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Lucia De Santis

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Research Innovation Views Embryology Reproduction

Editor in Chief

Lucia De Santis

Department of Obstetrics/Gynaecology, IVF Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy

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MediMay Communication Srl

Via Giovanni Antonelli, 47

00139 - Rome - Italy

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Managing Editor

Ferdinando Maggio

maggio@medimay.it

Managing Office

Anna Kamola

kamolka78@gmail.com

editorial-office@river-journal.com

Language Editor

Catherine Wrenn

Graphic Design & Layout

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EDITORIAL

Dear Colleagues and Friends,



Through basic research, it is possible to obtain information with important clinical and therapeutic implications.

The application of assisted reproduction techniques has clearly benefited the treatment of infertility, but many aspects that regulate the biology of reproduction remain totally unknown or still little investigated.

The most important characteristic of research in modern medicine is precisely its translational nature and its ability to discover molecular mechanisms and scientific approaches that can be used in fields other than those for which they were initially studied. A striking example is the birth, in virology, of m-RNA vaccines, whose use has paved the way for applications in the field of oncology.

In the first of the five concise reviews published in this issue of RIVER, *The therapeutic promise of mesenchymal stem cells and their exosomes in treating polycystic ovary syndrome*, Casoli and Bevilacqua explore the therapeutic promise, in polycystic ovary syndrome, of treatments with exosomes and mesenchymal stem cells.

Knowledge of the oviduct and its various districts is the basis of all studies investigating aspects of reproduction ranging from the meeting of gametes to the early stages of preimplantation embryo development, on whose foundations culture media have been formulated to obtain these steps *in vitro*. Belda-Perez and Tatone, *The oviduct: a key to unlocking reproductive science*, take us on a journey that offers possible keys for opening up the world of reproductive sciences.

Reproductive health is also known to be linked to the wellbeing of

the individual and the environment in which they live. Nevertheless, much emerging evidence suggests that even though the human species today enjoys greater quality of life, broadly understood in terms of general well-being (and greater longevity) than in the past, some aspects of progress expose the organism to substances whose effects are anything but physiological. I therefore invite you to read the review by Bianchi and Di Emidio, *Implication of nano and microplastics in reproduction: understanding oocyte vulnerability, who explore the topic of vulnerability of the female gamete linked to exposure to nano- and microplastics*.

Two interesting reviews, by Anastasi *et al.*, *Male fertility preservation in cancer patients*. Reasoned opinion, and Capodanno *et al.*, *Female fertility preservation: why it does not always mean preservation of fertility*, then tell readers about the approaches currently available for fertility preservation in both males and females, also highlighting aspects that, although going beyond the primary indication of preservation, must be considered when addressing the topic of fertility as a whole.

The issue closes with two short but significant case reports. In the first case report, *Lymphocytic hypophysitis associated with ovarian hyperstimulation syndrome during assisted fertilization*, Barbato *et al.* describe the onset of a serious problem and its relative management and resolution during an episode of ovarian hyperstimulation syndrome. In the second case report, *Successful in vitro fertilization pregnancy (IVF) after conservative management of endometrial cancer*, Sorce and Castello describe a case of successful IVF treatment following conservative surgery for endometrial cancer.

All the articles included are stimulating and topical, and I hope you enjoy reading the issue.

Lucia De Santis
Editor in chief, RIVER Journal

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

Giovanni Casoli¹, Arturo Bevilacqua²

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

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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

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.

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 κ B (NF- κ B) signaling pathway ^[20]. NF- κ B 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- κ B 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-1 β , IL-6 and tumor necrosis factor (TNF- α). Furthermore, hUC-MSC-Exos inhibited the phosphorylation and nuclear translocation of NF- κ B p65, suggesting that suppression of the NF- κ B 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-1 β) and TNF- α ^[30]. Treatment of H295R cells with hBM-MSC secretome significantly downregulated gene expression of IL-1 β 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].

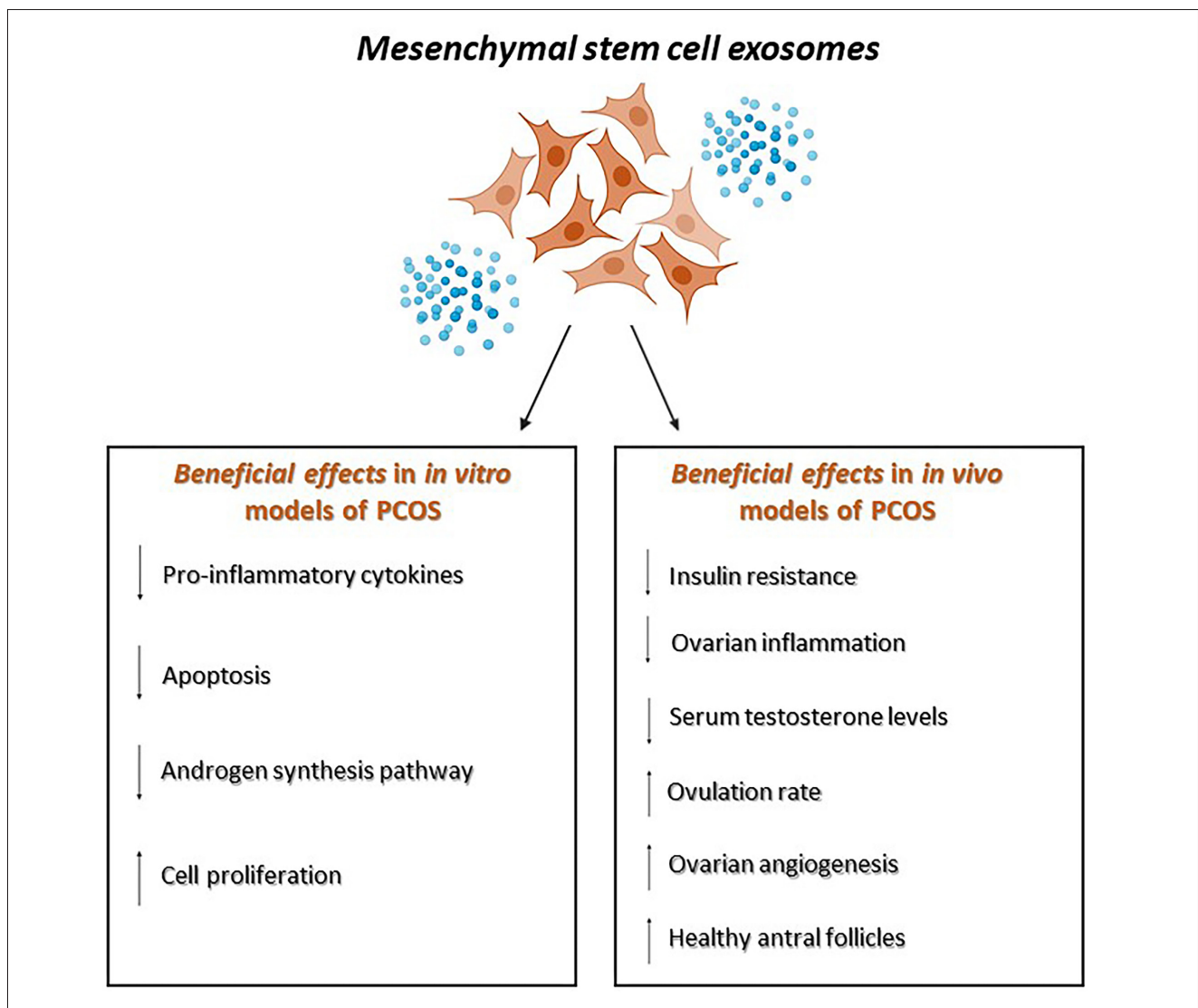
The efficacy of letrozole (LTZ), a competitive aromatase inhibitor, and dehydroepiandrosterone (DHEA), a testosterone precursor, is well-recognized in inducing hyperandrogenism, a key feature of PCOS, in mouse models. These models are particularly effective for studying PCOS pathophysiology and potential 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 model⁸. 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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The oviduct: a key to unlocking reproductive science

Ramses Belda-Perez, Carla Tatone

Department of Life, Health and Environmental Sciences, University of L'Aquila, L'Aquila, Italy

ABSTRACT

Despite significant advances, assisted reproductive techniques (ARTs) remain suboptimal due to challenges linked to embryo development and quality, often attributed to the epigenetic reprogramming process and environmental factors. The aim of this review is to summarize the function of the oviduct, the organ where fertilization takes place. This organ plays a crucial but often overlooked role in natural fertilization, where it selectively allows only a few sperm to reach the egg, contrary to what happens in *in vitro* conditions, where oocytes are exposed many sperm, without this always resulting in fertilization. The oviduct environment is vital for fertilization, since it creates a reservoir for sperm, extending their viability and readiness for fertilization. In addition, the environment provided by this organ is optimal for the early stages of embryo development. Interactions between embryos and oviduct cells affect gene expression, improving embryo quality. Mimicking the conditions of the oviduct, by using oviductal fluid in culture media, for example, or developing advanced 3D cultures and microfluidic systems, can improve ART outcomes and embryo quality. In conclusion, the oviduct is essential for creating the optimal environment for fertilization and embryo development. Replicating its conditions can enhance ART success and the health of the offspring, highlighting its importance in reproductive processes.

KEYWORDS

Oviduct, ART, reproduction.

Introduction

As is well known, the problem of infertility has grown in recent years, leading to an increase in the use of assisted reproductive technologies (ARTs), resulting in more than two million children in Europe being born through use of these techniques ^[1].

Since their creation, ARTs have undergone continuous improvements and refinements. However, despite the advances achieved, ARTs are still suboptimal. Not all oocytes successfully develop into embryos, and the ones that do reach the blastocyst stage may not exhibit the same quality as their *in vivo* counterparts, showing reduced developmental capacity in bovine ^[2] and swine ^[3] species, for example. This suboptimal quality can primarily be attributed to the epigenetic reprogramming process that occurs during the early stages of mammalian embryo development when the embryo consists of just a few cells ^[4], making it susceptible to environmental factors. For instance, maternal malnutrition has been observed to affect imprints on the offspring's epigenome ^[5]. Furthermore, this susceptibility to the environment makes embryos exceptionally vulnerable to suboptimal *in vitro* conditions, which notably differ from the natural conditions of the female reproductive tract. These differences in environmental factors, such as oxygen levels or temperature, can influence the developmental trajectory of the embryo ^[6].

The purpose of this review is to compile and highlight the functions of the fallopian tube or oviduct, the organ where life begins and fertilization takes place. Often overlooked in dis-

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Contact

Ramses Belda-Perez; ramses.beldaperez@univaq.it
Department of Life, Health and Environmental Sciences,
University of L'Aquila, L'Aquila, Italy

cussions about reproductive health, the fallopian tube plays a crucial role in the early stages of conception.

The forgotten organ

While millions of ejaculated sperm can be counted in the vagina or cervix after mating, depending on the species, only a few hundred manage to reach the oviduct, the organ where fertilization takes place ^[7]. However, even though the vast majority of sperm are lost during their journey, the few that reach the oviduct are capable of *in vivo* fertilization. In *in vitro* conditions, on the other hand, we have a different situation, and even though the oocytes are exposed to thousands/millions of spermatozoa, fertilization is not always successful. These discrepancies between natural and laboratory environments can be explained by the strong selection that spermatozoa undergo in the female genital tract. For instance, in some species, cervical mucus is hypothesized to serve as a selective barrier for sperm, as it is highly effective at impeding defective spermatozoa that

are unable to swim normally or possess a poor hydrodynamic profile due to morphological anomalies^[8], eliminating around 70–85% of the sperm during this step^[9]. After overcoming this barrier, spermatozoa enter the uterus, where another portion of the sperm population appears to be attacked by neutrophilic granulocytes, which are present in significant numbers following their post-insemination migration into the uterus^[10]. Subsequently, the spermatozoa reach the utero-tubal junction, the part of the oviduct that connects with the uterus. While in humans this part does not play a significant role in sperm selection due to the strong anatomical continuity between the oviduct and the uterus, in certain species such as ruminants, pigs, rats, hamsters and mice, it does indeed play a role in sperm selection, being considered the second main selective barrier after the cervix. In those species, the utero tubal-junction only allows live^[11], unreacted^[12] and motile^[13] spermatozoa to pass. Although the mechanisms of sperm selection at this point are still unknown, a single molecule-dependent interaction has been suggested, since the sperm of ADAM3 knockout mice are unable to get beyond this point^[14].

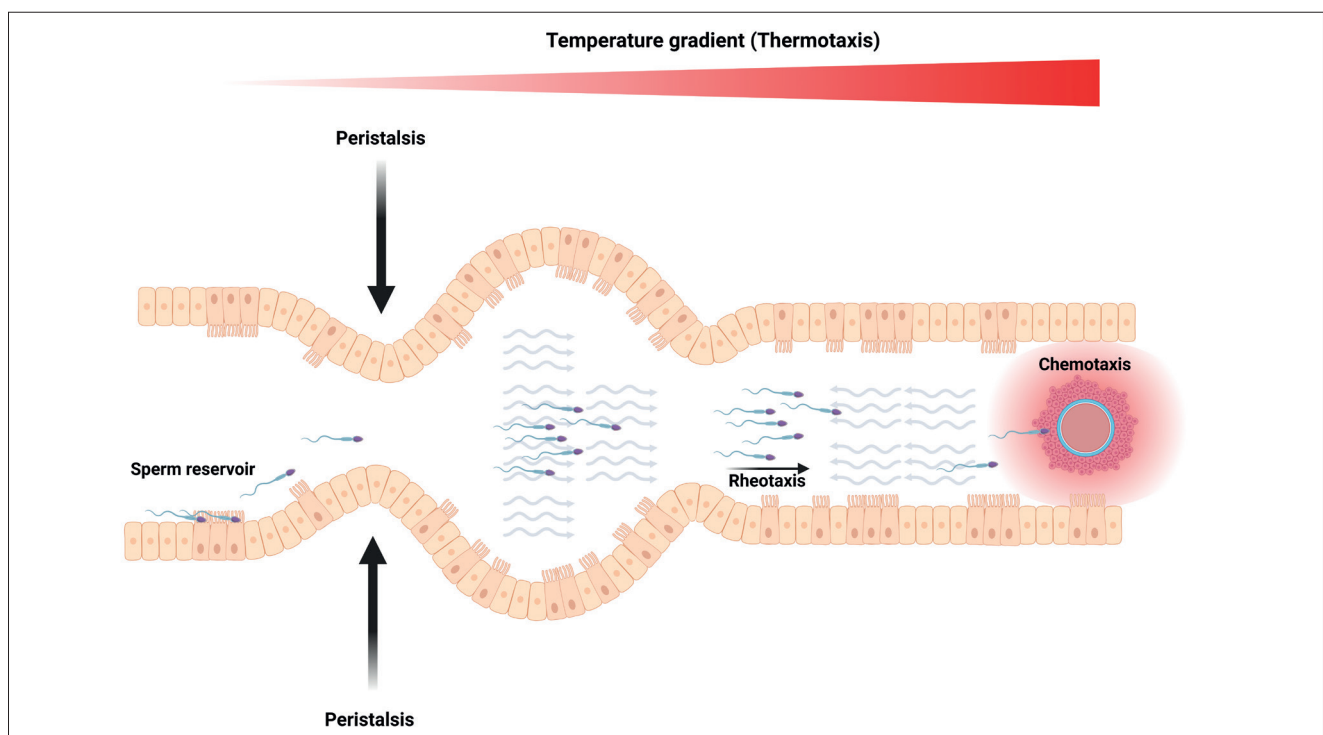
Finally, after this nightmarish journey, the few fortunate sperm that have overcome all the barriers enter the oviduct, where the environment is much more favorable. Here, where there seem to be no leukocytes waiting to attack them^[15], they instead bind to the carbohydrates of the ciliated cells in the oviduct epithelium through lectin-like protein present in the head of the sperm (reviewed by Coy *et al.*^[16]), forming the sperm reservoir. These ciliated cells “take care” of spermatozoa, delaying the process of sperm capacitation^[17], and extending their viability from hours to months, depending on the species^[18]. This process ensures that sperm are ready at the time of ovulation, when they are released, and acquire their fertilization capacity^[19].

However, once released, they still need to navigate through a long and intricate passage, overcoming all the folds of the oviduct until they reach the oocyte. For this reason, it has been proposed that the meeting of the spermatozoa and the oocyte is not a coincidence^[20]. *In vitro* studies have shown that spermatozoa can respond to temperature gradients. This phenomenon is not limited to a single species; it has been documented in rabbits^[21] and humans^[22], and is recognized as a common trait among mammalian sperm^[23]. In conjunction with the finding in certain animals, such as rabbits^[24] and pigs^[25], that after ovulation, the temperature in the place of fertilization is higher than in the sperm reservoir, researchers propose that thermotaxis could be one of the mechanisms guiding sperm to the site of fertilization.

On the other hand, it has been shown that spermatozoa are able to align against the fluid flow, a phenomenon called rheotaxis. Again, this effect has been observed in several species, including humans^[26,27]. In view of this ability of the sperm, together with the fact that there is a current flow that goes from the ovaries to the uterus^[28], rheotaxis has been proposed as another mechanism serving to guide the spermatozoa on its journey to the oocyte. Lastly, sperm are capable of moving in response to chemical gradients of certain substances. One of the first chemoattractants reported was follicular fluid in humans^[29], and since then the phenomenon has been observed in species such as mice^[30] or cattle^[31]. However, follicular fluid is not the only fluid that could have chemoattractants. The cumulus cells accompanying the oocyte produce progesterone^[32], a steroid capable of inducing chemotaxis in humans and rabbits, among others^[33].

Although these proposed mechanisms (summarized in Figure 1) have been heavily questioned, as peristaltic movements

Figure 1 Representation of the mechanisms of sperm guidance in the oviduct.



may play a more significant role in the *in vivo* oviduct ^[34], they have proven to be useful *in vitro*, improving sperm selection and *in vitro* production (IVP) outcome ^[35].

After fertilization, the first division of the embryo takes place. It has been observed that the presence of the embryo can induce distinct gene expression patterns in the oviductal epithelial cells (OECs) ^[36]. This diverse gene expression could play essential roles, such as facilitating embryo transport (e.g., ciliary motility) or creating an optimal environment for early embryo development. In a bidirectional way, it has been observed that co-culture of embryos with OECs promotes a different gene expression profile of genes involved in embryo quality ^[37]. In fact, when the embryos are produced in *ex vivo* oviducts, even in a heterologous manner, they show higher quality, ^[38]. The aforementioned effects have been observed in both *in vitro* and *in vivo* experiments.

Traditionally, the oviduct has been regarded simply as a tube, mainly because pregnancy can be achieved without exposing gametes or embryos to the oviduct environment. However, as we have discussed, its functions should not be underestimated; we are dealing with a highly complex system that is even capable of reacting differently depending on the sperm's sex, since the presence of X- or Y-sperm populations can change the oviductal transcriptome ^[39].

Mimicking the physiological environment

Numerous research groups are currently trying to improve the ARTs, mainly by mimicking the physiological environment of the oviduct. One of the most common strategies is the use of oviductal fluid (OF) as a supplement in the culture media, which has been used in different species, improving the yield of the IVP system ^[40]. Canovas *et al.*, in the swine model, showed that the quality and methylation pattern of embryos produced *in vitro* with OF as an additive in the culture media were closer to their *in vivo* counterparts than to embryos produced *in vitro* without OF ^[41]. Even after birth, the growth of those piglets was more similar to that of piglets born through artificial insemination than that of piglets produced without OF ^[42].

Due to ethical concerns surrounding studies of this type in humans, determining the impact of these techniques remains challenging. However, the fact that the embryo culture medium can influence perinatal outcome ^[43] and children's growth ^[44] suggests that the potential consequences of a suboptimal non-physiological environment should not be underestimated. In fact, the use of OF has been already proposed, and other reproductive fluids, such as uterine fluid, have already been used as supplements in culture media, leading to the birth of healthy offspring ^[45].

It is important to highlight that the abovementioned *in vitro* studies focusing on the phenomena that occur in the oviduct have predominantly relied on monolayer cultures. Those standard 2D growth techniques have played an important role in advancing our understanding of how OECs respond to different stimuli. Nevertheless, 2D cell cultures may not always represent the optimal approach for conducting studies, since they fail to closely mimic the physiological conditions. As Watson *et al.*

pointed out, *in vitro* studies of this kind give us results describing the 2D life, where the cells “talk only to cells of like mind, live in the dark and in their own excrement, and don't bury their dead”. A solution to this problem could be to adopt a 3D approach, providing us with a more realistic perspective on how these responses might translate to an *in vivo* environment ^[46].

In recent years, several studies have been published in which the 3D culture approach was used to study different areas of the female reproductive tract ^[47]. Meanwhile, there is a growing interest in the development of microfluidics, a field that focuses on the control and manipulation of fluids at microscopic level to recreate the dynamic *in vivo* flow. Applying that technology to 3D cultures, it has even been possible to recreate the menstrual cycle *in vitro* ^[48]. By combining both 3D cultures and microfluidics technology, it has proved possible to create “oviducts on a chip”, which represent a significant advance in improving IVP ^[49]. These innovative scaffold devices accurately replicate the physiological conditions of the oviduct, providing a more realistic environment for sperm-egg interaction. This technology has been shown to enhance bovine epigenetic reprogramming, making it more similar to the profiles of *in vivo*-derived embryos ^[49]. In humans, a similar device has been created and, although used with endometrial cells, was found to improve the blastocyst rate by mimicking the physiological environment ^[50].

Conclusion

In conclusion, the oviduct plays crucial roles beyond merely sperm selection and capacitation; it is not simply a tube for gamete encounter or embryo transport. Instead, this organ is responsible for creating the optimal environment for both gametes and the embryo, and its functions should not be underestimated. Mimicking the oviduct environment has proven to be a valuable strategy for enhancing ART yields and a potential tool for ensuring the health of the offspring.

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Implication of nano and microplastics in reproduction: understanding oocyte vulnerability

Andrea Bianchi, Giovanna Di Emidio

Department of Life, Health and Environmental Sciences, University of L'Aquila, L'Aquila, Italy

ABSTRACT

The widespread environmental dispersion of nano- and microplastics (NMPs) has sparked serious concerns about their potential impact on human and animal health. NMPs are the result of plastics breaking down due to various chemical, physical, and biological processes. Numerous studies have identified and confirmed the harmful effects of NMPs on the female reproductive system. Specifically, NMPs trigger apoptosis and pyroptosis in granulosa cells by activating the NLRP3/caspase pathway and disrupting the Wnt signaling pathway. These processes contribute to uterine fibrosis and reduce the number of ovarian follicles. Additionally, some research suggests that NMPs may impair oocyte maturation, reduce oocyte quality, and compromise overall ovarian function. This review aims to synthesize the currently limited knowledge on the effects of NMPs on granulosa cells and oocytes.

KEYWORDS

Nano-microplastics (NMPs), oocyte, granulosa cells, oxidative stress, inflammation, apoptosis.

Introduction

The widespread release of nano- and microplastics (NMPs) into the environment is raising serious concerns about their potential effects on human and animal health. NMPs are formed when plastics degrade into particles smaller than 5 micrometers and 100 nanometers, respectively micro- and nanoplastics^[1], under various chemical, physical, and biological conditions. The pervasive presence of NMPs raises serious concerns about their potential impacts on human health, as these particles are extremely challenging to remove from the environment^[2,3].

Research has shown that NMPs can cross biological barriers, such as the placenta^[4], and induce toxic effects in various organs and tissues. Numerous studies indicate that NMPs can adversely affect oocytes and granulosa cells (GCs) through mechanisms involving oxidative stress and endocrine disruption, ultimately impairing fertility and development. In females, NMPs have been shown to accumulate in the ovaries, thereby reducing follicle count and ovarian size, with dramatic consequences on fertility in terms of reduced embryo production and pregnancy rates.

Exposure to NMPs tends to have more pronounced reproductive effects in females than in males^[5]. This raises serious concerns for future generations, as transgenerational effects in offspring have also been observed^[6]. Despite these findings, the underlying mechanisms of NMP toxicity in oocytes and GCs, particularly in mammals, remain poorly understood. The present work examines the mechanisms underlying NMP toxicity in the ovarian microenvironment and its long-term implications.

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Contact

Giovanna Di Emidio; giovanna.diemidio@univaq.it
Department of Life, Health and Environmental Sciences,
University of L'Aquila, L'Aquila, Italy – Phone: +39 0862 433576

Search methods

The PubMed database was examined for peer-reviewed original articles. The following keywords were searched: 'Nanoplastics', 'Microplastics', 'Human granulosa cells', 'Oocytes', 'Fertility', 'mammals', and 'oxidative stress'. These keywords were combined with other search phrases relevant to the topic.

The effects of NMPs on oocytes

Research on the impact of NMPs on the mammalian female reproductive system is limited, with the majority of studies focusing primarily on rodent models such as mice and rats. NMPs have been found to accumulate in mouse ovary, uterus, and blood^[7-9].

Exposure to NMPs has been linked to microstructural abnormalities in the mammalian female reproductive system, including dilated oviducts, an increase in corpus luteum and ovarian cysts, a thinner granular layer of secondary follicles, and a decrease in the number of developing follicles^[7,10-12].

Rat ovarian GCs are permeable to bigger NMPs, which

causes an increase in fibrosis markers ^[7] and a marked drop in serum levels of anti-Müllerian hormone and E2 expression. Moreover, follicle growth appeared to be slowed and abnormalities in the estrous cycle have been reported. After exposure to NMPs, mice showed dilated oviducts, ovarian cysts, a higher number of corpora lutea, a thinner granulosa layer in secondary follicles, fewer growing follicles, increased ovarian collagen and fibronectin accumulation, and GC apoptosis ^[5]. NMPs were also found to influence oocyte maturation and function, leading to reduced fertility potential ^[13]. Finally, NMPs deeply affected the abundance of cytoskeletal proteins in rat ovaries, e.g. α -microtubulin, which plays a key role in oocyte-GC synchronization ^[8].

An important implication of NMPs is related to the observation that they can also be assumed indirectly through the diet. Indeed, it has been demonstrated that consumption of seafood containing NMPs ^[14] may negatively impact reproduction, food intake, and the endocrine system ^[15]. In 2019, Cong *et al.* found decreased oocyte production among the many consequences of 120 days of exposure to 10- μ m polystyrene microspheres in *Oryzias melastigma* ^[16]. Studies in *Oryzias latipes* ^[17] and oysters ^[18] have similarly revealed a decline in the quantity and caliber of eggs and oocytes. NMPs have also been shown to induce oxidative stress and damage to DNA. They also reduced the growth of human extravillous trophoblastic cells, triggered apoptosis, and produced cell cycle arrest at the G2/M phase ^[19].

NMP exposure induces alteration in ROS production and DNA damage in GCs and oocytes

Reactive oxygen species (ROS) are highly reactive oxygen-containing molecules, including hydrogen peroxide (H_2O_2), hydroxyl radicals ($\bullet OH$), and superoxide ($O_2\bullet^-$) ^[20]. These molecules are involved in various biological processes, acting both as mediators of cell signaling and as contributors to oxidative damage in cells and tissues. ROS are typically produced as byproducts of cellular metabolism during oxidative phosphorylation.

It has been observed that NMPs accumulate in organs and induce endocrine and reproductive system dysfunctions, typically by inducing apoptosis and oxidative stress ^[21-23]. Numerous investigations in rainbow trout (*Oncorhynchus mykiss*) and zebrafish have demonstrated that NMPs raise oxidative stress in the gonads, with detrimental effects on the reproductive system ^[24-26]. When mouse oocytes were exposed to NMPs, a decrease in calcium and reduced glutathione abundance in the endoplasmic reticulum were reported in association with an increase in ROS ^[9].

Furthermore, it was noted that antioxidant enzymes like catalase (CAT) and superoxide dismutase (SOD) were less active ^[8] after NMP exposure. In mouse ovaries, NMPs induced oxidative stress, an inflammatory response demonstrated by increased levels of IL-6, IL-10, and TNF- α , which leads to decreased egg quality, in turn demonstrated by a reduced number of ovulated oocytes showing the first polar body and a decline in oocyte survival ^[9] (Figure 1).

ROS signaling is important for female reproductive functions, affecting oocyte quality and early embryo development in particular ^[3]. Considering that exposure to NMPs leads to an increase in ROS content at systemic level ^[27], NMP detrimental effects are also related to the imbalance in redox homeostasis in the reproductive system ^[9]. ROS increase can cause changes in the expression levels of various proteins and inflammatory factors, which in turn activate related signaling pathways, ultimately leading to ovarian dysfunction and a decrease in oocyte quality. Indeed, increased ROS production, as well as histological abnormalities, e.g. the presence of micronuclei in the oocyte nucleus and cytoplasmic vacuolization in somatic and germinal cells, has been described in ovaries exposed to NMPs ^[17,28]. Accordingly, GCs isolated from rats exposed to NMPs were characterized by reduced activity of the antioxidant enzymes, that is SOD, CAT, and GPx (glutathione peroxidase), which may be considered a consequence of ROS accumulation ^[29].

Grechi *et al.* observed that bovine oocytes exposed to NMPs during *in vitro* maturation were characterized by a reduced IVM rate and presented damage in the zona pellucida ^[30]. Applying proteome analysis to examine oocytes exposed to NMPs, these authors found an upregulation in the expression of proteins implicated in the oxidative stress response, including TXN, GPX1, ATP5IF1, PADI6, and SNRPD2 ^[30], and the up-regulation of proteins crucial for spindle formation, nuclear maturation, and the arrangement of microtubules and organelles. This evidence suggests that NMP exposure induces oxidative stress and DNA damage during oocyte growth and maturation *in vitro*.

WNT pathway activation caused by NMPs induces fibrosis

In ovaries of mice exposed to NMPs, the establishment of a condition of oxidative stress induces the expression of proteins involved in the Wnt/ β -Catenin signaling pathway and of fibrosis markers ^[7]. The Wnt/ β -Catenin signaling pathway controls the processes of embryonic development, cell division, and apoptosis — all vital processes for maintaining tissue and organ homeostasis. Recent literature has demonstrated that NMPs induce ovarian fibrosis through the Wnt/ β -Catenin signaling pathway, which in turn increases the expression of fibrotic markers, namely, transforming growth factor- β (TGF- β), fibronectin, and α -smooth muscle actin, or α -SMA, in ovarian GCs ^[7]. Activation of the Wnt/ β -Catenin signaling pathway has been also associated with a variety of morphological and functional alterations in the reproductive system, including lower oocyte counts, impaired follicular growth, GC mortality, decreased ovarian reserve function, and ovarian and uterine fibrosis (Figure 1).

Moreover, NMPs cause uterine fibrosis through TLR4 and Notch signaling pathway activation. When these pathways are activated, more uterine fibrotic collagen and proteins are produced, which results in the creation of ROS ^[31]. Furthermore, NMPs increase the expression of HMGB1 and acetyl-HMGB1, which stimulates the TLR4/NOX2 receptors and encourages oxidative stress ^[31,32]. These results are corroborated by Wu *et al.*,

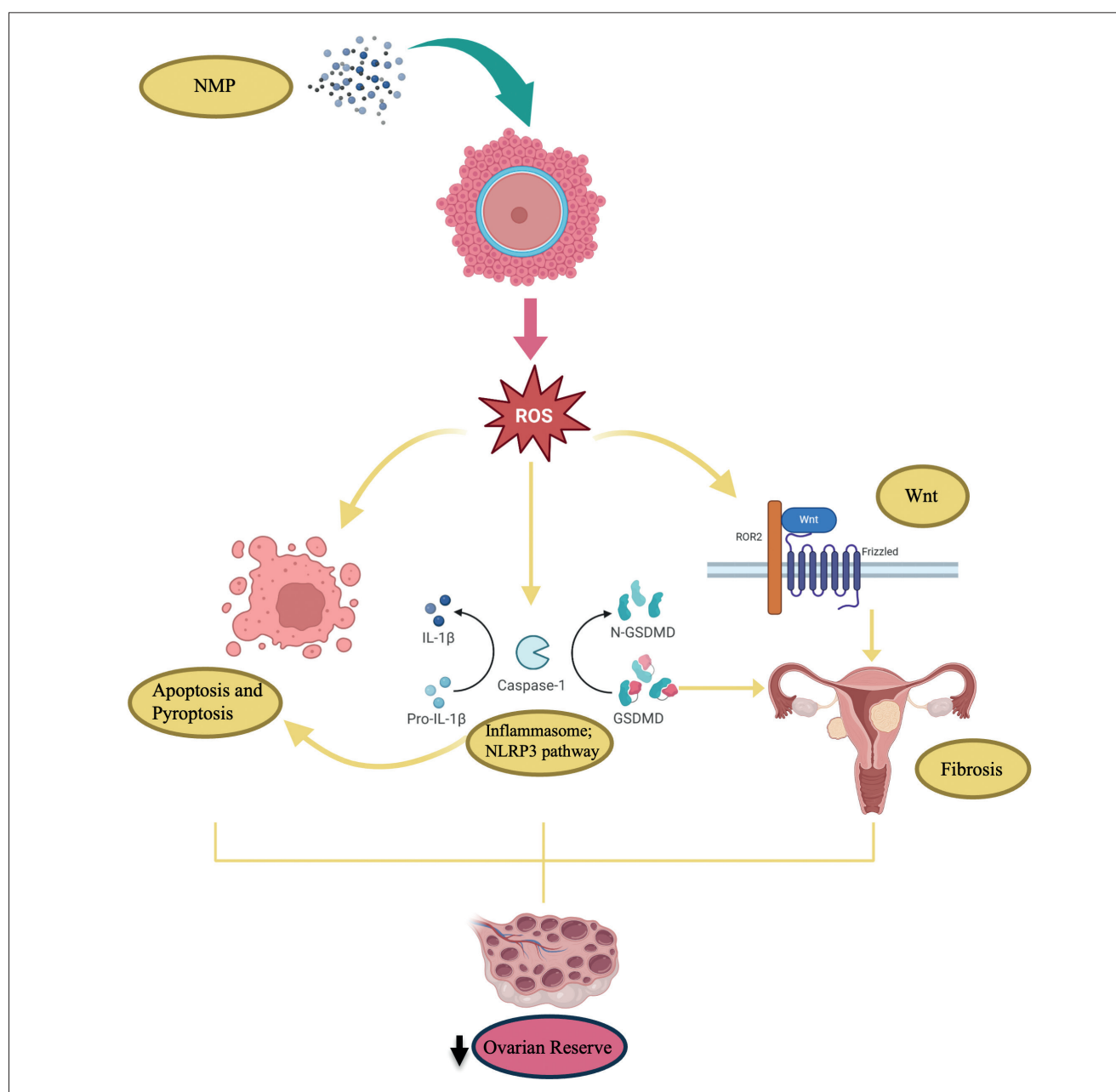
who showed that ROS-induced endometrial thinning and uterine fibrosis in female mice treated with NMPs was mediated by the Notch and TLR4 signaling pathways. Moreover, in these animals, fibrotic tissue development was facilitated by TGF- β [31].

NMP exposure induces apoptosis and pyroptosis in human granulosa cells and oocytes

Ovaries of mice exposed to NMPs are also characterized by increased expression of pro-apoptotic proteins in concomitance with lower levels of anti-apoptotic factors [7]. In this context,

the multiprotein complex NOD-like receptor thermal protein domain-associated protein 3 (NLRP3) is implicated in apoptosis and inflammation and acts as a defense mechanism against endogenous damage, microbial invasion, and toxic stimuli. Recent literature has demonstrated that in GCs from mice exposed to NMPs, a condition of oxidative stress is responsible for activation of not only elements of Wnt/ β -Catenin signaling [7], but also the NLRP3/Caspase-1 [10] pathway [33], and these changes have been described as the possible mechanism underlying the decline of ovarian reserve. Moreover, ovarian dysfunction and GC apoptosis [7,10] seem to be related to a rise in the apoptosis-associated speck-like protein [34], which is part of the NLRP3/caspase-1 signaling pathway and has been found to

Figure 1 NMPs enter the oocytes and cause an increase in ROS. An excessive increase in ROS leads to cell death, activation of the NLRP3 pathway, which activates the inflammasome and induces cell death by pyroptosis, and activation of the Wnt pathway. The Wnt/NLRP3 pathways cause fibrosis in the female reproductive system. All these pathways lead to a decrease in the ovarian reserve.



increase upon oxidative stress and NMP exposure ^[10].

Zhang *et al.* found that injection of NMPs lowered oocyte quality and hindered oocyte maturation by increasing ROS levels and inducing apoptosis ^[13]. Another study revealed that after 28 days of exposure, NMPs induced apoptosis and damage to DNA in the germ cells of *C. elegans*. Furthermore, it was noted ^[13,35] that there was upregulation of HUS-1 expression as well as pro-apoptotic genes such as *cep-1*, *egl-1*, *p53*, *ced-3*, and *ced-4*, which are implicated in germ line apoptosis ^[35,36]. Similarly, human GCs exposed to NMPs displayed an increase in apoptosis. Finally, numerous lines of evidence suggest that NMPs activate the inflammasome, which in turn initiates pyroptosis, as a consequence of increased activation of the NLRP3/Caspase-1 signaling pathway ^[10,37]. Pyroptosis is an alternative program of cell death ^[38], which occurs during inflammatory processes and causes the flattening of the cytoplasm ^[39]. The latter is related to the formation of pores in the plasma membrane that allow the passage of IL-1 β /IL-18 ^[40]. Through ion influx, oxidative stress induces cell lysis and releases IL-18, IL-1 β , and other inflammatory cytokines ^[41,42]. Interleukins that are inert, including IL-1 β and IL-18, become active when the NLRP3 signaling pathway is activated ^[33]. In the final event, water enters through the pores, causing cell swelling, osmotic lysis, and the subsequent release of IL-1 β and IL-18. While activation of caspase 1 is crucial for the host defense systems because it inhibits pathogen growth and host survival, its overstimulation can lead to pathological inflammation ^[41,43]. In human GCs, it has been demonstrated that NMP-induced oxidative stress may activate the NLRP3/Caspase-1 signaling pathway ^[10]. Therefore, it can be speculated that the primary causes of the decline in ovarian reserve are oocyte apoptosis and GC pyroptosis ^[10] (Figure 1).

Conclusion

NMPs are among the most widespread and dangerous contaminants, with severe toxic effects in animals and humans ^[44], due to their ability to accumulate in all organs, including the ovaries. Moreover, NMPs play a crucial role in the toxicity to GCs and oocytes, with dramatic consequences on fertility. In particular, the exposure to NMPs causes an increase in ROS generation, apoptosis and pyroptosis in the ovarian microenvironment, which seem to be the causative mechanism underlying diminished ovarian reserve.

However, very few studies have been conducted so far, thus current understanding of the reproductive toxicity associated with NMPs is still limited and requires further investigations.

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Male fertility preservation in cancer patients. Reasoned opinion

Attilio Anastasi¹, Francesco Capodanno², Francesca Bonesi³, Andrea Gallinelli²

¹ Procreazione Medicalmente Assistita (PMA), Azienda Ospedaliero Universitaria Careggi, Florence, Italy; ² UOC di Fisiopatologia della Riproduzione Umana, AUSL di Ferrara - Ospedale del Delta di Lagosanto (FE), Italy; ³ Istituto Bernabeu Venezia, Martellago (VE), Italy

ABSTRACT

Every day about thirty cancers are diagnosed in young Italian patients. Of these, 37.4% are cases of young men mainly affected by testicular, colon-rectal or thyroid cancer, melanoma or Hodgkin's lymphoma. The risk of infertility in some types of cancer is very high, but for others the risk is much lower. Due to numerous confounding factors, it is difficult to clarify a role of cancer itself in male fertility. Semen cryopreservation may represent the only chance of future genetic parenthood for many patients. Rapid freezing of semen in liquid nitrogen vapors, before cancer treatment is started, is considered the gold standard for male fertility preservation; nevertheless, no more than 10% of patients ask to use frozen sperm to obtain a pregnancy. Despite the efficacy of male fertility preservation methods, the rate of men achieving pregnancy with samples frozen prior to cancer treatment is about 3.9%. In this reasoned opinion, different reasons are given, which suggest the need for a personalized approach to male fertility preservation.

KEYWORDS

Male fertility, fertility preservation, cryopreservation, sperm freezing, male cancers, fertility preservation efficacy, cancer patients.

Introduction

Thirty cancers are diagnosed daily in Italian patients < 40 years of age. Of these, 37.4% are cases of young men mainly affected by testicular, colon-rectal or thyroid cancer, melanoma or Hodgkin's lymphoma^[1-4]. Cancer itself and the related treatments can affect gonadal function leading to an irreversible impairment of spermatogenesis, although this scenario is observed only in some cases, being dependent on various factors: the patient's age, the disease localization, the treatment duration and regimen, the sperm quality before treatment, and individual susceptibility.

Since not all cancer patients are at risk of infertility, a customized approach considering the type of disease and therapy is required in order to optimize male fertility preservation efficacy and cost-effectiveness. Sperm cryopreservation is the only established option for male oncological patients wishing to preserve their fertility.

Cancer and infertility

Male infertility is associated with many cancer co-factors such as obesity, cigarette smoking, lack of physical exercise, poor nutrition, alcohol consumption, and stress^[5]. Due to these confounding factors, it is difficult to clarify a role of cancer itself in male fertility. However, testicular cancer seems to be associated with a worsening of sperm concentration, as do lymphomas, whereas leukemia seems mainly to be responsible for a reduction

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Contact

Attilio Anastasi; att.anastasi@gmail.com
Procreazione medicalmente assistita (PMA),
Azienda Ospedaliero Universitaria Careggi, Florence, Italy

in sperm motility and changes in sperm morphology^[6,7]. Conversely, there is a paucity of data on the impact of gastrointestinal, prostate, and many other cancers on spermatogenesis.

Modern cancer treatments are not always associated with male infertility. The impact of the therapy on spermatogenesis depends on the length of treatment, drug/irradiation dosages, the patient's age, and the type of drug^[1]. In fact, while, on the one hand, some data confirm an association of cisplatin, cyclophosphamide, procarbazine, and chlorambucil with azoospermia, on the other, cancer treatments with fluorouracil, vincristine, vinblastine, dacarbazine, interferon alpha, bleomycin, and others do not seem to induce irreversible infertility. Similarly, testicular irradiation seems to induce azoospermia only at dosages >6 Gy. The toxic effect of molecular targeted therapy on male gonads is still unclear^[1].

Sperm freezing

Semen cryopreservation may offer the only chance of future genetic parenthood for many patients. Time constraints,

treatment delays, and difficulty accessing resources are some of the factors that give most concern.

Following the achievement of the first human live birth, sperm cryopreservation quickly became widespread as a male fertility preservation method [8,9]. Nowadays, cryopreservation of ejaculated sperm is the only established technique strongly recommended for all post-pubertal patients of reproductive age before cancer treatments, as stated by the Scottish Intercollegiate Guidelines Network (SIGN) and American Society for Medical Oncology (ASCO). Sperm cryopreservation can be successfully performed in “high-risk” pubertal males before the initiation of customized therapy. Rapid freezing in liquid nitrogen vapors before starting cancer treatment is the gold standard for male fertility preservation [10].

Efficacy of male fertility preservation

“Efficacy” means the patient’s chance of achieving one live and healthy baby from sperm frozen before cancer treatment.

Although less than 10% of cancer patients ask to use sperm frozen during fertility preservation, almost 50% of them obtain one live baby [11]. Moreover, the clinical pregnancy rate and live birth rate per cycle observed in IVF treatments with sperm frozen for oncological indications are similar to those observed in non-cancer patients submitted to IVF using fresh sperm, independently of the IVF technique used [12].

The high number of healthy babies born from sperm banked for fertility preservation today allows sperm freezing to be considered a highly recommended established technique.

Conclusions

The main conclusions are listed in Box 1.

Sperm freezing is an effective and efficient technique for fertility preservation in male oncological patients, does not involve the need to delay starting cancer treatment, and allows the achievement of pregnancy rates and live birth rates per IVF cycle similar to those observed in non-cancer patients using fresh semen. In our opinion, two main factors explain this: (i) the strong resistance of sperm to the cryopreservation and thawing processes; and (ii) the fact that partners of male cancer patients are usually young (< 37 years old) and fertile, submitted to ovarian stimulation and IVF due to the impairment

of spermatogenesis subsequent to their male partner’s cancer experience; this allows an optimal ovarian response to be obtained and gives these couples a good IVF prognosis [13,14].

Despite the efficacy of male fertility preservation, more than 60% of surviving patients do not use banked semen, which results in a pregnancy rate per patient submitted to sperm banking of 3.9% [11]. There are several possible reasons for this, such as recovery of spermatogenesis, the patient’s personal choice to postpone fatherhood or not have children, and the patient’s death. Moreover, in some cases sperm banking is performed in very young patients, and more than 10–15 years may elapse before the sperm is used. Of note, the proportion of patients asking to discard their banked samples is less than 20%, and modern cancer treatments allow an overall survival rate of 90% in patients undergoing fertility preservation techniques. So, sperm banks usually contain a high number of unused samples from surviving patients, which entails high costs for the public health system for many years. Moreover, the utilization rate recently reported by Ferrari *et al.* confirms data reported by van Casteren *et al.* in 2008, which seems to indicate that insufficient advances have been made in terms of policies designed to encourage the utilization or discarding of frozen sperm after cancer treatment.

In conclusion, a customized approach and follow up after cancer treatment should be suggested in order to: i) better select patients eligible for fertility preservation, ii) optimize the effectiveness of male fertility preservation, and iii) obtain better management of the cryopreserved material (use, destruction, transport, etc.). This is especially important in countries, like Italy, where the cost of fertility preservation in cancer patients is totally supported by the national health system.

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Box 1

MALE FERTILITY PRESERVATION – MAIN CONCLUSIONS
Sperm cryopreservation before antineoplastic treatments always needs to be suggested in patients of reproductive age.
Testicular cancer, seminoma, and hematological malignancies seem to be associated with a reduction in sperm concentration, and progressively worse motility and morphology.
Freezing of ejaculated sperm is an effective method in terms of live birth rate.
Customized counseling on the management of the cryopreserved material (use, destruction, transport, etc.) is highly recommended in order to optimize the cost-effectiveness of the procedure.

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Female fertility preservation: why it does not always mean preservation of fertility

Francesco Capodanno¹, Attilio Anastasi², Francesca Bonesi³, Marialuisa Cinti⁴, Andrea Gallinelli¹

¹UOC Fisiopatologia della Riproduzione Umana, AUSL di Ferrara - Ospedale del Delta di Lagosanto (FE); ²Procreazione Medicalmente Assistita (PMA), Azienda Ospedaliero Universitaria Careggi, Florence, Italy; ³Istituto Bernabeu Venezia, Martellago (VE), Italy; ⁴ARC-STER Centro Studi per la Terapia della Sterilità della Coppia, Mestre (VE), Italy

ABSTRACT

The risk of cancer-related infertility is high for some diseases, but still unclear for others. Due to numerous confounding factors, it is difficult to clarify the impact of cancer itself on female fertility. Oocyte and ovarian tissue cryopreservation offer many cancer patients an opportunity to achieve genetic parenthood, but the real efficacy of the method in terms of live births is still unknown. Since not all women with cancer are at risk of infertility, a customized approach, taking into account the type of disease and therapy, is required in order to optimize female fertility preservation method choice, efficacy and cost-effectiveness. This mini-review focuses on the efficacy of female fertility preservation by oocyte and ovarian tissue cryopreservation, in order to provide some useful tips for oncofertility counseling.

KEYWORDS

Female fertility, fertility preservation, vitrification, oocytes, male breast cancer, fertility preservation efficacy, ovarian tissue.

Introduction

Every year, nearly 5000 Italian women of reproductive age get a cancer diagnosis. Breast, thyroid, cervical, and colon-rectal cancers and melanoma are the most frequently diagnosed types^[1]. Cancer itself and the related treatments can affect ovarian function leading to an irreversible worsening of oogenesis, although this scenario is observed only in some patients, depending on their age, the site and stage of the disease, the treatment duration and regimen, the pre-treatment ovarian reserve, and the individual susceptibility. Since not all women with cancer are at risk of infertility, a customized approach, taking into account the type of disease and therapy, is required in order to optimize female fertility preservation method choice, efficacy and cost-effectiveness. More importantly, a realistic assessment of reproductive chances after fertility preservation should be provided during fertility preservation counseling in order to avoid false hopes.

Cancer and infertility

Understanding the role of cancer in infertility is very challenging because of several confounding factors. First, the parameter most frequently used to evaluate ovarian function in cancer patients is the number of oocytes collected after ovarian stimulation, an analysis that should be restricted to young and fertile cancer patients undergoing fertility preservation. Moreover, cancer shares several predisposing factors with infertility, namely a higher body mass index, smoking, lack of physical exercise, poor diet, alcohol consumption and stress^[2], and this makes it almost impossible to understand whether infertility is due to these factors, cancer, or both. However, a series of studies

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Contact

Attilio Anastasi; att.anastasi@gmail.com
Procreazione medicalmente assistita (PMA),
Azienda Ospedaliero Universitaria Careggi, Florence, Italy

reviewed by Friedler *et al.* in 2012 seems to suggest a worsening of ovarian response in cancer patients versus controls, even though the analysis was probably affected by several biases, in particular, the lower gonadotropin administration in the cancer group^[3]. Conversely, more recent data show that the presence of cancer itself does not seem to be an independent factor of impaired ovarian response during stimulation^[4-11], even though more specific analyses suggest that ovarian cancer and/or high-stage/grade disease could significantly affect ovarian function in terms of collected oocytes^[6,9,10,12]. Papers examining the correlation between cancer and ovarian response are listed in Table 1.

A correlation between infertility and cancer treatment is not always documented. The impact of chemotherapy and radiotherapy on ovarian activity depends on the duration of treatment, the drug/irradiation dosages, and the class of drug used. In fact, since regimens such as cyclophosphamide/methotrexate/fluorouracil (CMF), cyclophosphamide/doxorubicin/fluorouracil (CAF), and cyclophosphamide/epirubicin/fluorouracil (CEF) seem to be related to a high risk of amenorrhea (> 80%), other treatments such as doxorubicin/bleomycin/vinblastine/dacarbazine (ABVD), cyclophosphamide/doxorubicin/vincristine/prednisone (CHOP) and methotrexate, do not show this correlation. Similarly, infertility after radiotherapy has been described only for some irradiation regimens^[11].

Finally, the main factor influencing cancer-related infertil-

Table 1 Papers correlating cancer and ovarian response after stimulation for oocyte freezing.

REFERENCES	OVARIAN RESPONSE WORSENING	NOTES
Pot, 1998	Yes	Included in Friedler, 2012
Oktay, 2006	Yes	Included in Friedler, 2012
Knopman, 2009	Yes	Included in Friedler, 2012
Klock, 2010	Yes	Included in Friedler, 2012
Quintero, 2010	Yes	Included in Friedler, 2012
Michaan, 2010	Yes	Included in Friedler, 2012
Robertson, 2011	Yes	Included in Friedler, 2012
Cardozo, 2015	No	
Quinn, 2017	No	
Tsampras and Roberts, 2017	No	Except for ovarian cancer
Cobo, 2018	No	
Lefebvre, 2018	No	
Dolinko, 2018	No	Except for diffuse diseases
Von Wolff, 2018	No	Except for ovarian cancer
Moraes, 2019	No	
Volodarky-Perel, 2019	Yes	Only in high stage/grade disease

ity risk is the patient's age. For instance, CMF, CAF and CEF are associated with a high risk of amenorrhea in women older than 40, but no effect on ovarian function is documented in patients under 30 years old. Radiotherapy-related infertility risk is also associated with the patient's age: 5-6 Gy irradiations could lead to infertility in women older than 40, whereas it takes > 30 Gy to result in permanent infertility in younger patients (under 26 years old).

Female fertility preservation strategies

Strategies for female fertility preservation are listed in Table 2.

Embryo freezing

Embryo freezing was the first technique to become established for this purpose, but it is rarely used because of the need for a male partner, and also because of the ethical issues involved. Vitrification at the blastocyst stage is suggested, allowing a more than 90% survival rate, and pregnancy and live birth rates similar to those obtained with replacement of fresh embryos.

Oocyte freezing

Oocyte freezing is the first choice for fertility preservation in post-pubertal women. The slow-freeze technique has been

Table 2 Papers correlating cancer and ovarian response after stimulation for oocyte freezing.

TECHNIQUE	CHARACTERISTICS	NOTES
Embryo freezing	– Male partner, ovarian stimulation and IVF required – Safe – Effective in terms of biological and clinical outcomes – Documented efficacy in fertility preservation	Established
Oocyte vitrification	– Ovarian stimulation and IVF required – No male partner required – Safe – Effective in terms of biological outcomes – Efficacy in fertility preservation still debated	Established
Ovarian tissue cryopreservation	– No ovarian stimulation, IVF or male partner required – Useful in pre-pubertal girls – Safety not fully documented – Invasive – Effective in terms of ovarian function and menstrual cycle resumption – Efficacy in fertility preservation still debated	Open clinical application
Ovarian suppression with LHRH analog	– No ovarian stimulation, IVF or male partner required – Safe – Effective in terms of ovarian function and menstrual cycle resumption – Efficacy in fertility preservation documented in breast cancer patients	Established for patients with breast cancer
Ovarian transposition	– No ovarian stimulation, IVF or male partner required – Risk of ovarian cyst formation – Invasive – Effective in terms of ovarian function and menstrual cycle resumption – Efficacy in fertility preservation still debated	Established
<i>In vitro</i> maturation	– Ovarian stimulation and IVF required – No male partner required – Useful in pre-pubertal girls – Safe – Effective in terms of biological outcomes – Efficacy in fertility preservation still debated	Experimental

replaced by vitrification, allowing an almost 90% oocyte survival rate and an 80% fertilization rate. The necessary ovarian stimulation is safe, since no impact on cancer prognosis has been demonstrated, even in patients affected by estrogen receptor-positive breast cancer [13]. Moreover, several strategies are available that allow collection of a higher number of oocytes in a short time, avoiding a significant delay in the cancer treatment [14,15]. Oocyte freezing by vitrification is established and strongly recommended for all post-pubertal patients of reproductive age before cancer treatments [16].

Ovarian tissue cryopreservation

Ovarian tissue cryopreservation is a useful strategy to restore endocrine function in women with cancer who have a high risk of premature ovarian failure due to gonadotoxic cancer treatments, and it is the only choice in pre-pubertal patients [17]. Briefly, small strips of ovarian cortex are removed by laparoscopy and cryopreserved by slow freezing [18]. Ovarian tissue can be collected at any stage of the menstrual cycle and does not require ovarian stimulation, which means that the procedure does not delay cancer treatment. Age and ovarian reserve, the latter shown by anti-Müllerian hormone (AMH) level and antral follicle count (AFC), are the most important patient selection criteria [19]. After thawing, strips are analyzed to exclude the presence of cancer cells, and an orthotopic transplantation is performed on residual ovary or in the pelvic peritoneum. Heterotopic transplantation into pockets created in the subcutaneous space of the abdominal wall, with the aim of obtaining endocrine recovery, has also been reported [20].

LHRH analog administration

The role of luteinizing hormone-releasing hormone (LHRH) analog administration in fertility preservation has been extensively investigated. It could reduce the gonadotoxic effect of cancer treatment in several ways, for example by suppressing FSH secretion and follicular recruitment, activating LHRH ovarian receptors, and, finally, reducing uterine and ovarian perfusion. Many studies have assessed the effect of LHRH analog therapy in breast cancer patients, and several meta-analyses have been published, albeit without consistent conclusions. Conversely, a more recent meta-analysis including five randomized studies, suggests a significant effect of LHRH analog administration, both on menstrual cycle recovery and on pregnancy rate in breast cancer patients with respect to controls [21]. LHRH analog administration is therefore considered an efficient fertility preservation strategy in breast cancer.

Ovarian transposition

Ovarian transposition is the removal of ovaries from the irradiation field. A laparoscopic intervention is required and the efficacy in terms of fertility preservation is still debated. Possible reported risks are the formation of ovarian cysts, as well as difficulty in ovarian metastasis visualization.

Oocyte *in vitro* maturation

Oocyte *in vitro* maturation involves the collection of germinal vesicle-stage oocytes, either without ovarian stimulation, or following short ovarian stimulation and triggering. Oocytes are incubated in specific media to reach the MII stage and vitrified; the main advantage of this method is its rapidity.

The competence of *in vitro* matured oocytes is still an open question, and few data have been published about the efficacy of this technique in terms of live birth rates in oncological settings. For these reasons, *in vitro* maturation is not widespread as a fertility strategy itself, although it could be useful after *ex vivo* oocyte collection in association with ovarian tissue cryopreservation [22].

Efficacy of female fertility preservation

The efficacy of fertility preservation is measured as the patient's chance of achieving one live, healthy baby after cancer treatment. Among the female fertility preservation techniques listed in Table 2, only oocyte vitrification and ovarian tissue cryopreservation are routinely used [19-23].

As regards the efficacy of oocyte vitrification, high survival and fertilization rates are reported, but these outcomes do not reflect the goal of fertility preservation. In fact, the clinical efficacy of female fertility preservation is reflected in the live birth rate per frozen oocyte. Unfortunately, this is difficult to estimate as the rate of oncological patients seeking pregnancy with their oocytes frozen prior to the cancer treatment is less than 8% (Table 3), and this is directly reflected in the low number of babies obtained in this specific patient group (Table 4).

Another key point is that oocyte vitrification is a less efficient technique, since many oocytes are needed to get a baby. The number of oocytes required is strongly associated with the patient's age: for instance, in women < 35 years old, at least 8 oocytes are needed to obtain a cumulative live birth rate of about 35%, whereas the same number of oocytes in women > 35 years old seems to provide a cumulative live birth rate of

Table 3 Patients seeking oocyte thawing according to the reason for cryopreservation.

REFERENCES	PATIENTS SEEKING OOCYTE THAWING (%)	OOCYTE FREEZING REASON
Hodes-Werts, 2013	6%	Non-oncological
Garcia-Velasco, 2013	1.2%	Oncological
Martinez, 2014	2.9%	Oncological
Alvarez, 2018	2%	Oncological
Balkenende, 2018	7%	Both oncological and non-oncological
Cobo, 2018	7.5%	Oncological
Cobo, 2018	12.2%	Non-oncological
Diaz-Garcia, 2018	4.8%	Oncological
Specchia, 2019	4.5%	Oncological

about 29%. More recent data seem to confirm this, describing a live birth rate of about 40% with at least 10 frozen oocytes in patients < 35 years old [7,11]. Taken together, the literature data suggest that no fewer than 8–15 mature oocytes should be banked to provide a realistic chance of motherhood in cancer patients [7,11,23], and this estimate is confirmed by a recent Italian paper where the mean number of stored oocytes in oncological patients was 9.5 ± 6.1 [24].

Regarding the efficacy of ovarian tissue cryopreservation, recently published data suggest that this technique could provide endocrine recovery in more than 60% of patients, a clinical pregnancy in 58% of patients, and a live birth rate of about 18–30%. Moreover, more than 130 live births have been reported [25–27]. Importantly, since natural conception has been reported in 60% of transplanted patients, pregnancy and live-birth rates in patients submitted to IVF seem to be significantly lower than in the general IVF population, corresponding to patients with poor ovarian re-

serve [28]. Recently, many authors have suggested that ovarian tissue cryopreservation fulfills the criteria for an established method rather than an experimental one [29], and in some centers the ovarian tissue cryopreservation is formally considered an “open clinical application” procedure [30]. Nevertheless, there is a need for more data about the efficacy in pre-pubertal patients and the safety of transplantation, and for an ovarian tissue cryopreservation world registry to definitively remove the experimental label from this fertility preservation technique. Moreover, the real efficacy of ovarian tissue cryopreservation is not known because of the low number of surgical operations aimed at preserving ovarian tissue and the low number of transplants performed.

Discussion

The main conclusions of this mini-review are listed in Box 1.

Table 4 Babies born from oocytes cryopreserved for oncological reasons

REFERENCES	TECHNIQUE	STORED OOCYTES (N°)	LIVE BIRTHS (N°)	GESTATIONAL AGE (WEEKS)	BIRTH WEIGHT	REPORTED CONGENITAL DISEASES
Yang, 2007	SF	13	1	37	3062	No
Porcu, 2008	SF	7	2	38	2100 2400	No No
Sanchez-Serrano, 2010	Vit	16	2	34	1650 1830	No No
Kim, 2011	Vit	7	1	35	2410	No
Garcia-Velasco, 2013	Vit	4	1	39	3440	No
Alvarez, 2014	Vit	14	1	38	2650	No
Martinez, 2014	Vit	4	1	40	3440	No
		5	1	40	2850	No
		3	1	40	3220	No
		8	1	38	2920	No
Alvez Da Motta, 2014	Vit	28	1	At term	2970	No
Doyle, 2016	Vit	N.R.	1	N.R.	N.R.	No
Druckemiller, 2016	SF/Vit	N.R.	2	33	2087 1452	No No
			1	40.8	2858	No
			1	38.6	3357	No
			1	39	3311	No
Perrin, 2016	Vit	5	1	37.5	3180	No
Specchia, 2019	Vit	$6.5 \pm 3.5^*$	1	41	3720	No
			1	38	2660	No

Data expressed as mean $\pm$ standard deviation. **SF**: slow freeze; **Vit**: vitrification; **NR**: not reported

Box 1

FEMALE FERTILITY PRESERVATION – MAIN CONCLUSIONS
Literature data, while not definitive, do not seem to support a generic effect of cancer on ovarian function
Ovarian cancer and high stage/grade disease could reduce the ovarian function
More data are needed to improve the efficacy of female fertility preservation by oocyte and ovarian tissue cryopreservation
More appropriate terminology should be adopted during counseling, in order to avoid generating false hopes

The first choice for female fertility preservation is oocyte vitrification. Several reports confirm that ovarian stimulation in cancer patients is safe, since no significant worsening of oncological prognosis has been proven in patients submitted to fertility preservation compared with controls. The safety of pregnancy has also been documented — indeed, relapses have not been found to be significantly increased in patients becoming pregnant after cancer. Nevertheless, less than half of young oncological patients are informed about the possibility of banking oocytes prior to cancer treatment, no more than 8% of oncological patients seek pregnancy with oocytes, and to date few babies have been born in this population. Cultural, social and economic factors are responsible for the low rate of possible candidates for fertility preservation who receive specific counseling, whereas the low utilization rate of oocytes could be associated with other reasons, such as recovery of ovarian function (observed especially in young patients), patients choosing to delay starting a family or deciding not to have children (due to the risk of oncogenetic transmission) and, finally, patient death. Again, in some cases more than 10 years may elapse prior to oocyte thawing, due to the young age at the time of freezing or to the prolonged duration of cancer treatment (in the case of estrogen receptor-positive breast cancer). Some open questions remain about the use of vitrification in the oncological setting: the most important issue is the lack of a long-term follow up of babies conceived from vitrified oocytes, and the effect of high concentrations of cryoprotectant agents on oocyte competence; the most effective vitrification tools and freezing/warming protocols are also debated. For all these reasons, it is not possible to clarify the real efficacy of female fertility preservation by oocyte vitrification.

When oocyte freezing is not feasible, ovarian tissue cryopreservation could be considered. The first successes with ovarian tissue cryopreservation, dating back to 2004, generated considerable confidence and optimism around this technique. However, ovarian tissue cryopreservation is still not established and many difficulties prevent its standardized application. Since a great advantage of ovarian tissue cryopreservation is its capacity to restore endocrine function, it could be offered as an alternative to non-experimental techniques, very useful in the case of young women at high risk of premature ovarian failure due to sterilizing high-dose chemotherapy.

Importantly, as suggested by several authors, the efficacy of ovarian tissue cryopreservation seems to depend strongly on patient selection ^[19,27].

Conclusions

Oocyte vitrification and ovarian tissue cryopreservation are routinely used as fertility preservation strategies, even though their real efficacy can be only estimated. Data reported in this mini-review lead us to deduce that fertility preservation does not always mean preservation of fertility. In our opinion, this should be reflected in the fertility preservation counseling and decision-making process; in short, we agree with Grynberg and Sermondade, who suggest that the terminology used should be reconsidered in order to avoid generating false hopes about fu-

ture motherhood ^[31]. During counseling the term “fertility preservation” should be replaced with a more appropriate one such as “gamete preservation”, “oocyte banking”, or “gonadic tissue banking” in order to clearly express the concept that storing biological samples of reproductive cells does not always mean that fertility will be preserved. Evidence-based medicine can provide clear information about the real chances of conceiving after cancer. This evidence should help doctors to provide more correct information, which in turn would prevent patients from harboring false hopes about their chances of having a child.

Finally, better follow up after cancer treatment could help patients to decide whether to use or dispose of their banked oocytes.

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Lymphocytic hypophysitis associated with ovarian hyperstimulation syndrome during assisted fertilization

Francesco Barbato¹, Cristina Stradella², Mariano Galdiero³, Anna Maione⁴, Roberto Allocca¹, Antonietta Topa¹, Simone Aramini¹, Federica Matrone¹, Sandra Perillo¹, Giorgio Bosso^{1,5}, Fabio Giuliano Numis^{1,5}

¹ Department of Emergency and Urgent Medicine, Stroke Unit, Santa Maria Delle Grazie Hospital, Naples, Italy; ² Department of Obstetrics and Gynecology, P.O. Santa Maria delle Grazie Asl Napoli 2 Nord, Naples, Italy; ³ Department of Medicine PO A Rizzoli, ASL Napoli 2 Nord, Lacco Ameno, Naples, Italy; ⁴ Fertility Unit, Maternal-Child Department, AOU Federico II, Naples, Italy; ⁵ Department of Emergency and Urgent Medicine, Emergency Medicine, Santa Maria Delle Grazie Hospital, Naples, Italy

ABSTRACT

Lymphocytic hypophysitis is a rare autoimmune disease, often presenting with symptoms of glandular enlargement and endocrine dysfunction. Despite its low prevalence, it remains a significant diagnostic challenge, often requiring tissue biopsy for confirmation. This report describes the case of a 34-year-old woman who developed lymphocytic hypophysitis following ovarian hyperstimulation syndrome (OHSS) during fertility treatment. Initial symptoms included severe headache, uncontrollable vomiting, and behavioral changes. Radiological and neurological evaluations revealed an enlarged pituitary gland with symmetrical hyperintensity on MRI, and laboratory tests showed elevated ACTH and prolactin levels. Further gynecological examination confirmed OHSS. The patient was treated with supportive therapy and high-dose dexamethasone, leading to complete resolution of the symptoms. This case highlights the need for awareness of hypophysitis as a potential complication in women undergoing fertility treatments and suggests the importance of comprehensive diagnostic evaluations to distinguish it from other conditions. Further research is needed to explore any potential link between OHSS and lymphocytic hypophysitis.

KEYWORDS

Lymphocytic hypophysitis, ovarian hyperstimulation syndrome, CSF lymphocytosis.

Introduction

Lymphocytic hypophysitis is a rare autoimmune disease, but the most frequent histopathological subtype of hypophysitis^[1,2]. Clinically, it can manifest with symptoms related to glandular enlargement, such as headache and meningism, and/or symptoms of endocrine dysfunction^[3]. The prevalence of hypophysitis is low, with an incidence of about 1 in 9 million patients^[1], although it is thought to be underestimated^[3]. Lymphocytic hypophysitis continues to be a diagnosis of exclusion, with tissue biopsy being the gold standard for a definitive diagnosis^[3]. However, clinical, laboratory, and imaging data can assist in diagnosis^[4]. Traditionally, lymphocytic hypophysitis has been considered an autoimmune condition that predominantly affects women over men, with an 8:1 ratio^[2]. In women, it occurs mainly in the third trimester of pregnancy and in the postpartum period^[5]. While other autoimmune diseases are associated in 20-50% of cases^[6], it is not typically reported in association with ovarian stimulation^[4].

Ovarian hyperstimulation syndrome (OHSS) is characterized by a wide spectrum of clinical and laboratory manifestations caused by the exogenous administration of substances used for the induction of ovulation^[7] or, in the assisted reproductive

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Contact

Francesco Barbato; francesco.barbato@aslnapoli2nord.it
Department of Emergency and Urgent Medicine, Stroke Unit,
Santa Maria Delle Grazie Hospital, Naples, Italy

technology (ART) setting, for the induction of multiple follicular growth^[8,9]. It is mainly characterized by the displacement of serum from the intravascular space to the third space, primarily to the abdominal cavity. In its severe form, OHSS is life-threatening because it can cause venous or arterial thromboembolic events, including stroke, but clearly not autoimmune diseases^[9]. We describe a case of lymphocytic hypophysitis plus OHSS in a young woman undergoing ART procedures.

Clinical case

A 34-year-old woman was admitted to the Emergency Department (ED) with an around four-day history of worsening headache, associated with uncontrollable vomiting, behavior-

al alterations, and verbal stereotypies. She only repeated “it hurts” while touching her head. Initial clinical and radiological examinations, including CT of the skull and CT angiography of the neck and cerebral vessels, were negative. No fever, leukocytosis, or alterations in other acute-phase reactants were noted in the ED. Neurological evaluation also revealed stiff neck and photo/phonophobia, so she was admitted to the Stroke Unit. An EEG was performed, showing a monomorphic diffuse theta rhythm, suggesting diffuse slowing without epileptic abnormalities. Transcranial Doppler (TCD) was negative. The patient’s history was reviewed with family members, who reported the intake of 150 mcg corifollitropin alfa fifteen days earlier, followed by menotropin and ganirelix, in preparation for a fertilization procedure. The patient had been diagnosed with diminished ovarian reserve, and so the family was undergoing medically assisted procreation. Her baseline AMH was 1.8 ng/mL. In the days prior to admission to hospital, during ovarian stimulation, a regular clinical and laboratory check-up had been carried out, with hormone dosage. No drugs had been administered, other than corifollitropin alfa, menotropin and ganirelix. Cabergoline had not been administered. Given this history, pituitary hormone assays were performed, revealing a marked increase in ACTH (487 pg/mL), PRL (79 ng/mL), cortisol (56 mcg/dL), and estradiol (3236 pg/mL). Beta HCG was 2.3 mIU/mL. Lumbar puncture showed an opening pressure of 32 cmH₂O, pleiocytosis (447 cells/microL), and elevated protein (87 mg/dL). Morphological examination confirmed the presence of lymphocytes only. Since lymphocytic hypophysitis was suspected, brain and pituitary magnetic resonance imaging (MRI) with gadolinium enhancement was performed, showing an enlarged pituitary gland with homogeneous T1 hyperintensity after gadolinium administration (see figure 1). There was no deviation of the pituitary stalk. Subsequently, a complete

gynecological evaluation with ultrasound revealed multifollicular ovaries of increased size, measuring 64x38mm on the right and 55x48mm on the left. The ovaries were mobile relative to the surrounding tissues and slightly painful upon movement of the exploratory probe, with a small transonic layer in the Douglas pouch measuring 2.2 cm (see figure 2). A diagnosis of OHSS was made. Blood tests showed leukocytosis (14,000/ μ L white blood cells), CRP 1.2 mg/dL, hypoalbuminemia (2.7 g/dL), and hemoconcentration. Renal function was normal (eGFR 73 mL/min). After five days in which the patient underwent daily hormonal checks and supportive therapy for OHSS, ACTH and prolactin levels returned to normal values. Supportive therapy included albumin, low molecular weight heparin, and progesterone. Given the evidence of ovarian hyperstimulation associated with a case of lymphocytic hypophysitis, steroid therapy with high doses of dexamethasone (8 mg thrice a day) was administered followed by slow tapering. A week later, the patient displayed complete resolution of the symptoms and a significant clinical improvement.

Discussion

In this case, the diagnosis of hypophysitis was made through clinical, radiological, and laboratory criteria. Gadolinium-enhanced MRI of the pituitary gland is the method of choice and can easily distinguish lymphocytic hypophysitis from a pituitary adenoma. In a pituitary adenoma, MRI shows asymmetrical pituitary enlargement, often with deviation of the pituitary stalk [3]. In lymphocytic hypophysitis, the pituitary gland and stalk are symmetrically enlarged without deviation. MRI shows, as in this case, a homogeneously intense pituitary gland with increased dura [3,4]. Diagnosis is not always straightforward, so

Figure 1 (A) Volumetric increase of the pituitary gland, with a globular appearance and protrusion of upper profile of the sellar diaphragm. Preserved physiological hyperintensity of the neurohypophysis signal. After gadolinium (B), widespread impregnation of the gland and the peduncle is observed. Pituitary gland is on axis.

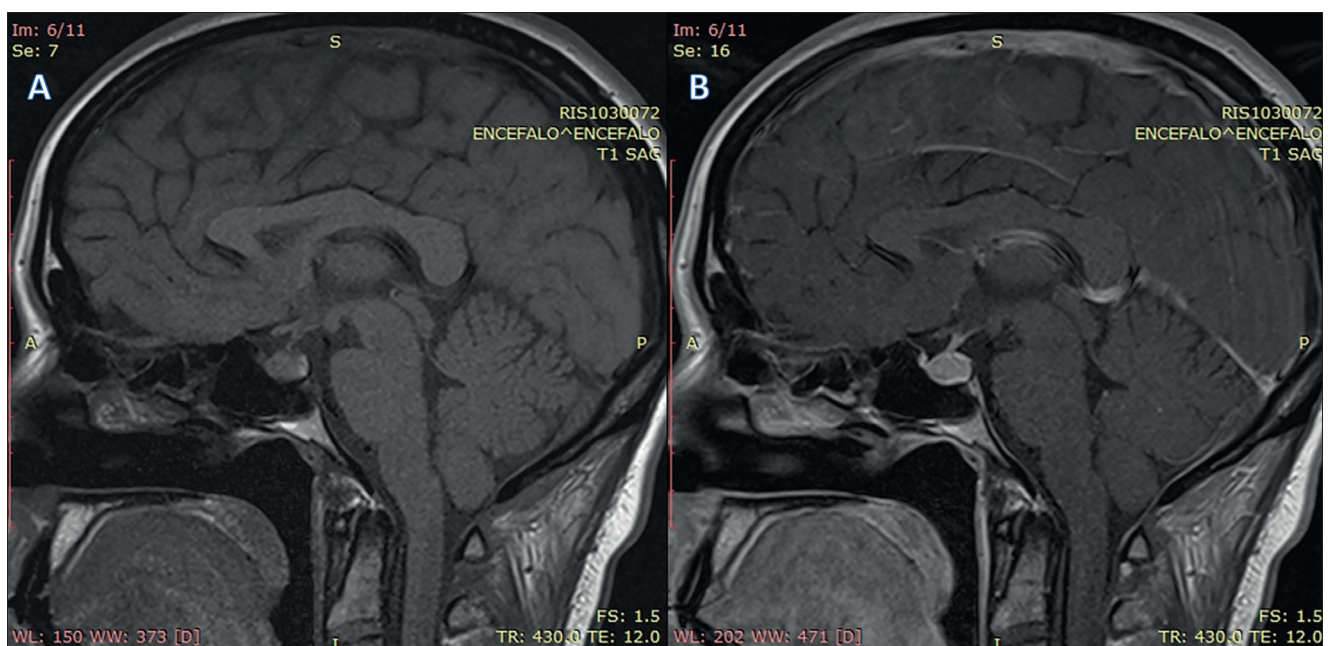
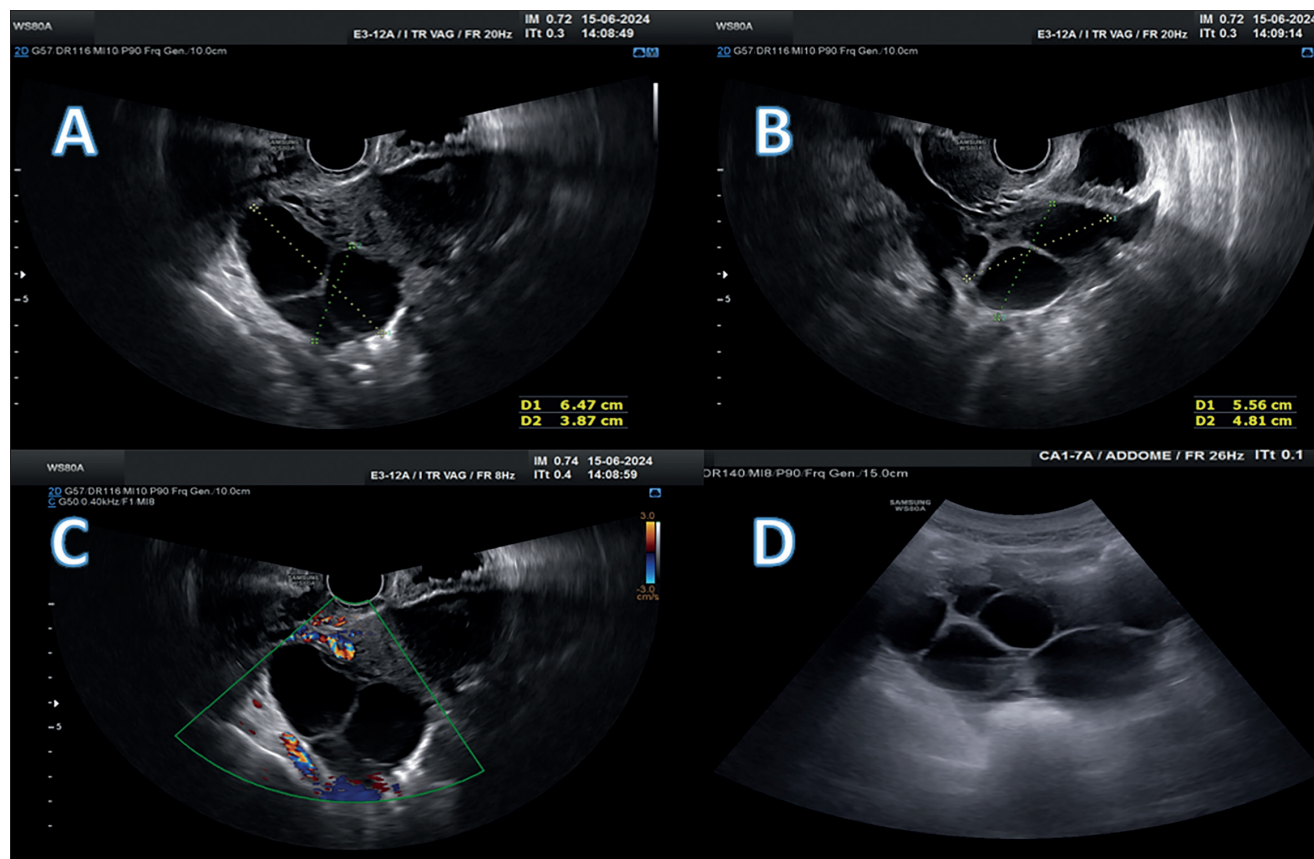


Figure 2 Multifollicular ovaries of increased size, measuring 64x38mm on the right (A, C) and 55x48mm on the left (B, D). The ovaries were mobile relative to the surrounding tissues and slightly painful upon movement of the exploratory probe, with a small transonic layer in the Douglas pouch measuring 2.2



a scoring system known as the Gutenberg score has been developed to accurately identify lymphocytic hypophysitis [12]. A total score greater than 1 indicates a pituitary adenoma, while a score of zero or less indicates lymphocytic hypophysitis, with a specificity of 99%, a sensitivity of 92%, and positive and negative predictive values of 97% [12]. In this case, the score was -2. The finding of numerous lymphocytes in cerebrospinal fluid (CSF) examination and elevated blood prolactin and ACTH levels supported this diagnosis. This hormonal profile can be part of an acute hypophysitis clinical picture, where there is an initial increase in PRL and ACTH levels. This is likely related to inflammation, which preferentially affects ACTH- and PRL-secreting cells [10]. Therefore, biopsy of the pituitary gland was not considered, both because of the invasiveness of the procedure in a critically ill patient, and because the clinical and instrumental data were extremely clear. Differential diagnoses were considered; similar CSF findings can occur in HaNDL (headache with neurological deficits and CSF lymphocytosis), but it was excluded due to the presence of meningeal signs and headache preceding neurological worsening [11].

Findings of raised intracranial pressure [12] are also incompatible. No focal neurological signs were found in this patient, so the involvement of a vessel and consequently cerebral ischemia was never suspected [13]. A pituitary adenoma was excluded due to the progressive and spontaneous normalization of hormone levels and the clinical and radiological findings [14]. Neurological symptoms, such as headache and confusion,

were completely resolved after steroid therapy, while supportive therapy was administered for ovarian hyperstimulation syndrome as described in the literature [17]. No autoantibodies or involvement of additional glandular structures were documented, reasonably excluding pathologies such as antiphospholipid syndrome, IgG4-related disease, Crohn's disease, and Takayasu's arteritis [15]. It is interesting that while ovarian hyperstimulation syndrome can be related to hormone therapy with exogenous gonadotropins, lymphocytic hypophysitis clearly cannot [15].

Conclusion

Since an association has been described between hypophysitis and physiological hormonal changes in women, we assume that there could also be an association with pharmacological ovarian hyperstimulation. A single case is not decisive, of course, in establishing a causal relationship, but it may represent a starting point for further studies.

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Successful *in vitro* fertilization pregnancy (IVF) after conservative management of endometrial cancer

Silvia Sorce, Claudio Castello

S.C. PMA, ASL Città di Torino, Turin, Italy

ABSTRACT

Objective: To report a successful IVF pregnancy in a couple after conservative treatment of endometrial cancer. **Design:** Case report and literature review. **Setting:** ASL città di Torino – SC PMA, Valdesse dpt. **Patient(s):** A 32-year-old white woman. **Main Outcome Measure(s):** Successful pregnancy after conservative management of endometrial cancer. **Intervention(s):** Grade 1 endometrial adenocarcinoma diagnosed at hysteroscopy, followed by dilatation and curettage (D&C). On follow-up D&C, pathologic examination was normal after high-dose progesterone therapy. The patient subsequently underwent an IVF cycle with transfer of one embryo. **Result(s):** The patient delivered a baby boy with a vaginal delivery at 37 weeks of gestational age. She is currently undergoing follow-up, and hysterectomy and bilateral salpingo-oophorectomy have not yet been performed. **Conclusion(s):** In carefully chosen situations, deferring surgery in infertile patients with endometrial cancer may be a viable option permitting subsequent successful pregnancy.

KEYWORDS

IVF, endometrial cancer, save fertility, pregnancy conservative management.

Introduction

Endometrial cancer (EC) ranks among the most prevalent gynecological malignancies globally ^[1,2], mainly affecting postmenopausal women. However, a notable proportion of EC cases, approximately 7.1%, occur in women aged 20 to 44 years, and strikingly, over 70% of these women are nulliparous at the time of diagnosis ^[3,4]. The characteristic presentation often includes vaginal bleeding ^[5,6], enabling early detection and intervention, contributing to a favorable five-year survival rate exceeding 95%.

EC has been categorized into two primary types, Type I and Type II, each with distinct clinical and pathological features.

Type I EC is predominantly characterized by genetic mutations, notably microsatellite instability, and is often linked to lifestyle factors such as obesity, hormonal imbalances (like those seen in polycystic ovarian syndrome), and a hyperestrogenic state ^[7]. The latter associated with a high body mass index (BMI) is, in part, explained by the peripheral conversion of androgens to estrogen in adipose tissue via aromatase.

Type II ECs, on the other hand, are typically associated with older age at diagnosis, advanced disease stage at presentation, higher tumor grade, and non-endometrioid histopathological subtypes, including serous, clear-cell, and undifferentiated carcinomas. These cancers tend to have a relatively poor prognosis ^[8].

Understanding the risk factors associated with EC is essential for both prevention and management. Key risk factors include:

- high BMI: excess body weight is a significant contributor to

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Contact

Claudio Castello; claudio.castello@aslcitytorino.it
S.C. PMA, ASL Città di Torino, Turin, Italy

the hyperestrogenic state, increasing EC risk;

- hyperestrogenic states: beyond BMI, conditions such as nulliparity, estrogen-releasing tumors, early menarche, late menopause, and exposure to unopposed estrogen further elevate the risk of EC.
- systemic diseases: conditions like hypertension and diabetes mellitus are linked to an increased risk of EC ^[4,9,10].

The incidence of EC among women of reproductive age is on the rise, necessitating fertility-sparing management strategies. Preserving fertility while effectively treating EC is a delicate balance, requiring careful consideration ^[11].

Fertility-sparing approaches

Various fertility-sparing approaches have been explored for young women with EC who wish to preserve their reproductive potential.

Hormonal therapies. These therapies aim to induce regression or stabilization of the tumor. Progestin-based treatments, such as medroxyprogesterone acetate and megestrol acetate, are commonly employed and have shown success in achieving

complete or partial response in many cases ^[4,12,13].

In addition to oral therapy, another viable option for administering progesterone treatment is through the use of a temporary levonorgestrel-releasing intrauterine device (LNG-IUD). This approach is particularly beneficial when seeking to avoid the systemic adverse effects associated with high-dose oral progestins. One significant advantage is the localized nature of the therapy. By delivering the progestin directly to the uterine lining, the LNG-IUD minimizes systemic absorption. This, in turn, reduces the risk of the systemic side effects commonly associated with high-dose oral progestins.

These side effects, which can include mood swings, weight gain, and changes in appetite, are less likely to occur with the LNG-IUD due to its localized action. Moreover, the LNG-IUD provides the convenience of long-term treatment without the need for daily medication. It can typically remain in place for several years, ensuring a sustained and consistent release of hormone.

The choice between oral progestin therapy and LNG-IUDs, as well as other treatment options, should be based on careful consideration of the patient's specific medical condition, preferences, and individual clinical factors. The suitability of LNG-IUDs for fertility preservation in patients with EC will depend on various factors, including the patient's overall health and the stage and characteristics of the cancer. Therefore, it is crucial for patients to engage in thorough discussions with their healthcare providers to determine the most appropriate and effective treatment strategy tailored to their unique circumstances and goals ^[14,15].

Hysteroscopic resection. In select cases, hysteroscopic resection can be considered to remove small tumors or polyps while preserving the uterus ^[3].

Combination therapies. Some approaches combine progestin-based therapies including LNG-IUD with hysteroscopic resection for improved outcomes ^[14].

Patient selection criteria

Selecting suitable candidates for fertility-sparing management is paramount. Factors that guide patient selection include:

- age: a younger age at diagnosis is generally more favorable for fertility preservation;
- cancer stage: early-stage EC (Stage I) is more amenable to fertility-sparing approaches;
- histological type: patients with endometrioid-type EC may be better candidates than those with non-endometrioid histologies;
- fertility desires: the patient's preferences with regard to future childbearing play a crucial role in decision-making ^[3,4].

Follow-up and surveillance

Post-treatment follow-up and surveillance are essential to assess treatment effectiveness and monitor for any disease recurrence. Regular imaging, biopsies, and clinical assessments are standard components of follow-up care.

Challenges and limitations

Managing EC in young women seeking fertility preservation is challenging for several reasons. These include the rarity of EC in this demographic, the need for individualized treatment plans, and the limited long-term data on the outcomes of fertility-sparing approaches.

Future directions

Ongoing research aims to refine and expand fertility-sparing options, improve patient selection criteria, and enhance our understanding of the long-term outcomes and safety of these approaches. Novel treatments and tailored management strategies are areas of active investigation.

Women younger than 40 years of age tend to have well-differentiated tumors and early-stage disease. Women of reproductive age who wish to preserve their fertility may not want to undergo definitive surgical treatment. We describe the case of a patient who had a successful IVF cycle resulting in a pregnancy after conservative treatment of endometrial cancer.

Case report

In September 2020, a 32-year-old non-obese (BMI 20.6), white, nulligravid woman presented at our institution with a diagnosis of endometrial intraepithelial neoplasia. This had been diagnosed incidentally during an ultrasound examination, which revealed a heterogeneous endometrium. Her medical, surgical, and social history was unremarkable. She had a unicornuate unicollis uterus with a suspected accessory right cornus. She had no family history of diabetes, hypertension, or cancer, and she did not smoke. The patient declined standard surgical management and requested conservative treatment in an attempt to preserve her fertility. A gynecologic oncologist evaluated the patient and explained the risks of conservative management. Progestin therapy was recommended; the patient was prescribed oral megestrol acetate (medroxyprogesterone acetate, *Farlutal*®, 20 mg) once daily, and an LNG-IUD (*Mirena*® Bayer Health Care Pharmaceutical Inc, Wayne, NY, USA) was inserted. The IUD was removed when the patient was ready to attempt pregnancy.

Follow-up: Subsequent hysteroscopies showed pseudo-decidualization of the endometrium, consistent with exogenous hormone therapy, but no evidence of residual adenocarcinoma. It was decided to use IVF treatment to optimize the patient's potential for fertility, and attempt to achieve a viable pregnancy without further delay. Hysterectomy was recommended following fertility treatment.

The patient underwent her first IVF cycle in November 2021. Nine oocytes were obtained (6 in metaphase II, 2 in metaphase I, and one germinal vesicle), three of which were fertilized. Two embryos and one blastocyst were obtained. On February 8, 2022, after removing *Mirena*® in December 2021, one blastocyst was transferred, resulting in a pregnancy. Unfortunately, in March, at 8 weeks and 5 days of gestational age,

a miscarriage was diagnosed. Hysteroscopy after the miscarriage, performed in May, showed the absence of malignant neoplasm, so the patient underwent her second transfer. On June 6, 2022, one grade-A day-3 embryo was transferred, but no pregnancy resulted. On August 2, 2022, the last embryo (also grade-A, day-3) was transferred and this resulted in a full-term pregnancy. The patient successfully delivered a healthy baby boy at 37 weeks and 2 days of gestational age, after experiencing premature rupture of the membranes. The patient had a normal postpartum course, was breastfeeding, and took a progestin-only pill for contraception. It is reassuring to learn that the patient has undergone two hysteroscopies that did not reveal any neoplasms. The patient's healthcare team will continue to assess her condition and determine the most appropriate course of action based on her individual circumstances.

IVF cycle protocol: As mentioned, the IVF cycle was initiated in November 2021; nine eggs were retrieved, three of which were fertilized. The patient's peak estradiol level on the day of hCG administration was 670.4 pg/mL. She had an initial hCG level of 1283.2 mIU/mL 13 days after the last embryo transfer (ET). 21 days after ET, she had an hCG level of 2235.9 mIU/mL, and an ultrasound examination confirmed the pregnancy. The patient had a normal postoperative course, was breastfeeding, and took a progestin-only pill for contraception.

IVF cycle protocol: The stimulation cycle was performed using alpha follitropin (Ovaleap®, 300 IU/d) in addition to the patient's therapy as prescribed by her oncologist (Mirena® and Farluta®). Human chorionic gonadotropin (Gonasi®), 10,000 U, was administered when at least two follicles with a mean diameter of 18 to 20 mm were present, and the estradiol level was greater than 500 pg/mL. Transvaginal retrieval of oocytes was performed 36 hours after hCG administration. ET was done on the patient's natural cycle triggered by HCG 5000 UI prior to measurement of P4. After the transfer, the patient continued taking levothyroxine 25 mcg (prescribed during preconceptional medical check-ups), folic acid 400 mcg (1 tablet daily), and started progesterone 400 mg (2 vaginal suppositories daily), cardioASA100 mg (1 tablet daily), and methylprednisolone 4 mg (1 tablet daily).

Conclusions

Fertility-preserving treatment can be a viable option for younger patients diagnosed with stage IA EC who express a desire to become pregnant. However, it is important to acknowledge that while this approach holds promise, the available evidence to support its efficacy remains somewhat limited.

The first step in pursuing fertility preservation in EC patients is to conduct a thorough evaluation and ensure an accurate diagnosis. Not all patients are suitable candidates for this approach, so individualized assessments are essential to determine eligibility.

Several treatment options exist for fertility preservation in EC, and they can be tailored to the patient's specific circumstances. These options include oral progestin therapy, the use of LNG-IUDs, hormone replacement therapy (HR), gonadotropin-releasing hormone agonist therapy, and the use of

aromatase inhibitors, either alone or in combination with other treatments.

Combination therapy involving HR following medical treatment may offer an improved response rate. Nevertheless, further research is required to establish the most effective dosage, route of administration, and treatment duration for these combination approaches.

Once a complete response is achieved, it is advisable for women wishing to conceive to attempt pregnancy as promptly as possible. Assisted reproductive technologies may be considered, in order to expedite the conception process compared with spontaneous conception.

Regular follow-up with oncology specialists, typically scheduled at six-monthly intervals, is an integral part of the treatment plan. This ongoing monitoring is essential to track the patient's progress and evaluate the response to treatment.

Crucially, the treatment approach must be personalized to accommodate the individual patient's characteristics, such as age, BMI, tumor grade, and response to treatment. Recognizing and addressing these unique factors is essential to optimize outcomes and enhance the chances of successful fertility preservation in EC patients.

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