The therapeutic promise of mesenchymal stem cells and their exosomes in treating polycystic ovary syndrome

Giovanni Casoli [1], Arturo Bevilacqua [2]

[1] University of L'Aquila- L'Aquila- Italy, Dept. of Life- Health and Environmental Science, L'Aquila, Italy; [2] Sapienza University of Rome- Italy, Dept. of Dynamic, Clinical Psychology and Health Studies, Rome, Italy; Systems Biology Group Lab; The Experts Group on Inositols in Basic and Clinical Research (EGOI), Research Center in Neurobiology Daniel Bovet (CRiN), Rome, Italy.

ABSTRACT

Polycystic ovary syndrome (PCOS) is a common disorder of the female reproductive system. It is significantly associated with decreased fertility, and characterized by metabolic abnormalities such as hyperinsulinemia, insulin resistance, obesity, and hyperandrogenism. It is also associated with increased risk of type 2 diabetes, hypertension, and cardiovascular disease. Current therapies have limitations and are often ineffective, making it essential to explore new therapeutic approaches. Exosomes are extracellular membrane vesicles, released by cells, which play crucial roles in cell-to-cell communication. They transport various types of cargo, including lipids, proteins, mRNAs, miRNAs, and other noncoding RNAs. Since the cytokine effects of stem cells and their derived exosomes can offer protection against metabolic diseases, in this review we evaluate their potential use in the treatment of PCOS.

KEYWORDS

PCOS, exosomes, infertility, mesenchymal stem cells.

Introduction

Polycystic ovary syndrome (PCOS) is a complex condition impacting 8–13% of women of reproductive age and often leading to infertility [1]. Diagnostic criteria for PCOS include oligo-ovulation or anovulation, hyperandrogenism, and polycystic ovarian morphology (PCOM) as observed via ultrasound. The Rotterdam criteria require two of these features to be present for a PCOS diagnosis, and therefore create four phenotypes: A, all features present; B, presence of hyperandrogenism and oligo-anovulation; C, presence of hyperandrogenism and PCOM; and D, presence of oligo-anovulation and PCOM [2,3]. This syndrome is also often associated with metabolic disorders, insulin resistance, diabetes, and cardiovascular disease [4]. Despite being the leading cause of anovulatory infertility in women, the exact pathogenesis of PCOS remains unclear.

Current treatment modalities primarily focus on symptom management, and standard treatment typically includes oral contraceptives to manage menstrual irregularities and hyperandrogenism [5]; this underscores the need for novel therapeutic approaches that address the underlying pathophysiological mechanisms. Recent studies have explored the potential of mesenchymal stem cells (MSCs) and their exosomes as promising therapeutic agents for PCOS [6-8], due to their self-renewal and differentiation capabilities [9]. We here review the potential use of MSCs in the treatment of PCOS, without focusing on any particular diagnostic phenotype.

MSCs can be harvested from various tissues, including bone marrow, umbilical cord, menstrual blood, endometrial tissue, and adipose tissue [10]. Numerous preclinical and clinical trials have demonstrated the efficacy of MSCs in treating a variety of conditions, including cardiovascular disorders, diabetes, neurological disease, renal fibrosis, and female reproductive disorders [11,12]. However, stem cell therapy can present challenges, such as transplant rejection, issues with transportation and storage, commercialization difficulties, and, in the absence of adequate monitoring, safety concerns [13].

Extracellular vesicles (EVs) are lipid bilayer particles released by cells into their microenvironment; they act as messengers by transporting cargo such as proteins, microRNAs, lipids, and cytokines. EVs are involved in several biological processes, such as immune modulation, tissue repair, and tumor progression. Their potential applications in diagnostics and therapeutics are significant since, while exhibiting low immunogenicity, they can serve as disease biomarkers, deliver drugs, and facilitate regenerative strategies [14].

MSC-derived EVs offer numerous advantages over their parent MSCs, including improved biological stability, easier delivery to target tissues with enhanced therapeutic effectiveness, and lower likelihood of eliciting immune responses.

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Contact
Giovanni Casoli; casoli.giovanni@guest.univaq.it
Department of Life - Health and Environmental Science
University of L'Aquila, L'Aquila, Italy

Additionally, EVs do not require the extensive culture conditions necessary for live cells, making them simpler to produce and manage in clinical settings [15]. In recent years, many studies have focused on the therapeutic efficacy of exosomes, the smallest extracellular vesicle subtype [16]. This review compiles findings from significant studies, highlighting the various advantages of MSC-based therapies in managing PCOS.

Positive effects of mesenchymal stem cells in *in vitro* models of PCOS

Recent studies have shown that PCOS patients experience chronic low-grade inflammation characterized by elevated levels of pro-inflammatory factors, which suggests that these factors may be crucial in the pathogenesis of PCOS [17].

Granulosa cells (GCs) are crucial components of the follicular fluid microenvironment. They surround the developing oocyte, supplying it with nutrients and growth regulators, and secreting paracrine signals that regulate germ cells [18]. As critical sensors of follicle and oocyte health, the state of GCs can significantly impact oocyte quality. It has been shown that elevated levels of inflammatory cytokines in GCs can impair oocyte development, resulting in poor oocyte quality [19], and that inhibiting pro-inflammatory factors in the GCs of PCOS patients can improve oocyte development and quality, and pregnancy outcomes [20]. However, while there has recently been some investigation of the role of various cytokines, including IL-8, IL-12, and IL-18, in oocyte maturation, fertilization, and embryo development, further research is required in order to understand the specific functions of these molecules [21].

Zhao *et al*. investigated the therapeutic potential of human umbilical cord mesenchymal stem cell-derived exosomes (hUC-MSC-Exos) in inhibiting the inflammatory response in GCs through the nuclear factor κB (NF-κB signaling pathway [20]. NF-κB is a critical protein complex that regulates various biological processes, including immune responses and inflammation. It becomes activated in response to stimuli, leading to the transcription of pro-inflammatory genes. Exosomes can carry specific microRNAs and proteins that inhibit the NF-κB signaling pathway in recipient cells. This inhibition helps promote an anti-inflammatory environment, supporting tissue repair and homeostasis [22].

Human umbilical cord mesenchymal stem cells (hUC-MSCs) are already good candidates for the treatment of different diseases, such as pulmonary disease [23]. However, research focusing on the effects of MSCs in PCOS is limited. A study by Xie *et al*. demonstrated that treatment with hUC-MSCs could alleviate ovarian dysfunction by reducing both local and systemic inflammatory responses in PCOS [24]. Despite these findings, the mechanism by which MSCs affect ovarian inflammation in PCOS patients remains largely unknown, current research being primarily restricted to mouse models.

Since recent studies have highlighted the significant role played by exosomes in communication between MSCs and the ovarian microenvironment [25], Zhao *et al*. focused on GCs from the follicular fluid of PCOS patients and on the KGN-cell line, a human immortalized GC line, in order to further elucidate the impact of hUC-MSC-Exos on the inflammation of these cells and analyze the underlying molecular mechanisms [20]. In this study, it was demonstrated that hUC-MSC-Exos have anti-inflammatory effects on GCs, significantly reducing the mRNA and protein levels of pro-inflammatory cytokines, including IL-1β, IL-6 and tumor necrosis factor (TNF-α). Furthermore, hUC-MSC-Exos inhibited the phosphorylation and nuclear translocation of NF-κB p65, suggesting that suppression of the NF-κB signaling pathway mediates these anti-inflammatory effects. Additionally, treatment with hUC-MSC-Exos improved cell viability and proliferation in GCs, as shown by CCK-8 and EdU incorporation assays. There was a notable increase in cell proliferation following exosome treatment. The study also found a significant reduction in apoptosis in treated GCs, as evidenced by flow cytometry and TUNEL staining, indicating that exosomes promote cell survival.

The therapeutic effects of hUC-MSC-Exos were also studied on a human immortalized ovarian theca-like cell line (H295R), which was used to analyze initial steroidogenic pathways [26]. Park *et al*. measured the levels of genes that regulate androgen synthesis, such as Cyp17a1, Cyp11a1, and DENND1a, which are overexpressed in PCOS theca cells [27]. The treatment of H295R cells with MSC-derived conditioned media or purified MSC-derived exosomes produced a significant decrease in the levels of all the genes mentioned. Interestingly, the effect of purified exosomes was comparable to that of whole conditioned media, suggesting that exosomes are the primary regulators of androgen production in this *in vitro* model. Overall, these results demonstrated that MSC-derived exosomes were as effective as the entire secretome in decreasing androgen synthesis pathways.

H295R cells have also been used, together with primary human theca cells, to establish the potential therapeutic role of the human bone-marrow mesenchymal stem cell (hBM-MSC) secretome in the treatment of PCOS [28]. Similar to the effects obtained by Chugh *et al*., the hBM-MSC secretome decreased steroidogenesis-related gene expression and androgen production in H295R cells. Chronic inflammation significantly impacts the ovarian microenvironment in patients with PCOS, leading to increased ovarian androgen production [29]. This process involves two key pro-inflammatory cytokines: interleukin-1 beta (IL-1β) and TNF-α [30]. Treatment of H295R cells with hBM-MSC secretome significantly downregulated gene expression of IL-1β and TNF-α, indicating a decreased inflammatory response after treatment.

In view of the observed anti-inflammatory effects of the hBM-MSC secretome, the impact of the anti-inflammatory cytokine IL-10, known to be released by MSCs, was investigated. IL-10 has immune-suppressive and anti-inflammatory properties in various disorders, including PCOS [31]. Chugh *et al*. examined the effect of IL-10, secreted by hBM-MSCs, on steroidogenesis-related gene expression, androgen production, and inflammatory state in H295R cells: IL-10 decreased steroidogenesis-related gene expression and androgen production (testosterone, CYP17A1, CYP11A1, DENND1A) and exerted an anti-inflammatory effect [28]. These data suggest that IL-10 is a key mediator of the effect of the hBM-MSC secretome on *in vitro* human cell PCOS models.

Mesenchymal stem cells improve the PCOS phenotype in mouse models

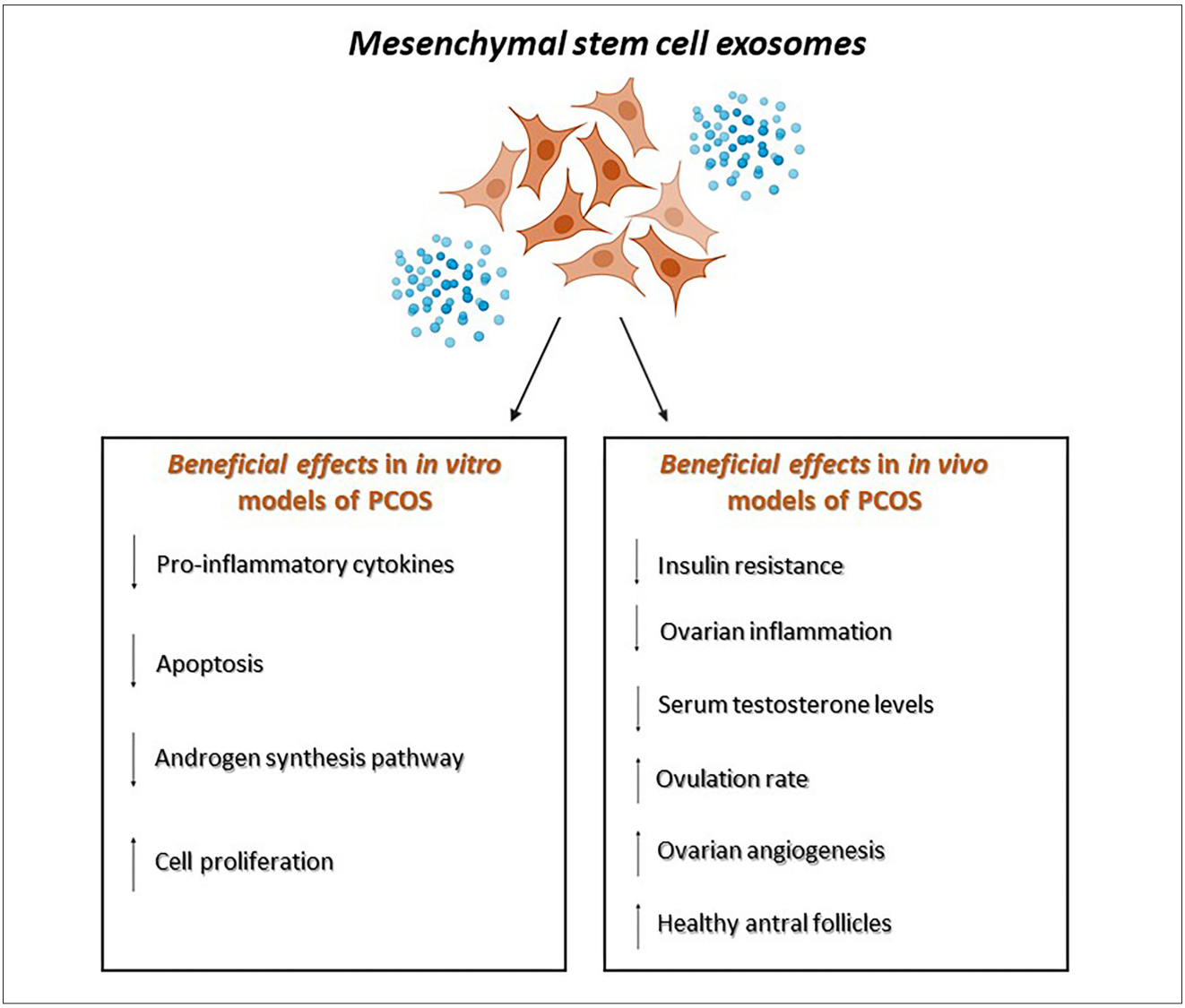
PCOS mouse models are extensively used in research to study the pathophysiology of and potential treatments for PCOS. These models are valuable because they replicate many of the symptoms and underlying mechanisms of human PCOS, enabling researchers to explore disease progression and therapeutic interventions in a controlled environment [32].

The efficacy of letrozole (LTZ), a competitive aromatase inhibitor, and dehydroepiandrosterone (DHEA), a testosterone precursor, is well-recognized in inducing hyperandrogenism, a key feature of PCOS, in mouse models. These models are particularly effective for studying PCOS pathophysiology and potential treatments because, in addition to inducing hyperandrogenism, they also replicate other typical characteristics of PCOS, such as ovarian dysfunction and metabolic changes [32,33].

Chugh *et al*. observed the anti-inflammatory effects of the hBM-MSC secretome in an LTZ-induced PCOS mouse model. MSC therapy significantly restored estrous cycles, increased ovulation rates, and improved fertility in PCOS mice, leading to higher pregnancy rates compared with untreated PCOS mice. Histological examination showed a notable decrease in the number and size of ovarian cysts in MSC-treated PCOS mice [28,33]. The treatment also increased the number of antral follicles, indicating improved ovarian function. Additionally, hBM-MSC secretome therapy led to significant improvements in metabolic dysfunction commonly associated with PCOS [34]. Treated mice showed reduced insulin resistance and improved glucose tolerance. Furthermore, serum testosterone levels were significantly lower in MSC-treated PCOS mice, indicating a reduction in hyperandrogenism [35].

The potential therapeutic effects of the hBM-MSC secretome have also been studied in a DHEA-induced PCOS mouse model[8]. BM-MSC-Exos treatment enhanced angiogenesis in ovarian tissues, as shown by increased expression of angiogenic markers, such as vascular endothelial growth factor and CD31, a key marker for vascular dynamics in PCOS [36,37]. BM-MSC-Exos treatment also inhibited apoptosis in ovarian tissues, with a significant reduction in the expression of pro-ap-

Figure 1 Summary of the beneficial effects of human mesenchymal stem cell exosomes in *in vitro* and in *in vivo* models of PCOS.



optotic markers, such as Bax and caspase-3, and an increase in the anti-apoptotic marker Bcl-2. Moreover, the treatment resulted in a marked decrease in ovarian inflammation. Levels of pro-inflammatory cytokines, including TNF-α and IL-6, were significantly reduced in BM-MSC-Exos-treated mice.

Advances in MSC-exosome therapy for PCOS: future directions and conclusions

The reviewed studies collectively highlight a promising role for MSCs and their exosomes in addressing both the reproductive and the metabolic aspects of PCOS. Their ability to modulate inflammation, improve metabolic functions, and restore ovarian health offers a comprehensive approach to treating this multifaceted syndrome.

Future treatments might include systemic administration (e.g., intravenous injection) or direct intraovarian injection, depending on the severity of the condition and the patient's needs. Since MSC exosomes are derived from cells and do not contain cellular material, they are, as already pointed out, generally considered low in immunogenicity, which may make them a safer and more tolerable option for long-term management of PCOS.

MSC exosomes could potentially also serve as diagnostic markers, given their unique cargo that reflects the parent cells' origin and health status, and they could also be explored as a "theranostic" approach, meaning that they could be used both for therapy and for monitoring the progression of PCOS and the response to treatment.

The clinical use of MSC exosomes requires further study to clarify optimal dosage, delivery methods, and long-term safety. Improving their stability and compatibility for medical applications, as well as bioengineering for better targeting, is essential. While they hold promise as diagnostic biomarkers, more validation is needed. Although preclinical results are promising, additional clinical trials are crucial to fully assess the efficacy and safety of MSC exosomes for PCOS treatment, with further investigations seeking to enhance their therapeutic properties.

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