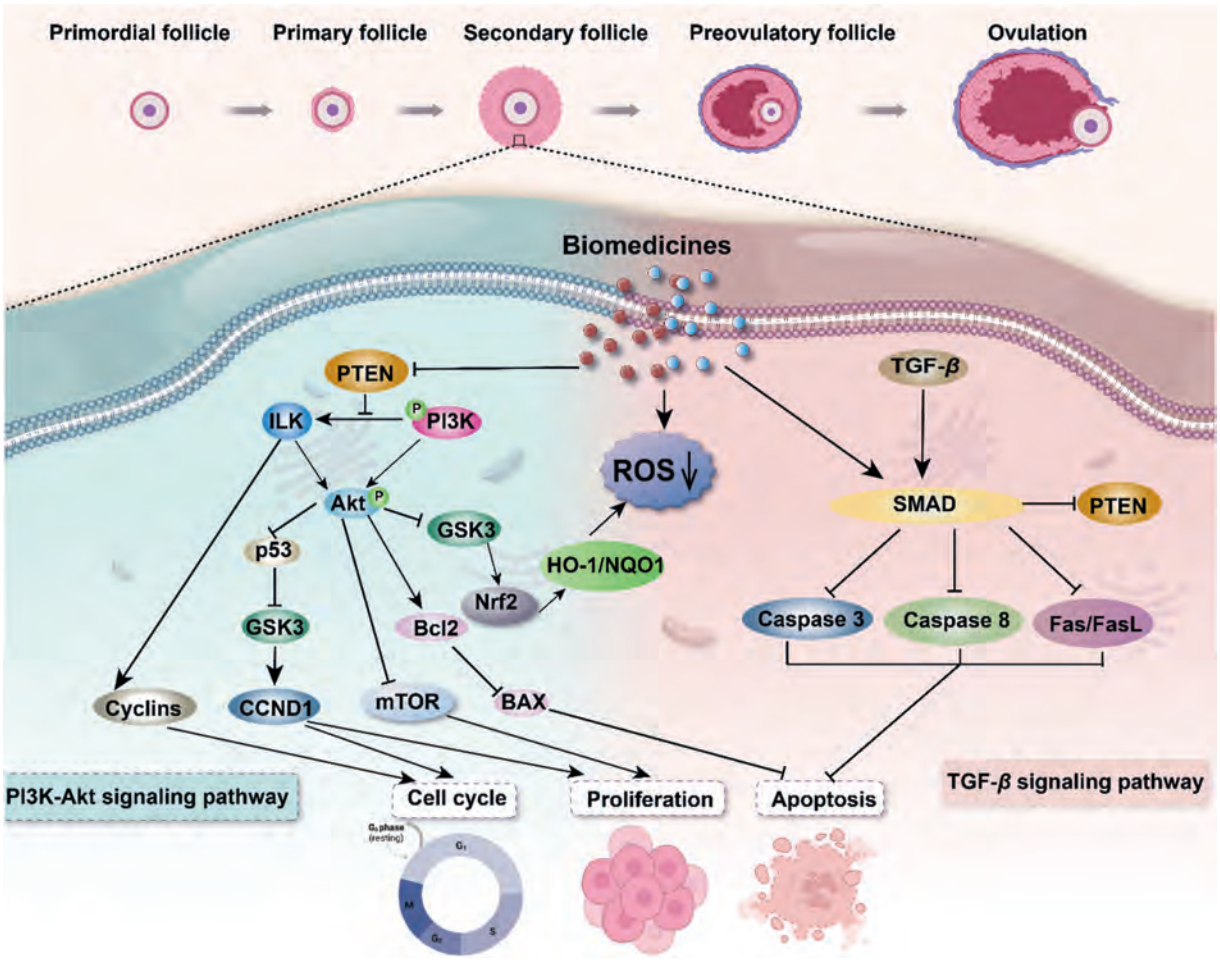

Figure 3 The classic therapeutic mechanisms of OD: ROS scavenging and apoptotic/proliferative pathway regulating.

regulatory activities, to treat OD. These materials directly regulate biological processes through their physicochemical characteristics, independent of drug delivery mechanisms. For instance, metal-based NPs and natural biomedicines can scavenge ROS, reduce inflammation, and restore hormonal balance, thereby addressing the causes of OD. In the past few years, metal-based NPs[62-65], selenium (Se) NPs[66-69], and natural biomedicines with carrier-free[70-72] have been increasingly applied in OD therapy. Although these biomedicines primarily function through intrinsic properties, their potentials can be further enhanced through integration with DDSs.

3.1.1. Metal-based biomedicines

In terms of the antioxidant and anti-inflammatory mechanisms, cerium dioxide (CeO_2) NPs have attracted widespread attention due to their mixed valence (Ce^{3+}/Ce^{4+})[107]. It could effectively improve metabolic disorders by scavenging free radicals, reducing the level of oxidative stress markers (*e.g.*, malondialdehyde), and increasing the activity of glutathione and SOD[108]. Notably, the size effect of CeO_2 NPs significantly influenced their biological activity. A smaller CeO_2 NPs could increase the Ce^{3+} fraction, thereby enhancing their oxygen vacancy mediated ROS scavenging ability[109]. Yang et al.[62] demonstrated that CeO_2 NPs reduced ROS levels and endoplasmic reticulum stress (Fig. 4A), reversed the high-fat diet induced OD mice, and restored meiosis and metabolic homeostasis in mice oocyte (Fig. 4B), offering a new therapeutic strategy for obesity related OD.

Silver NPs (Ag NPs) have been found to alleviate chronic inflammation and oxidative stress[110,111]. In hormone regulation and tissue repair, Ag NPs have shown great potentials in modulating hormonal imbalances, and mitigating inflammation associated with PCOS[63]. Specifically, Ag NPs significantly reduced serum testosterone levels, and increased estradiol concentrations in PCOS models by inhibiting the secretion of pro-inflammatory cytokines such as TNF-α and interleukin-6 (IL-6)[63]. Moreover, Ag NPs synthesized from cinnamomum zeylanicum have shown efficacy in reducing inflammatory markers in PCOS rats, further highlighting their anti-inflammatory properties[64].

Additionally, magnesium oxide (MgO) NPs promoted the conversion of androgen to estrogen by upregulating the expression of the cytochrome P450 family 19 subfamily A member 1 (*Cyp19a1*) gene. Meanwhile, they work synergistically with antioxidants to repair ovarian tissue structure and reduce cyst formation[65]. Similarly, zinc oxide (ZnO) NPs exhibited antioxidant and anti-inflammatory effects, creating a protective environment that minimizes cell damage, and supported the maintenance of cellular homeostasis[112]. ZnO NPs have dual advantages in treating diabetic OD by enhancing insulin sensitivity, and regulating glucose metabolism[113]. ZnO NPs could not only reduce ovarian tissue oxidative damage markers (*e.g.*, malondialdehyde (MDA)),

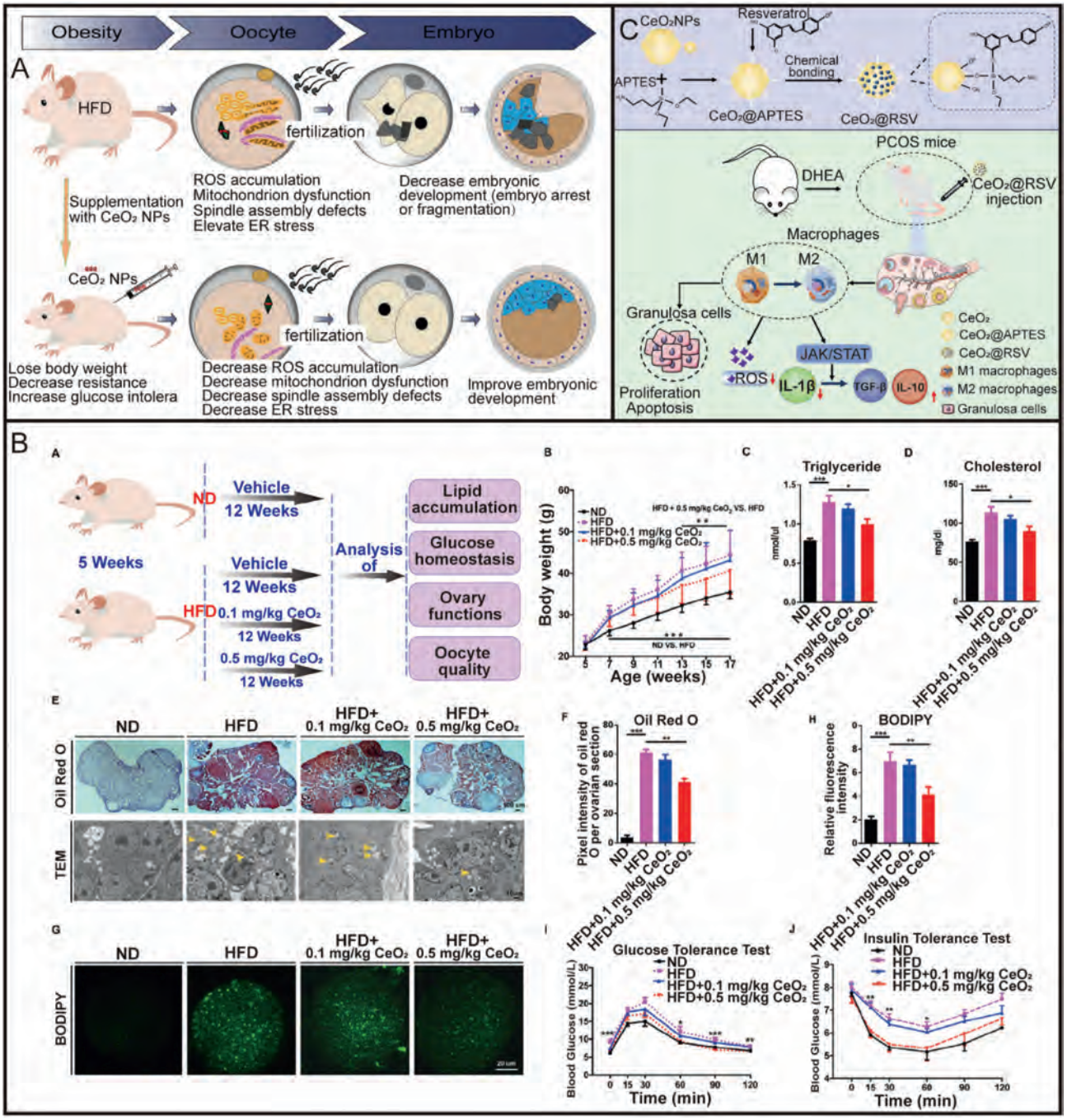

Figure 4 CeO_2 NPs reduced ROS levels through antioxidant and anti-inflammatory effects, and improved the endocrine disorder and ovarian morphological abnormalities in the OD model. (A) Treatment with CeO_2 NPs attenuated high fat diet induced obesity, dyslipidemia and hyperglycemia in mice. Reprinted with the permission from Ref. 62. Copyright 2021 @ Elsevier Ltd. (B) The proposed mechanisms for CeO_2 NPs to ameliorate obesity induced oocyte dysfunction and retard early embryonic development. Reprinted with the permission from Ref. 62. Copyright 2021 @ Elsevier Ltd. (C) The synthesis process of CeO_2 carrying resveratrol (CeO_2@RSV), and its anti-inflammatory functions in PCOS C57BL/6 mice models. Reprinted with the permission from Ref. 77. 2021 @ Elsevier Ltd.

but also promote follicular development, and restore sex hormone balance[112]. These characteristics make ZnO an ideal candidate for improving insulin resistant PCOS.

3.1.2. *Selenium NPs*

Selenium (Se) is a trace element, and its deficiency is associated with the risk of PCOS[114]. As an important micronutrient, Se has antioxidant, anti-inflammatory, and immunomodulatory functions[115]. By utilizing these characteristics, Se NPs have become a promising therapeutic agent for OD[67]. Their unique physicochemical properties and biological activities enhanced the bioavailability and therapeutic efficacy of Se through nanoscale effects.

In the letrozole induced PCOS rat models, Se NPs significantly improved PCOS related indicators, including decreased body weight, ovarian weight, testosterone, LH, and insulin levels, while

enhancing antioxidant activities, and reducing inflammatory factors such as IL-6, TNF-α, and IL-1β[66]. These findings suggest that Se NPs can improve the metabolic and endocrine abnormalities associated with PCOS through antioxidant and anti-inflammatory mechanisms[66]. Another study showed that Se NPs restored the estrous cycle, reduced body mass index (BMI) and insulin resistance, improved dyslipidemia and serum testosterone levels, and inhibited androgen synthesis by regulating steroidogenic related genes, and decreasing AR expression in PCOS rats induced by high fat diet and letrozole[68]. Moreover, Se NPs enhanced insulin sensitivity by regulating the PI3K/potein kinase B (Akt) pathway, which is crucial for treating PCOS complications[69] *via* restoring mitochondrial function, and enhancing anti-inflammatory activity[69]. Notably, the combination of Se NPs and metformin has a better therapeutic effect on PCOS-IR patients than single drug use, indicating a synergistic effect[69].

3.1.3. Natural biomedicines

In recent years, researchers have been focusing on exploring natural bioactive substances, such as collagen, low molecular weight chitosan (LMWC), and sodium alginate (SA) as potential interventions for POF and PCOS. Collagen is a key component of human tissues, with high biocompatibility and biodegradability, making it and its derivatives (*e.g.*, gelatin) widely used in biomedicines[116]. Research has demonstrated that collagen extracted from the swim bladder of sturgeon could protect cyclophosphamide induced POF mice through a variety of mechanisms[70]. These mechanisms include antioxidant effects, such as scavenging free radicals, regulating antioxidant enzyme activity, and reducing oxidative stress. Additionally, collagen peptides could activate the PI3K/Akt and Bcl-2/Bcl-2 associated X protein (Bax) pathways, and inhibit the mitogen-activated protein kinase (MAPK) pathway, thereby reducing apoptosis. Furthermore, these peptides improve ovarian tissue structure by increasing the number of growing follicles, and reducing the number of atretic follicles[70].

Like collagen, LMWC can also function with carrier-free, avoiding the potential risks of complex delivery systems, and demonstrating good biocompatibility and therapeutic efficacy. Recent studies have found that LMWC can enhance the phagocytic ability of macrophages by upregulating the expression of phagocytic molecules, and regulating the M1/M2 polarization ratio. This regulation reduces the accumulation of senescent cells in the ovary, decreases the levels of inflammatory factors, improves the ovarian microenvironment, and ultimately delays the aging process[71].

Under the relevant background, sodium alginate (SA) significantly reduced the body weight, blood glucose, blood lipid, and androgen levels in PCOS patients through its unique gel forming ability and dietary fiber properties[72]. These mechanisms provide an effective therapeutic option for PCOS, broadening biomedicine applications of OD.

3.2. Biomedicines with traditional drug carriers

The development of biomedicines has transformed traditional DDSs in reproductive medicine. Biodegradable polymers such as poly(lactic-*co*-glycolic) acid (PLGA)[73-76], and metal-based delivery system[77,78], have emerged as promising DDSs due to their unique physicochemical properties and biological activities. These biomedicines show significant potentials in lowering oxidative stress, inflammation, and hormonal imbalances. It is worth further exploring its mechanism and therapeutic effect in traditional DDSs.

3.2.1. PLGA carriers

The degradation products of PLGA, lactic acid and glycolic acid, are byproducts of human metabolism. Therefore, PLGA has high biosafety in DDSs[117]. As a drug carrier, PLGA has been widely used in preclinical studies of cancer therapy. For instance, Byeon et al.[118] prepared PLGA encapsulating both paclitaxel and focal adhesion kinase small interfering RNA (siRNA), which demonstrated significant anti-tumor effects in HeyA8-MDR and SKOV3-TR cells and drug-resistant xenograft ovarian tumor models.

In reproductive medicine, PLGA also shows great potentials[119]. The lactic acid produced by PLGA degradation can serve as a metabolic substrate to support the antioxidant capacity of oocytes and cumulus cells cultured *in vitro*[120]. In addition, a PLGA sponge scaffold containing magnesium hydroxide has been subcutaneously implanted in human embryonic stem cell-derived mesenchymal progenitor cells (hESCMPCs) of POF mice models. This scaffold provided a three-dimensional physical scaffold for cell attachment and proliferation[73]. And PLGA has demonstrated the significant potentials in encapsulating siRNA, thereby enhancing its stability and delivery efficiency *in vivo*[121]. Research demonstrated that PLGA NPs encapsulating miR-146a effectively silenced the expression of p38 mitogen-activated protein kinase 14 (p38-Mapk14) gene, and alleviated oxidative stress and inflammation, thereby alleviating symptoms of POF[74]. Furthermore, the delivery of miR-503 mediated by PLGA increased the apoptosis rate of ovarian endometriosis cells, and inhibited cell proliferation, providing a novel therapeutic strategy for the treatment of ovarian endometriosis[75]. In the treatment of PCOS, PLGA has also shown promising applications[76]. A study compared the therapeutic effects of oral and local ovarian administration of metformin and its formulation in a rat model of PCOS. The results demonstrated that PLGA effectively encapsulated metformin (metformin@PLGA), enhancing its stability and bioavailability. Local ovarian injection of metformin@PLGA directly delivered the drug to ovarian tissue, increased drug concentration at the target site, reduced systemic side effects, and significantly improved PCOS symptoms[76].

3.2.2. Metal-based drug carriers

CeO_2 NPs can serve as independent biomedicines for treating OD, as well as effective drug carriers, utilizing their unique physicochemical and biological properties to treat reproductive system diseases, and improve therapeutic outcomes[122]. The composited biomedicines formed by CeO_2 and resveratrol (CeO_2@RSV) have been shown to improve endocrine disorders and ovarian morphological abnormalities in PCOS mice by regulating macrophage polarization (promoting M2 anti-inflammatory phenotype/inhibiting M1 pro-inflammatory phenotype), and reshaping the ovarian immune microenvironment (Fig. 4C)[77].

Superparamagnetic iron oxide NPs (SPIONs) serve as efficient DDSs to overcome the limitations of curcumin, a phenolic compound with anti-inflammatory and antioxidant properties[78]. Although curcumin has shown potentials in improving hyperglycemia, hyperlipidemia, hyperandrogenism, and insulin resistance in PCOS patients[123], its poor solubility and bioavailability limit its therapeutic efficacy. By encapsulating curcumin, SPIONs significantly enhance its bioavailability, and enable the targeted delivery. This system exhibits dose-dependent anti-apoptotic effects, and

protects the structural integrity of preantral follicles in PCOS mice, demonstrating its potential as a promising therapeutic strategy for PCOS[78].

3.3. Hydrogel drug carriers

Hydrogel-based biomedicines, such as chitosan[79-82], sodium alginate[83,84], collagen[85-87], and hyaluronic acid (HA)[88,89], serve as effective delivery systems for treating OD. They enable sustained release, enhance cell retention, and improve therapeutic outcomes by promoting angiogenesis, reducing fibrosis, and restoring ovarian function and hormone levels.

3.3.1. Chitosan-based drug carriers

Chitosan is a deacetylated derivative of chitin, and the second most abundant natural polymer, derived from crustaceans, insects, bacteria, algae, and plants[124]. As a biocompatible and biodegradable material, chitosan typically has positive surface charges, and mucosal adhesion properties, allowing it to bind to negatively charged biofilms, and facilitate controlled drug release[125,126]. This characteristic renders chitosan an ideal drug carrier for enhancing the stability, bioavailability, transportation, and permeability of drugs for therapeutic purposes[80].

In the PCOS rat model, chitosan loaded with flaxseed extract (FSE) significantly reduced testosterone levels, and improved the LH/FSH ratio, demonstrating excellent therapeutic efficacy (Fig. 5A)[79]. Moreover, chitosan-fennel seed extract nanocomposites (FEC@NBC) have been shown to significantly increase FSH and high-density lipoprotein cholesterol (HDL-C) levels in the PCOS model[81]. This effect is attributed to the targeted delivery properties of chitosan, and the multi-target biological activity of fennel seed extract (Fig. 5B)[81]. In addition to fennel seed extract, chitosan has also been employed to encapsulate curcumin by chemical modification, introducing arginine and *N*-acetylhistidine to form amphiphilic chitosan conjugates[82]. This approach significantly enhanced the solubility and bioavailability of curcumin. The resulting curcumin loaded chitosan demonstrated therapeutic efficacy in PCOS rats by reducing serum levels of LH, prolactin, testosterone, and insulin, increasing progesterone, and restoring normal ovarian morphology and ovulation function[82]. These findings emphasize the potential of chitosan delivery systems in regulating PCOS related hormones and metabolic disorders, demonstrating their therapeutic efficacy in targeting PCOS mechanisms.

3.3.2. Sodium alginate-based drug carriers

Sodium alginate (SA) is a non-toxic polysaccharide rich in brown algae and bacteria, easy to process, and widely used in two dimensional (2D) and three dimensional (3D) mammalian cell cultures[127]. As a natural gelling agent, SA supports cell adhesion, proliferation, and retention at transplantation sites, demonstrating excellent drug delivery capabilities[128]. Recent studies have confirmed that SA hydrogel combined with acellular bovine ovarian ECM could support the culture of isolated human follicles *in vitro*[129]. The soft and porous hydrogels facilitate the diffusion of nutrient and growth factor, highlighting its potentials in drug delivery and regenerative medicine[130].

Human amniotic epithelial cells (hAECs) hold promises for treating POF due to their regenerative potentials, but their effectiveness is limited by low survival rates, and restricted proliferation after transplantation[131]. The composited hydrogel of SA and bioactive glass (BG) significantly promotes angiogensis by upregulating the expression of angiogenesis related factors in endothelial cells, thereby accelerating skin tissue regeneration[132]. In a recent study, SA-BG hydrogel encapsulated with hAECs was transplanted into mice with chemotherapy induced ovarian injury[83]. It significantly improved the survival rate and function of hAECs by providing biological support, and stimulating the secretion of pro-angiogenic factors such as VEGF, insulin-like growth factor binding protein-2/3 (IGFBP-2/3), and tissue inhibitor of metalloproteinase-1 (TIMP-1)[83]. These effects promoted angiogenesis, inhibited granulosa cell apoptosis by reducing caspase-3 activity, and stimulated cell proliferation *via* the upregulation of Ki-67 and proliferating cell nuclear antigen (PCNA), ultimately restoring ovarian function[83]. In terms of drug delivery, clinical studies showed that SA-based progesterone vaginal gel increased the serum progesterone levels and endometrial thickness in anovulatory PCOS patients, and the pregnancy rate (46.66%) is significantly higher than that of the Progest® vaginal capsule group (26.67%)[84]. In addition, SA enhances the bioavailability of metformin microspheres by improving mucosal adhesion, and achieving controlled release, offering a new approach for PCOS treatment[133].

3.3.3. Collagen-based drug carriers

Collagen, is the most abundant ECM protein, widely used in drug and cell delivery due to its excellent biocompatibility, biodegradability, low immunogenicity, and minimal inflammatory response[134,135]. Joo et al.[136] reported that type I collagen hydrogel could promote the development of rat follicles. With the increase of collagen concentration, the matrix elasticity and fibrous structure were enhanced, and the survival rate of follicle exceeded 90%[136].

In POF rat models, Su et al.[85] confirmed that the collagen scaffolds, as cell delivery systems, significantly increased the retention rate of human adipose derived stem cells (hADSCs) in the ovary, restored ovarian function, and promoted proliferation and angiogenesis of follicular granulosa cells. Similarly, Yang et al.[86] utilized collagen scaffolds to encapsulate and deliver hUCMSCs into POF mice models, improving follicular growth, and alleviating POF symptoms. Ding et al.[87] further highlighted the role of collagen in cell delivery, indicating that collagen-hUCMSCs transplantation could activate forkhead box O3a (FOXO3a) and forkhead box O1 (FOXO1) signaling pathways, promoting the activation of primordial follicles. Notably, the efficacy of collagen as a DDS had been validated in clinical trials, where two POF patients successfully became pregnant following this approach[87]. These findings highlight collagen's potential as a versatile platform for drug and cell delivery, offering bioactive support and enhanced cellular function. The collagen-based system utilized its biocompatibility and ability to maintain cell viability[137], providing a promising therapeutic strategy for ovarian restoration and OD treatment.

3.3.4. Hyaluronic acid-based drug carriers

Hyaluronic acid (HA)-based hydrogel are frequently employed as DDSs due to its biological functions, abundant reactive functional groups, and degradability[138,139]. HA is particularly abundant in the ovarian ECM, and plays an essential role in maintaining the microenvironment required for follicular development[140].

A study by Shin et al.[73] compared the effect of porous sponge and HA hydrogel in delivering hESCMPCs to POF models. The

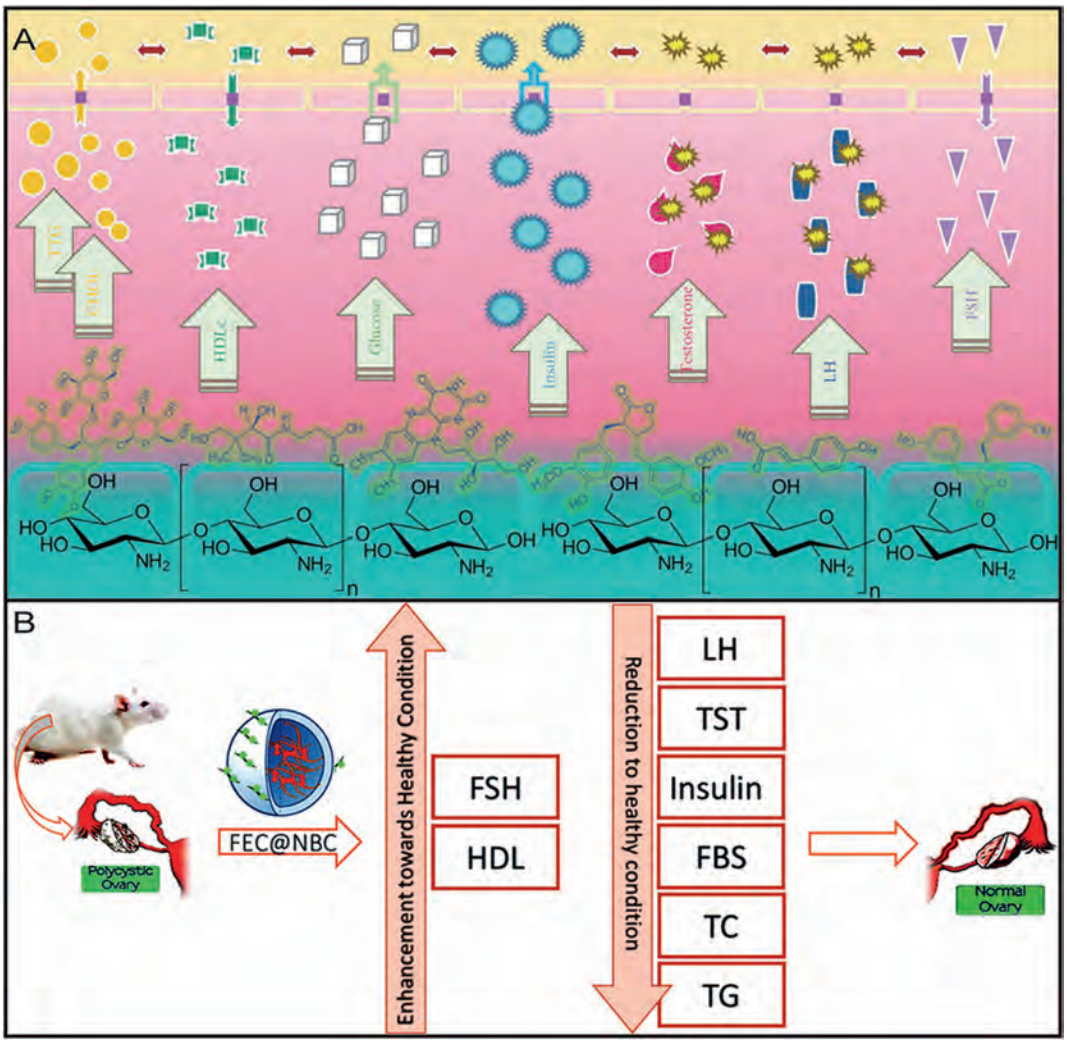

Figure 5 Chitosan in drug delivery applications. (A) The effects of chitosan loaded flaxseed extract on OD therapy in a rat model of PCOS. Reprinted with the permission from Ref. 79. Copyright 2024 @ Wiley Ltd. (B) The overall effects of chitosan-fennel seed extract (FEC@NBC) on the biochemical and hormonal factors in PCOS rat models. Reprinted with the permission from Ref. 81. Copyright 2021 @ Elsevier Ltd.

HA-based hydrogel DDS exhibited superior ovarian function, and significantly increased the follicle reserve, estradiol and AMH levels. And the estrous cycle of mice became regular, and the quality of follicle and embryos was improved[73]. Similarly, Jiao et al.[88] studied the combination of hUCMSCs and HA hydrogel in POI mice models. Compared with the control group without HA, the HA-based DDS extended hUCMSCs' retention in the ovary to 7 days, enhanced secretory function, and improved follicle survival and ovarian recovery[88]. In addition, in a chemotherapy induced POF model, exosomes (Exos) derived from lipopolysaccharide pretreated hUCMSCs (hUCMSCs-Exos) were encapsulated in HA methacryloyl (HAMA) microcarriers prepared using microfluidic electrospray technology[89]. This HA-based DDS increased CD34 positive blood vessels, enhanced ovarian angiogenesis, and reduced collagen deposition, indicating the decreased fibrosis and improved ovarian repair[89]. In summary, HA has shown significant potentials as DDS in ovarian function recovery. It promotes angiogenesis, reduces fibrosis, and prolongs cell retention time, providing a new strategy for the treatment of POF and POI.

3.4. Biobased drug carriers

In recent years, biomedicines derived from EVs, such as Exos, microvesicles, and apoptotic bodies, have gained attentions for treating OD. EVs originate from embryonic stem cells (ESCs), human induced pluripotent stem cells (iPSCs) and MSCs[141,142], serving as efficient DDSs to transport active molecules such as nucleic acids, proteins, and lipids to regulate targeting cell functions[143]. Compared to traditional systems like viral vectors[144], EVs possess advantages such as low immunogenicity, precise targeting, and high biocompatibility[145], making them a core tool for cell-free therapeutic strategies. 3D culture increases the MSC-EV yield by 5.6 times, enhancing therapeutic potentials of ovarian repair through improving cell survival, hormonal balance, and reducing fibrosis[146]. This breakthrough supports the large scale application of EVs. The current research highlights their multi-target regulatory potentials in OD.

In POI mice models, hADSC derived Exos (hADSCs-Exos) significantly improved ovarian function, restored primordial, primary, secondary, and antral follicles to 96%, 97%, 93%, and 87%

of normal levels, respectively. The serum hormone levels were also enhanced, with E2 and AMH returning to 98% and 97% of normal levels, while FSH levels returned to normal[90]. *In vitro* experiments, hADSCs-Exos increased granulosa cell proliferation to 76%, and reduced apoptosis to 4% by regulating the transforming growth factor-beta (TGF-β)/mothers against decapentaplegic homolog (SMAD) signaling pathway[90]. Similarly, MSC-EVs have shown the therapeutic potentials in PCOS by modulating androgen production related genes, reducing inflammation, and improving insulin resistance[91]. In POF models, hUCMSC-EVs activated the PI3K/Akt and AKT/P38 signaling pathways, promoted granulosa cell proliferation, and inhibited apoptosis[92,93]. And BMSCs-Exos improved ovarian morphology in PCOS mice by increasing corpus luteum, and regulating angiogenesis and follicle development related factors[94]. Additionally, AF derived Exos injected directly into ovarian tissue reduced fibrosis, increase healthy follicles, and regulated hormone levels by modulating the TGF-β/SMAD pathway, ultimately improving fertility in POI rats[95].

In addition to its unique therapeutic effects, EVs can serve as an effective DDS to deliver bioactive molecules like chemokines, cytokines, and growth factors to the injured sites, promoting tissue regeneration, and avoiding the risk of tumorigenesis associated with live cell transplantation[147]. In ovarian disorder models, EVs regulate signaling pathways, and restore ovarian function by delivering key molecules, demonstrating therapeutic potential[96].

In the chemotherapy induced POF model, hUCMSCs-EVs significantly restore ovarian hormone levels (increased E2 and decreased FSH) *via* carrying clusterin (CLU) protein, the key component activating PI3K/AKT pathway, and anti-apoptotic proteins (BCL-XL) (Fig. 6A)[96]. These effects improved the follicular microenvironment, and delayed ovarian reserve depletion. The therapeutic effect of EVs is closely related to the miRNAs they carry. For example, iPSC-MSCs-EVs carrying miRNAs targeting phosphate and tension homology deleted on chromosome ten (PTEN) could inhibit granulosa cell apoptosis and promote their proliferation by activating the integrin linked kinase-PI3K/AKT (ILK-PI3K/AKT) signaling pathway, thereby effectively alleviating chemotherapy induced ovarian injury (Fig. 6B)[97]. Similarly, BMSCs-EVs enriched with miR-144-5p could target the inhibition of PTEN gene, and alleviate its negative regulation of ILK, thereby activating AKT phosphorylation, promoting granulosa cell proliferation, and inhibiting apoptosis[98]. Additionally, miR-21 carried by EVs regulates PTEN and caspase-3 apoptosis pathways, playing a key role in tissue regeneration[99]. Other studies have shown that adipose MSCs (AMSCs)-Exos carrying miR-323-3p improved fertility by regulating steroidogenesis and cumulus cell survival[100]. In animal studies, it revealed that AMSCs-Exos carrying miR-21-5p had beneficial effects on fertility recovery *via* improving metabolic status, indirectly promoting ovarian function recovery, and reducing ovarian cysts[101]. Similarly, Exos from BMSCs carrying miR-664-5p also could be applied for fertility recovery *via* inhibiting apoptotic genes[102]. Furthermore, MSCs-EVs deliver molecules such as miR-200c, miR-122 and miR-126 to regulate the PI3K/Akt/mammalian target of rapamycin (mTOR) pathway, thereby inhibiting follicular overactivation, and providing new insights for protecting ovarian function[103,104]. The above research emphasize the potential of EVs-based delivery system for treating OD, highlighting their clinical applicability through multi-target regulation and improved delivery efficiency.

4. Limitations and prospects

Although the existing biomedicines for ovarian tissue engineering is relatively limited compared to other tissues, advancements in biomedicines continue to contribute to further development of this field. However, due to the structural and functional complexity of the ovary, a reproductive organ containing specific hormones, the development of reliable biomedicines to promote structural regeneration and functional restoration of this gland remains challenging, with numerous obstacles to be overcome.

4.1. Limitations

4.1.1. Lack of intelligent delivery platforms

The current ovarian targeted DDSs lack the intelligent response required to address the dynamic pathophysiology of ovulation disorders. While hydrogel drug carriers exhibit biocompatibility and follicular support[73,88], they cannot adapt to enzymatic degradation or mechanical stress in the ovarian microenvironment,

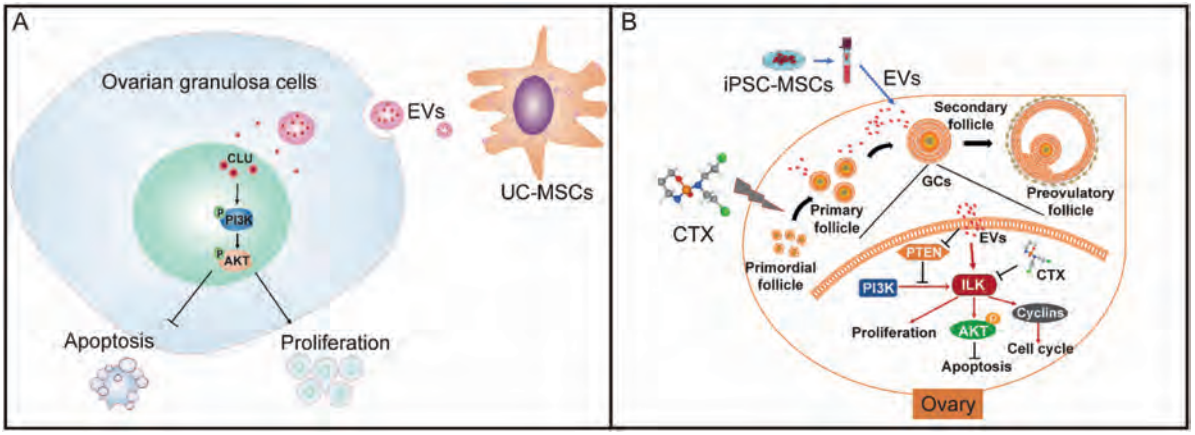

Figure 6 The role of EVs in the repair mechanism of POF. (A) Clusterin-carrying EVs derived from human umbilical cord MSCs restored the ovarian function of POF mice by activating the PI3K/AKT pathway. Reprinted with the permission from Ref. 96. Copyright 2024 @ BMC Ltd. (B) iPSC-MSC-EVs carrying functional miRNAs targeting PTEN reversed CTX induced downregulation of the ILK−PI3K/AKT pathway in granulosa cells, promoting proliferation and inhibiting apoptosis. Reprinted with the permission from Ref. 97. Copyright 2023 @ Zoores Ltd.

which indicates the broader limitation of passive drug release mechanisms. Even advanced strategies like SPIONs-curcumin system, have improved bioavailability, they still rely on external magnetic guidance rather than intrinsic pathological perception[78]. Similarly, the administration of LMWC and metabolic effects of SA operate through static pharmacological mechanisms[71,72], failing to autonomously adjust to ovarian physiological changes.

The etiology of OD is complex, involving factors such as endocrine disturbances, and abnormal follicular development. Current intelligent DDSs struggle to accurately identify these intricate pathological states. Targeted delivery to ovarian tissue requires highly specific biomarkers, but there is still a lack of reliable markers associated with OD. Intelligent DDSs for OD is still underdeveloped, and lacks a platform that can accurately respond to physiological changes in the ovaries, such as hormone levels and follicle development stages[148]. Although some studies have explored NPs and hydrogels, these materials have limited functions in intelligent response to changes in the ovarian microenvironment.

4.1.2. Complexity and safety concerns of biomedicines

Biomedicines, such as hydrogels, are complex compositions, and contain various bioactive factors. Chitosan-based drug carriers enhance drug stability and hormone regulation[79,82], yet their cationic properties may disrupt ovarian mucosal barriers during long-term use, and changes in deacetylation degrees affect reproducibility. In addition, decellularized ECM hydrogels are rich in collagen, glycosaminoglycans, and elastin, which may lead to unpredictable immune responses or biocompatibility issues. Thus, strict safety assessment is required before clinical application.

Some biomedicines may trigger immune rejection, particularly in allogeneic transplantation scenarios[149]. Although MSCs have low immunogenicity, their transplantation may trigger host immune recognition of allogeneic antigens, leading to rejection reactions. Long-term engraftment can persistently activate T cells/macrophages, and induce chronic inflammation[150]. Notably, stem cells have the risks of off target differentiation or abnormal expression of self-antigens, which may induce autoimmune diseases[151]. Particularly in pluripotent stem cells, the residual undifferentiated populations carries neoplastic risk[152]. Moreover, intravenously administered stem cells frequently exhibit poor ovarian orientation, which may impair non-target organs' function[151]. Another noteworthy issue is that stem cell derived paracrine factors, particularly exosome mediated epigenetic signaling, may exert long-term regulatory effects on host genomic stability[153]. While Exos' acellular nature minimizes acute immunogenicity, long-term administration may impair immune surveillance, increase susceptibility to opportunistic infections or carcinogenesis[154]. It is crucial that the current safety mainly focuses on murine models, and cannot summarize the complexity of human immunity. Thus, inferring the potential long-term toxicities from preclinical data remains challenging.

To address these immunological challenges, surface modification strategies have been developed to enhance the biocompatibility of biomedicines. By modifying biomedicines with PEGylation, protein adsorption, and cell adhesion, the immune recognition is reduced, thereby decreasing the immune response, and improving biocompatibility[155]. After modifying PEG with ECM peptides, it can simulate basement membrane interactions, promote follicular maturation, and preserve the ECM secreted by cells, thus restoring critical cell−matrix signaling[156]. Significantly, CD47 is a membrane protein widely expressed on the surface of normal autologous cells. It can bind to the SIRPα receptor on immune cells such as macrophages, transmitting a "don't eat me" signal to inhibit phagocytosis by immune cells[157,158]. This mechanism can be adopted by biomedicines through surface modification of CD47, enabling them to evade macrophage phagocytosis, and prolong their circulation time *in vivo*[159,160]. Immune evasion strategies such as PEGylation and CD47 modification haven't been applied in OD, and it is expected that they will reduce the immunogenicity of biomedicines, extend their *in vivo* retention time, and prevent premature clearance by the immune system.

Besides, some biomedicines, such as NPs, may accumulate in the body, leading to long-term biotoxicity. Recent studies highlight the need to assess the long-term biocompatibility of ovarian sustained release systems, with a focus on the cumulative toxicity from polymer degradation byproducts[161]. A study concerning exposure to ambient particulate showed the slow release of polymer byproducts may cause delayed toxicity, and require long-term evaluation to assess ovarian accumulation[162]. Current research suggests that scaffold degradation products may disrupt ovarian physiology *via* oxidative stress pathways, and lipid homeostasis dysregulation[163]. Notably, there is still a significant knowledge gap in the exposure of ovarian DDSs, particularly in terms of their extended tissue compatibility and safety. Systematic studies are needed about their tissue specific effects on oocyte competence and follicular reserve.

4.1.3. Discrepancies between animal models and human

Current studies rely on animal models, but there are significant differences exist between animal and human reproductive physiology. Although rodent models have provided fundamental insights into ovarian function, the key differences in physiology and ovulation mechanisms may limit the direct translational applicability of some findings to humans[26]. Some biomedicines promote follicular development in animal models, but their specific mechanisms in humans are not fully understood. The steroid hormone synthesis pathway in humans rapidly shifts from an estrogen dominant phase to a progesterone dominant phase during ovulation, with feedback regulation of inflammation and angiogenesis *via* progesterone receptors[28]. Conversely, in rodents, progesterone secretion is decoupled from the ovulation time window, and their luteal phase hormone regulation patterns are difficult to fully mimic the physiological states of humans[164]. In addition, regarding ECM remodeling and immune regulation in the ovulation microenvironment, the ECM of rodents is primarily composed of laminin, while humans form a dense basement membrane with a high proportion of fibronectin and elastin[165]. Glycosaminoglycans like hyaluronic acid mediate the interaction between human follicle stromal cells through CD44 receptors, and these species differences make it difficult for animal models to accurately reflect the dynamic stress response of human ovarian ECM during ovulation[165]. Furthermore, the dynamic interaction between pro-inflammatory and anti-inflammatory factors during human ovulation is more complex, involving unique subpopulations of immune regulatory cell such as regulatory T cells, which are difficult to fully summarize in current research models related to OD pathologies[166].

Besides, when considering interspecific differences in pharmacokinetics, additional complexities emerge. The clearance rate of certain biomedicines in rodent models is significantly faster than those in humans, leading to differences in dosage and efficacy[167]. The species-specific expression patterns of metabolic

enzymes, such as cytochrome P450 (CYP450) family, can lead to inconsistent responses to ovulation inducing drugs, have efficacies in animal models but fail to translate to humans, and even exhibit adverse effects[26]. Meanwhile, the drug response mechanisms in human follicles appear to be more complex, regulated by the follicular fluid microenvironment[168]. Compared with animal models, the production of LH surges in human seems to be regulated through more complex neuroendocrine circuits[26], and the responsiveness of mice ovaries to FSH is significantly higher than that of human[169]. When transitioning from animal studies to clinical trials, this interspecies variability requires rigorous pharmacological reassessment.

Future research may focus on comparing the RNA-seq data and other gene expression profiles of ovarian cells during the ovulation phase in humans, primates, and mice. This approach will facilitate the identification of conserved ovulation regulatory pathways, such as the LH surge signaling pathway, and species-specific differential expressed genes[26]. Special emphasis will be placed on examining cross species genes expression differences associated with inflammatory responses and apoptosis[170]. Subsequently, the proteome of ovarian tissue, including proteins secreted by granulosa cells, and the metabolome, including lipid metabolites in follicular fluid, will be integrated[171]. This integration will enable the construction of a pharmacological target network that highlights interspecies differences. For instance, the common abnormal metabolic pathways, such as arginine-proline metabolism, can be identified in PCOS patients and mice models[172]. These strategies hold promise in systematically addressing species-specific pharmacokinetic differences, and accelerating the development of precise biomedicines for OD therapy.

4.1.4. *Technical limitations and clinical challenges*

At present, there are limited ongoing or completed clinical trials investigating the use of biomedicines in the treatment of OD. A registered clinical study in 2016 explored the safety and efficacy of intra-ovarian injection of allogeneic hUCMSCs combined with injectable collagen scaffold in the treatment of POF, as well as the efficacy of improving ovarian function[173]. Additionally, a clinical study is currently underway to evaluate the efficacy of intravenous administration of human placental MSCs Exos for the treatment of POI[174]. Similarly, another clinical study aimed to investigate the effect of intra-ovarian injection of BMSCs-EVs on FSH and AMH levels, and menstrual cycle recovery in infertile patients with POF[175]. However, the above three clinical studies have not shown any published results. To date, one clinical trial reported the outcomes, in which 5 out of 8 patients with POF who received collagen/hUCMSCs transplantation exhibited follicular activity (62.5%). Among them, 1 patient (12.5%) conceived naturally, but chose to terminate the pregnancy at 24 weeks due to fetal trisomy 21 syndrome[87]. These findings are promising, but we still have little awareness of the safety, efficacy, and optimal dosage of biomedicines in clinic, let alone the broader impact on reproductive health.

Although these preliminary clinical findings show some promise, the preparation of some biomedicines is still complex and expensive, and there are technical difficulties in their storage and transportation. Similar challenges have also been observed in EVs-based therapies, where inconsistent isolation methods, unstable extraction efficiency, and undefined dosing regimen hinder clinical translation[96,146]. These technical barriers, coupled with the lack of standardized production and clinical validation, urge the need for improving engineering approaches, and conducting rigorous testing. The application of biomedicines requires precise surgery, such as the transplantation of hydrogels or stem cells into the ovary, which requires specialized medical teams and equipment. In addition, the invasiveness of this technique is also a reason for considering its clinical feasibility. Currently, the clinical application of such technologies faces significant obstacles[176]. Although some biomedicines show therapeutic potentials in experimental conditions, their clinical efficacy is still uncertain. In studies using stem cells DDSs to treat POF, some patients exhibited the improvements in follicular development and pregnancy rates, but the overall outcomes were inconsistent[177]. The significant differences in biomedicines treatment efficacies are attributed to varying degrees of OD, underlying causes, and individual patient conditions.

4.2. *Perspectives*

Firstly, developing more intelligent DDSs is an urgent task. It is suggested to integrate nanotechnology, biosensors, and biomedicines to create DDSs capable of real-time monitoring and intelligent response to changes in the ovarian microenvironment, such as hydrogels or NPs with pH responsive, temperature responsive, or hormone levels responsive. Nanotechnology can also be utilized to achieve precise targeting of ovarian tissues by the surface modification of NPs, to specifically recognize ovarian cell surface receptors, and improve drug delivery efficiency. Additionally, the development of smart materials that can response the physiological changes in the ovaries is a promising direction, such as NPs can release drugs in response to estrogen levels to support follicular development. Although the application of engineered stem cells, engineered microorganisms and artificial intelligence technology in OD therapy is still in its infancy, these technologies have shown great development potentials, and broad application prospects. Future efforts should also emphasize the interdisciplinary collaboration among materials science, biology, medicine, and engineering to develop novel intelligent controllable biomedicines, achieve more precise treatments, and promote the clinical translation of intelligent DDSs.

Secondly, it is worth noting that organoid technology, as a cutting-edge biomedical tool, can reproduce the heterogeneity and functional characteristics of ovarian cells by differentiating iPSCs into germ cells and ovarian somatic cells, and combining them with ECM-based ovarian organoid construction[178]. These ovarian organoids are key preclinical models for screening drugs that regulate follicular development or improve ovulatory function. Studies have demonstrated that patient derived organoids can determine effective therapeutic options for ovarian cancer patients, providing information for clinical decision making[179]. Similarly, organoid technology holds future potentials to predict the therapeutic efficacy of future biomedicines, and provides a robust platform for investigating the pathological mechanisms of OD[180].

Personalized treatment strategies are another critical area of future research. Tailored intelligent delivery solutions can be designed based on individual patient characteristics, such as genetic background and ovarian functional status. By detecting specific biomarkers, the unique needs of each patient can be identified, allowing for the selection of appropriate biomedicines and delivery strategies. The latest advances in biomedicines

indicate that the genome editing and bioengineering have synergistic potentials in personalized OD treatment. On the one hand, genome editing, especially clustered regularly interspaced short palindromic repeats and associated Cas protein (CRISPR/Cas), offers promising therapeutic methods for genetic diseases, notably monogenic diseases. However, the mechanisms behind OD are complex, involving multiple genetic and environmental factors, researches using CRISPR/Cas mainly focus on ovarian cancer[181]. Nevertheless, emerging studies suggest that activating the self-repair ability of endogenous ovarian germline stem cells (OGSCs) is a potential therapeutic strategy[182]. Integrating CRISPR/Cas technology to enhance OGSC activation may improve the efficacy of personalized ovarian function recovery. Specially, knocking out the Paxillin gene in granulosa cells using CRISPR/Cas improved fertility in mice, indicating that targeted gene editing may improve ovarian function[183].

On the other hand, the development of precision 3D scaffolds, such as alginate encapsulated with cumulus−oocyte complex, and artificial follicular walls containing granulosa cells and type I collagen, have been shown to markedly improve oocyte nuclear maturation and embryo quality[184]. These bioengineered constructs can support complete follicular development *in vitro*, and produce fertilized oocytes[185], particularly when cultured in microfluidic bioreactors that simulate physiological fluid dynamics to optimize bioenergy metabolism[186]. Significantly, by incorporating cytokines including IL-10 and VEGF into the 3D printing matrices to establish the dynamic follicular fluid microenvironment[187], this approach could provide a customized therapeutic platform to address both the structural and molecular deficiencies of PCOS or POF. The convergence of CRISPR-based gene regulation and patient tailored 3D bioprinted follicular architectures represents a potential transformative strategy for restoring ovarian function.

5. Conclusions

OD is the main cause of female infertility, and modern biomedicines provide effective treatment directions by reprogramming the ovarian microenvironment, promoting ovarian tissue repair, and regulating metabolism and immunity. However, due to the complex and diverse causes, varying diseases progression, and high heterogeneity of pathological features of OD, there is an urgent need for precise, controllable, and safe treatment strategies. Clinical translation now needs to be promoted in a dual way: to accelerate the phase I trials to evaluate the safety and efficacy of biomedicines therapy, and to establish the standardized protocols for biomarker identification to develop accurate targeted delivery systems. Future research should focus on interdisciplinary collaboration, especially by the combination of synthetic biology and artificial intelligence, to achieve intelligent driven, precise personalized, and biomimetic targeted strategies in OD therapy, promoting the development of reproductive regenerative medicine.

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Conflicts of interest

The authors declare no conflict of interest.

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