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EDITORIAL

The year 2025 marked a significant milestone in the field of gynecology and obstetrics as the International Society of Gynecology and Obstetrics and the Pelvic Floor Cosmetic Gynecology Association joined forces to host their annual congress together in Antalya, Turkey. This collaborative scientific gathering brought together an impressive assembly of 2,262 participants from across Turkey, including a few esteemed foreign scientists who also contributed with their presentations. Held under the inspiring motto **"Change through science and critical thinking,"** the congress provided an invaluable platform for the exchange of knowledge, clinical experience, and groundbreaking research.

The atmosphere throughout the event was one of engagement, inspiration, and progress. Esteemed scientists and clinicians, each distinguished in their respective specialities, shared their insights on the rapidly evolving landscape of women's health. The congress featured a dynamic blend of keynote lectures, panel discussions, interactive workshops, and oral presentations, all designed to shape the future of gynecology and obstetrics. The diversity of the topics covered, ranging from reproductive endocrinology to minimally invasive surgery and pelvic floor restoration, reflected the interdisciplinary nature of the field and underscored the importance of integrating evidence-based practices with innovative approaches.

In this spirit of scientific excellence and forward-thinking, the current issue of our journal brings together a selection of original articles and reviews that contribute meaningfully to our understanding of complex clinical challenges in women's health.

An in-depth review titled **"The Role of Androgens in Health and Disease in Females: A Narrative Review"** revisits the multifaceted influence of androgens in female physiology. Beyond their traditional role in reproductive function, androgens impact mood, metabolism, bone density, and cardiovascular health. This article elegantly summarizes the dualistic nature of androgens, both beneficial and pathological, and emphasizes the need for individualized therapeutic approaches.

The next featured article, **"The Importance of Exosomes in Gynecological Diseases,"** explores the emerging role of exosomes as biomarkers and therapeutic targets in gynecological disorders. As nanovesicles are involved in intercellular communication, exosomes have attracted significant interest for their potential in diagnosing and monitoring diseases such as endometriosis, ovarian cancer, and polycystic ovary syndrome (PCOS). This review provides a timely synthesis of current knowledge and outlines future directions for translational research.

Another important study, **"Randomized Controlled Trial Comparing Single Embryo Culture with Group Culture in a Micro-Well Dish: Impact on Embryo Development and Clinical Outcomes,"** offers new evidence in the realm of assisted reproductive technology. By comparing embryo development under different culture conditions, the authors shed light on how microenvironmental factors influence viability and implantation rates, potentially guiding embryologists toward more effective *in vitro* fertilization practices.

In a practical contribution to infertility treatment, the article **"A Novel Alternative to Sequential Protocol in Clomiphene Citrate-Resistant PCOS Patients with a Combined Regimen of Clomiphene Citrate and Recombinant Gonadotropin"** proposes a promising therapeutic strategy. The authors describe a combined protocol that demonstrated improved ovulatory responses and pregnancy rates, providing a potential solution for patients who do not respond to conventional clomiphene citrate therapy.

In **"A Double-Blind Randomized Controlled Trial Comparing Two Panty Liners with Different Surfaces with Respect to Microbial Colony per Square Centimeter,"** the authors address a relevant issue in everyday female hygiene. This meticulously designed trial assesses the microbiological safety of commonly used products, with implications for vulvovaginal health and consumer awareness.

The sixth contribution, **"A Case-Control Study of Female Genital Measurements of Polycystic Ovary Patients and Their Relation to Sexual Function and Genital Perception,"** addresses a less-explored dimension of PCOS. By comparing genital morphology and sexual health parameters between PCOS patients and healthy controls, this study provides novel insights into the physical and psychosocial aspects of the syndrome, offering a holistic view of patient care.

In **"Emergency Blood Transfusion in Gynecology Cases: A Multicenter Analysis,"** the authors present a comprehensive evaluation of transfusion practices in acute gynecological emergencies. By analyzing data across multiple centers, the study identifies key predictors of transfusion need, and transfusion-related complications, and highlights the importance of developing standardized protocols to enhance patient safety and clinical outcomes.

In the realm of rare and complex cases, **"Endometrioma Excision in a Patient with VACTERL Syndrome and a Rudimentary Uterine Horn: A Case Report"** illustrates the intersection of congenital anomalies and gynecological pathology. This report underscores the importance of multidisciplinary collaboration and individualized surgical planning when managing such rare and intricate clinical presentations.

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EDITORIAL

Each of these articles not only adds to the growing body of scientific literature but also aligns with the overarching theme of the 2025 congress-**progress through science and critical reflection.** As researchers and clinicians, we are reminded that continued advancements in our field are made possible by a willingness to question established norms, rigorously test new hypotheses, and integrate multidimensional perspectives into patient care.

We extend our gratitude to all contributors, reviewers, and editorial staff who made this issue possible. May the insights presented herein inspire further inquiry, collaboration, and innovation in the service of women's health worldwide.

Cemil Oğlak, MD
Associate Professor

Review

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The Role of Androgens in Health and Disease in Females: A Narratıve Review

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ABSTRACT

Androgens play a key role in maintaining women's and men's health. They act on the ovary, endometrium, vagina, and vulva to maintain a balanced reproductive system, and have regulatory and protective effects on almost every part and function of the body, such as the heart, brain, bone, muscle, skin, and metabolism. As with many other substances, the effects of androgens are dose-dependent and pathological when present in excessive amounts. Although the role of androgen therapies in pre- and postmenopausal women's female has been better understood in recent years with increasing studies, there is still a need for studies in terms of side effects, patient selection, and standardization in terms of laboratory tests.

Keywords: Androgens, women, reproductive health, menopause, testosterone therapy

INTRODUCTION

In discussions of women's reproductive health, estrogens are typically associated with women's health, while androgens are often contextualized within their related diseases. This perspective stems largely from the high clinical prevalence of androgen-related disorders. However, it is important to acknowledge the increasing recognition of the significance of androgenic well-being throughout a woman's life span. This review examines the role of androgens in both health and disease from an explanatory perspective.

Physiology of Androgens in Females

Androgen Synthesis and Measurement in Women

The main androgens in female are dehydroepiandrostenedione sulfate (DHEAS), dehydroepiandrostenedione (DHEA), androstenedione, testosterone and dihydrotestosterone (DHT), based on their concentration in serum.[1] DHEAS is a non-potent pro-androgen synthesized from zona reticularis in the cortex of the adrenal gland under the influence of adrenocorticotropic hormone. The secretion begins during adrenarche, peaks in mid-reproductive adulthood, and gradually declines before plateauing in later life. The rate of synthesis does not change during the menstrual cycle or is not affected by the transition to menopause.[2] DHEAS is an important source of androgen produced in the ovary.

DHEA is produced intracellularly, mainly from the zona reticularis by conversion in ovarian theca cells and from circulating DHEAS.[3] Serum concentrations decrease with age.[4,5]

Androstenedione is synthesized in the zona fasciculata of the adrenal cortex and stromal cells of the ovary. Serum concentrations are affected by circadian rhythm and menstrual cycle. It rises in the middle of the menstrual cycle in parallel with an increase in estrogen levels. A significant decrease in serum concentration has been observed in postmenopausal women undergoing oophorectomy.[1]

Testosterone is synthesized from both the zona fasciculata in the cortex of the adrenal gland and ovarian stromal cells. However, most is formed from androstenedione via peripheral conversion. It has been detected at higher concentrations in the ovarian vein than in the peripheral veins, and its serum concentrations decrease significantly in patients after oophorectomy. The level of testosterone is affected by the circadian rhythm.[6] The highest blood levels were observed in the early morning. It is the lowest in the early follicular phase of the menstrual cycle, peaks in the mid-cycle, and decreases in the luteal phase, although not as much as in the follicular phase.[7] Ten years after the onset of menopause, testosterone and androstenedione concentrations fell to half of those in the perimenopausal period.[1,8,9]

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DHT is derived from peripheral conversion of testosterone and is present in very low concentrations in the female body. A very small amount is produced by the adrenal zona fasciculata. Of note, testosterone can aromatize to estradiol, whereas DHT cannot.[1]

Liquid or gas chromatography and tandem mass spectrophotometry are reliable and reproducible methods for measuring total testosterone levels.[10] Direct immunoassay measurements are less reliable. However, salivary measurements are still far from being used clinically in terms of accuracy and they are considered investigational.[11]

Androgen Receptors in Women

Androgen receptors (ARs), which belong to the nuclear receptor family, are located in many organs of the female body, such as the ovaries, brain, endometrium, bone, and heart, and they mediate important metabolic activities. AR-ligand interactions have been observed in the prostate, breast, ovary, and pancreas, and AR deficiency leads to dysfunction in follicle development, ovulation, and fertility. Studies have also suggested that ARs are located in the endometrium of women and provide uterine hemostasis.[5,12,13]

Androgens and Reproductive Health

Ovarian Function

Understanding the specific role of androgens in the ovary is highly dependent on AR knockout animal models, as completely androgen-resistant individuals cannot arise through natural reproduction. These models have elucidated the stimulatory role of androgens in early follicle development and their subsequent use in communication between follicles to maintain follicle health and induce late-phase growth.[14] Therefore, an increasing number of studies in recent years have focused on the use of pro-androgenic or aromatase inhibitor drugs in patients who respond poorly to hyperstimulation in in vitro fertilization centers.[15] However, it should be remembered that the positive effects of androgen on follicular development are actually only possible within an optimal concentration range. At increasing concentrations, individuals may abruptly begin to show clinical symptoms similar to polycystic ovary syndrome (PCOS), and evidence has shown that AR-related signaling pathways are responsible for the development of PCOS.[16]

Endometrial Effect

Androgens can initiate both regulatory and reparative physiological mechanisms in the endometrium as well as pathological processes that can occur through AR-based pathways and aromatization to estrogen.[5] AR expression changes significantly throughout the menstrual cycle.[2,17] It is increased in epithelial cells during the proliferation phase and decreased in the secretory phase. It is suggested that ARs provide repair and durability in endometrial tissue. DHEA is thought to stimulate endometrial stromal fibroblasts in women of late reproductive age, causing decidualization, thus contributing positively to fertility.[12,18] In animal models, DHEA has also been shown to increase endometrial receptivity by exerting antioxidant effects on the endometrial stroma.[19,20]

There is no strong evidence that exogenous testosterone increases endometrial cancer. In a group of female-to-male patients, after long-term testosterone use, the endometrium underwent a process of atrophy similar to that observed in long-term progesterone users. In a large study comparing endometrial cancer risk according to the type of intrinsic androgen, pre-disease total and free testosterone levels were associated with endometrial cancer risk, whereas androstenedione and DHEAS levels were not.[21]

Vulvo-vaginal Effects

The efficacy of estrogen in vulvovaginal tissues by increasing superficial cells and lowering pH has long been known and has been used in the treatment of certain diseases such as the genitourinary syndrome of menopause (GSM). Recent studies in animal and human tissues have shown that ARs are also present in the labium and clitoris of the vulva, predominantly in all three layers of the vagina.[22,23] To understand whether these receptors work as predicted, it is necessary to understand the mechanisms of up- and down-regulation properties and more than 1000 receptor mutations have occurred. These mutations have mostly been found in androgen insensitivity syndrome or female-looking XY individuals. In animal studies, androgenic treatments have been shown to lead to positive improvements in many areas, including vaginal weight, which is a measurable parameter in mice.[24] However, androgens have not yet entered routine therapeutic use for these beneficial their effects on vulvovaginal tissue.[25] In addition, as with estrogen, there is no clear consensus on the benefits and harms of their systemic or local use.

Clinical Conditions Associated with Androgen Imbalance

Conditions Associated with High Androgen

High androgen levels in women are primarily associated with PCOS, which affects approximately 20% of young women. PCOS is characterized by several reproductive and metabolic abnormalities. From a reproductive perspective, women with PCOS often experience oligomenorrhea, ovulatory dysfunction, and infertility. The metabolic implications of PCOS are significant and largely driven by hyperandrogenism.[26] Elevated androgen levels predispose women with PCOS to obesity, insulin resistance, and metabolic syndrome. These metabolic disturbances can have far-reaching consequences. For instance, about half of obese women with PCOS develop metabolic syndrome, highlighting the strong interaction between androgens and insulin. Furthermore, metabolic derangements associated with PCOS may progress to more severe conditions, with approximately 40% of women with PCOS developing impaired glucose tolerance, which may eventually evolve into Type 2 diabetes mellitus. Additionally, women with PCOS often exhibit an unfavorable lipid profile, characterized by increased triglyceride and total and low-density lipoprotein cholesterol levels. Thus, high androgen

levels, particularly in PCOS, have significant implications for both reproductive function and metabolic health in women.[27,28]

Conditions Associated with Low Androgen

Screening for low androgen levels in women is not routinely recommended, as there is no well-defined syndrome of "female androgen insufficiency" that reliably correlates with serum androgen levels. The Endocrine Society advises against diagnosing "female androgen deficiency" or using testosterone to treat low-androgen states in women.[29] This is because low serum androgen levels do not consistently correlate with clinical symptoms, even among oophorectomized women.[30] Understanding the conditions that can lead to low androgen levels in women is important, however these conditions include reduced ovarian androgen production (caused by chemotherapy, radiation, ovarian failure or insufficiency, and oophorectomy), decreased adrenal androgen production (adrenal insufficiency), issues with the hypothalamic-pituitary axis (such as malnutrition, anorexia, and hypopituitarism), and the use of specific medications (corticosteroids, hormonal contraceptives, antiandrogenic agents, oral estrogen therapy, and opioids).[8] When considering these conditions, clinicians should focus on the patient's clinical presentation rather than solely relying on serum androgen levels, as the interpretation of these levels and their physiological effects are complex.

To address the necessity and timing of oophorectomy separately, the oophorectomy approach, which in the past was customarily performed prophylactically during hysterectomies for benign causes, is now considered unfavorable from the perspective of androgen metabolism and its effects.[31] Although many of the metabolic and cardiovascular disadvantages of oophorectomy-induced menopause in premenopausal women are mostly attributed to estrogen deprivation, oophorectomy in postmenopausal women causes similar findings. Therefore, studies have shown that in women with no ovarian indication and an average familial risk of ovarian cancer, the decision to perform prophylactic oophorectomy should be made with much more caution.[32]

Interpretation of Sex Hormone-Binding Globulin

Active testosterone circulates in the blood either free or bound to albumin. Testosterone measurements also measure the circulating inactive testosterone bound to sex hormone-binding globulin (SHBG) in the blood. SHBG is a protein synthesized in the liver and shows a high affinity for sex steroids. Factors that increase or decrease SHBG levels directly affect the amount of active testosterone circulating in the blood. For example, with menopause, SHBG decreases slightly, leading to increased levels of active testosterone in the blood. Exogenous estrogen therapy increases SHBG levels, which has the opposite effect. When transdermal estrogen preparations are used, this effect is not observed, unless very high doses are used.

SHBG functions beyond its role as a simple transport protein It serves as a metabolic marker. It is a parameter that warrants consideration in the evaluation of PCOS due to its direct association with hyperinsulinemia, Type 2 diabetes, and metabolic syndrome.[33] Low concentrations of SHBG in postmenopausal women without elevated testosterone levels have been associated with unfavorable lipid profiles, visceral fat and diabetes risk. Genetic factors (single nucleotide polymorphisms) in the investigation of low SHBG levels were only informative in a small group.[34] Fatty liver disease, especially in the presence of dietary fructose, was sufficient to significantly lower SHBG levels in animal models. This effect is thought to be insulin independent. The free androgen index (FAI), calculated using total testosterone and SHBG levels, is associated with increased metabolic syndrome and cardiovascular risk in postmenopausal women, whereas this cannot be said for testosterone alone.[35] The conclusion from these studies is that SHBG may be important for screening women of various ages for metabolic diseases.[36]

Androgens in Non-Reproductive Health

Cardiovascular Effects

Many studies have examined the effects of androgen levels or lifetime exposure, on cardiovascular health in women. Women exposed to low androgen levels during reproductive age have been observed to have an increased risk of cardiovascular disease in the postmenopausal period.[37] This effect was supported by findings in another study that indicated that appropriate androgen levels help maintain an antiatherogenic lipid profile in women.[38] At the other end of the equation are women with hyperandrogenemia, the most common example being women with PCOS. In these women, total androgen overload appeared to increase atherosclerosis in the postmenopausal period. FAI is one of the methods used to monitor the effects of androgens in such patients. Insulin resistance, hypertension, impaired lipid profile, and central obesity are commonly observed in women with increased FAI. Based on these findings, it may be conclude that one of the conditions required to maintain cardiovascular health in women is maintaining androgen levels at physiological levels.[39,40]

Metabolic Effects

The difference in fat distribution in the male and female bodies suggests that androgens act on fat cells. Androgens do this by affecting the differentiation of adipose stem cells into mature adipocytes. Testosterone also reduces lipolysis by inhibiting adipose-sensitive lipases in women. Visceral obesity increases insulin resistance, leading to hyperinsulinemia and increased insulin-like growth factor-1 synthesis, which in turn increases ovarian androgens and decreases SHBG synthesis from the liver.[41] Thus, increased androgen levels lead to insulin resistance, which in turn leads to increased androgens, resulting in a cycle of PCOS and metabolic syndrome. The same effect can occur after menopause when testosterone levels increase due to decreased estrogen and SHBG.[42]

Neuroprotective and Cognition Regulating Effects

Like many other organs, the brain is influenced by the withdrawal of ovarian hormones, particularly during menopause. Estrogen and testosterone exhibit anti-inflammatory and neuroprotective effects in the brain, contributing to the regulation of cognition and mood.[43] ARs are distributed throughout the central nervous system and play a role in processes such as sexual desire,

thermoregulation, sleep, visuospatial skills, and language. In addition, testosterone appears to reduce oxidative stress, limit the accumulation of amyloid beta, and accelerate nerve regeneration, highlighting its potential protective effects against neurodegenerative conditions, such as Alzheimer's disease.[44]

Although the majority of women transitioning through menopause do not experience major cognitive changes, some encounter significant disruptions that impair their quality of life, particularly in younger women undergoing oophorectomy.[32] Observational and interventional studies suggest a relationship between physiological concentrations of testosterone and improvements in verbal learning and memory in postmenopausal women when administered exogenously. The simulation of male testosterone levels in premenopausal women has been shown to enhance visuospatial performance; however, its effects on verbal learning and memory in this population remain unstudied.

Randomized controlled trials investigating the cognitive effects of testosterone therapy in postmenopausal women are limited and are often constrained by small sample sizes or concurrent estradiol therapy. The current data suggest that testosterone therapy has no adverse effects on cognition, mood, or overall well-being in postmenopausal women. Research has indicated that postmenopausal women who applied 300 μg/day of testosterone gel transdermally for a duration of 26 weeks demonstrated marked improvements in verbal learning and memory functions.[45] However, no noteworthy effects on overall well-being were observed. In a contrasting investigation, hysterectomized women, regardless of whether they had undergone oophorectomy, received intramuscular testosterone at both physiological and supraphysiological levels in conjunction with transdermal estradiol. This study reported no significant alterations in cognitive function among participants.[46]

Notably, the cognitive benefits of testosterone in postmenopausal women appear to be independent of aromatization to estradiol. While these findings suggest that testosterone therapy may enhance verbal memory or delay cognitive decline, current evidence does not warrant its routine use for these purposes.[47]

Osteoprotective Effect

ARs and estrogen receptors (ER) are both important receptors regulating bone metabolism.[48] Androgens and estrogen-aromatized helices trigger significant effects on osteoblasts and osteoclasts through AR and ER.[49] AR activation stimulates osteoblast proliferation and inhibits osteoclast activity. In addition, ER activation inhibits osteoclast proliferation and activates osteoclast apoptosis. This results in an inhibitory effect on bone resorption.[50] In the female body, the second estrogen-dependent mechanism is more effective. In one study, low free testosterone levels in women of late reproductive age were associated with a more rapid decrease in bone density in the future.[51] Another study found that, among older patients, those with lower endogenous testosterone levels also had lower lumbar and hip bone densities.[36] In the Women's Health Initiative observational study, higher endogenous bioavailable testosterone concentrations were associated with lower hip fracture rates, independent of estradiol and SHBG.[36] It is important to note that studies in which an effect was observed have always compared endogenous testosterone values. Studies into whether testosterone treatment increase bone mineral density have been inconclusive, and the findings are conflicting. Although there are studies showing better results in women given testosterone in combination with estrogen compared to estrogen alone[52], there are also studies showing the opposite, and androgen therapies are still far from being used primarily to contribute to bone density.[53]

Effects on Skin and Hair

Although the adrenal glands and ovaries are the main centers of androgen production, the skin is also an organ where potent androgens such as DHT are formed, and ARs are involved in this mechanism. ARs are found in sebocytes, dermal papilla cells, root sheaths of hair follicles, sweat glands, vascular endothelial smooth muscle cells, and epidermal and follicular keratinocytes.[54] AR activation causes proliferation and sebum production in sebaceous glands. In the frontal region and vertex of the head, it shortens the anagen phase of hair follicles in genetically predisposed individuals and causes hair loss, whereas in other parts of the body, it transforms the vellus into terminal follicles.[55] Although it plays a role in the pathogenesis of acne, patients with acne vulgaris are rarely hyperandrogenic. Hirsutism is caused by androgen action, and androgens are elevated in most hirsute women. In female pattern hair loss, the pathogenesis of AR disorders is being investigated, although the causes are not fully understood.[56] Endocrinological tests and a multidisciplinary approach are important to ensure treatment success in androgen-mediated skin diseases.[56,57]

Effects on Muscle Mass and Performance

It is well known that androgens increase muscle mass and performance in both sexes. Many professional and recreational athletes worldwide use testosterone derivatives as well as anabolic steroids to enhance athletic performance or physique. In addition to external use, a study among athletes with PCOS showed a significant correlation between muscle mass and androgen levels. This gives some athletes a significant advantage over others.[58,59] The athlete's biological passport is used for such cases, and androgen levels are recorded after taking ethnicity, menstrual status, and oral contraceptive use into account. In addition, studies on postmenopausal women using exogenous androgen derivatives have shown increases in both muscle mass and athletic performance.[60,61] These therapies are currently being used for the treatment of neuromuscular diseases, dystrophies, and myositis.[62]

Therapeutic Applications of Androgens

Genitourinary Syndrome of Menopause

Up to 70% of women who have gone through menopause experience GSM, a condition previously referred to as vulvovaginal atrophy. It encompasses urinary, genital, and

sexual dysfunctions resulting from declining sex hormone levels.[63] The clinical presentation typically encompasses dyspareunia, vaginal dryness, irritation, dysuria, increased urinary frequency and urgency, recurrent urinary tract infections, and a shift towards alkalinity in vaginal pH. While non-hormonal therapies, such as vaginal moisturizers and lubricants, can provide some relief, they do not restore genitourinary tissue integrity. Hormonal therapies, particularly vaginal estrogen and DHEA, are considered to be the most effective treatments for GSM.[39,64] The Food and Drug Administration has approved intravaginal DHEA 6.5 mg for GSM treatment, which has shown improvements in cell maturation, vaginal pH, dyspareunia, and sexual function with neutral effects on the endometrium.[9,65]

Hypoactive Sexual Desire Disorder

Women's sexual behavior is inherently multifactorial and influenced by many organic and psychological factors. In addition, there are many variations in androgens, their receptors, and their pathways, and it is almost always very difficult to perform true androgen measurements in the laboratory. Despite this, it is now well-established that sexual function in women is regulated by androgens.[52,66] Testosterone and its precursors significantly affect sexual function, desire, and arousal.[52,67,68] There is a relationship between testosterone levels and sexual function pre- and post-menopause.[69] Although hypoactive sexual desire disorder (HSDD) is a controversial condition in some communities, it has become the focus of research for testosterone treatment in some countries.

Although research indicates potential sexual health advantages, testosterone formulations have not been approved for women in many countries. Based on existing safety information and adverse effect profiles, the preferred method of administering testosterone to women is through short-term, low-dose transdermal applications. The Endocrine Society Guideline recommends six months of transdermal testosterone for HSDD.[29] When prescribing testosterone to women, clinicians should use caution and typically prescribe a tenth or less of the recommended male dose to avoid supraphysiological dosing. Topical products should be applied to the inner thigh, buttocks, abdomen, or vulva, avoiding the breasts and arms.[25] Oral testosterone is discouraged due to first-pass metabolism in the liver and associated side effects.[70] Intramuscular and pellet therapies should also be avoided because of their potential for prolonged exposure and supraphysiologic dosing.[70] Although safe and successful results have been obtained in the short term, long-term studies are required.[9,67,71]

Monitoring During Treatment

Once testosterone therapy is initiated, careful monitoring is essential. The Endocrine Society and global consensus position statement recommend checking baseline testosterone levels before starting therapy. Monitoring of follow-up levels is recommended 3-6 weeks post-initiation and semi-annually thereafter to prevent side effects and excessive dosing.[25,29] It is essential to recognize that clinical response is not correlated with serum hormone levels; testing serves only to ensure treatment safety. Subsequent steps should focus on a clinical evaluation of perceived benefits versus risks, with the aim of enhancing sexual desire, arousal, orgasmic function, satisfaction, or responsiveness to sexual cues, while concurrently addressing sexual anxieties and distress. If there is no response after six months of consistent use, treatment should be discontinued. Regular monitoring should also include assessment of potential side effects, such as mild increases in acne or hirsutism, although significant adverse effects are rare when serum testosterone levels remain within normal physiological ranges.[9,67]

Androgens and Breast Cancer

Breast cancer is the most common cancer in women worldwide. ARs can be positive or negative in breast cancer tissue regardless of the ER. Those who are positive have shown better response to treatments and longer survival times.[72,73] The effect of androgen hormones on the development of cancer itself is still unclear. Some researchers suggest protective effects, while others suspect that they promote tumor growth.[25,73] Studies measuring androgen concentrations in the blood during the postmenopausal period have shown an increased incidence of cancer at higher concentrations, similar to estrogens. Currently, there are studies on the use of selective AR mediators in the treatment of some types of breast cancer.[73,74] For other reasons, the use of testosterone is not recommended in women with a history of breast cancer because of the risk of aromatization to estrogen.[25]

Emerging Research and Future Directions

Research on the effects of androgens on women's health will undoubtedly continue to be published and new studies will be planned in the coming years. We will continue to learn about the effects of androgens and their receptor behaviors on the health of individuals in reproductive and non-reproductive systems, in premenopausal and postmenopausal ages, and even in adolescence. On the reproductive side, studies examining androgens and ovarian eserve, receptor polymorphisms, and reproduction from conception to sexual behavior will continue to be examined in depth. On the non-reproductive side, we will see more oncological studies, especially in the endometrium and breast, and studies to discover the effect on pain perception, which has perhaps not received much attention so far. In the field of cosmetic gynecology, the results of studies examining androgens and their safety in complementary and preventive therapies will continue to be published, and undoubtedly more will be said about androgen excess and reproductive metabolic syndromes. Although androgens and their effects after gender-affirming surgery were not mentioned in this review, it seems that there will be results that will be of interest to both this group and non-transgender people.

CONCLUSION

Androgens play important roles in women's reproductive and systemic health, supporting ovarian function, endometrial repair, bone strength, neuroprotection, and metabolic balance. When dysregulated, they contribute to disorders: excess androgens, as in PCOS, lead to metabolic and reproductive

problems, while deficiency is linked to conditions like HSDD and GSM. Their systemic impact is shaped by interactions with estrogen and SHBG, influencing cardiovascular, cognitive, and musculoskeletal health. However, a major limitation to effective androgen therapy is the lack of reliable biomarkers to assess tissue-level androgen activity. Current treatments aim to maintain total testosterone within physiological ranges, but no strong evidence supports initiating therapy solely based on low levels. HSDD remains the only established indication for androgen replacement, underscoring the urgent need for standardized measures and long-term safety data.

Footnote

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REFERENCES

1. Burger HG. Androgen production in women. Fertil Steril. 2002;77 Suppl 4:S3-S5.
2. Rothman MS, Carlson NE, Xu M, et al. Reexamination of testosterone, dihydrotestosterone, estradiol and estrone levels across the menstrual cycle and in postmenopausal women measured by liquid chromatography-tandem mass spectrometry. Steroids. 2011;76(1-2):177-182.
3. Endoh A, Kristiansen SB, Casson PR, Buster JE, Hornsby PJ. The zona reticularis is the site of biosynthesis of dehydroepiandrosterone and dehydroepiandrosterone sulfate in the adult human adrenal cortex resulting from its low expression of 3 beta-hydroxysteroid dehydrogenase. J Clin Endocrinol Metab. 1996;81(10):3558-3565.
4. Gibson DA, Simitsidellis I, Collins F, Saunders PTK. Endometrial Intracrinology: oestrogens, androgens and endometrial disorders. Int J Mol Sci. 2018;19(10):3276.
5. Bianchi VE, Bresciani E, Meanti R, Rizzi L, Omeljaniuk RJ, Torsello A. The role of androgens in women's health and wellbeing. Pharmacol Res. 2021;171:105758.
6. Algburi HD Sr, Alhamza A, Mansour AA. Diurnal variation of serum total testosterone in women: a single-center study from Basrah. Cureus. 2023;15(10):e47677.
7. Huang G, Bhasin S, Pencina K, Cheng M, Jasuja R. Circulating dihydrotestosterone, testosterone, and free testosterone levels and dihydrotestosterone-to-testosterone ratios in healthy women across the menstrual cycle. Fertil Steril. 2022;118(6):1150-1158.
8. Davis SR, Wahlin-Jacobsen S. Testosterone in women--the clinical significance. Lancet Diabetes Endocrinol. 2015;3(12):980-992.
9. Smith T, Batur P. Prescribing testosterone and DHEA: the role of androgens in women. Cleve Clin J Med. 2021;88(1):35-43.
10. Shiraishi S, Lee PW, Leung A, Goh VH, Swerdloff RS, Wang C. Simultaneous measurement of serum testosterone and dihydrotestosterone by liquid chromatography-tandem mass spectrometry. Clin Chem. 2008;54(11):1855-1863.
11. Santoro N, Braunstein GD, Butts CL, Martin KA, McDermott M, Pinkerton JV. Compounded bioidentical hormones in endocrinology practice: an endocrine society scientific statement. J Clin Endocrinol Metab. 2016;101(4):1318-43.
12. Simitsidellis I, Saunders PTK, Gibson DA. Androgens and endometrium: new insights and new targets. Mol Cell Endocrinol. 2018;465:48-60.
13. Pihlajamaa P, Sahu B, Jänne OA. Determinants of receptor- and tissue-specific actions in androgen signaling. Endocr Rev. 2015;36(4):357-384.
14. Barad D, Gleicher N. Effect of dehydroepiandrosterone on oocyte and embryo yields, embryo grade and cell number in IVF. Human Reproduction. 2006;21(11):2845-2849.
15. Zhang M, Niu W, Wang Y, et al. Dehydroepiandrosterone treatment in women with poor ovarian response undergoing IVF or ICSI: a systematic review and meta-analysis. J Assist Reprod Genet. 2016;33(8):981-991.
16. Walters KA, Handelsman DJ. Role of androgens in the ovary. Mol Cell Endocrinol. 2018;465:36-47.
17. Evans SF, Hull ML, Hutchinson MR, Rolan PE. Androgens, endometriosis and pain. Frontiers in Reproductive Health. 2021;3:792920.
18. Wongwananuruk T, Sato T, Kajihara T, et al. Endometrial androgen signaling and decidualization regulate trophoblast expansion and invasion in co-culture: a time-lapse study. Placenta. 2016;47:56-62.
19. Qin A, Qin J, Jin Y, et al. DHEA improves the antioxidant capacity of endometrial stromal cells and improves endometrium receptivity via androgen receptor. Eur J Obstet Gynecol Reprod Biol. 2016;198:120-126.
20. Young SL. Androgens and endometrium: new lessons from the corpus luteum via the adrenal cortex? Fertil Steril. 2018;109(4):623-624.
21. Allen NE, Key TJ, Dossus L, et al. Endogenous sex hormones and endometrial cancer risk in women in the European Prospective Investigation into Cancer and Nutrition (EPIC). Endocr Relat Cancer. 2008;15(2):485-497.
22. Palacios S. Expression of androgen receptors in the structures of vulvovaginal tissue. Menopause. 2020;27(11):1336-1342.
23. Labrie F, Martel C, Pelletier G. Is vulvovaginal atrophy due to a lack of both estrogens and androgens? Menopause. 2017;24(4):452-461.
24. Traish AM, Kim SW, Stankovic M, Goldstein I, Kim NN. Testosterone increases blood flow and expression of androgen and estrogen receptors in the rat vagina. J Sex Med. 2007;4(3):609-619.
25. BRITISH MENOPAUSE SOCIETY Tool for Clinicians.; 2022.
26. Dumesic DA, Oberfield SE, Stener-Victorin E, Marshall JC, Laven JS, Legro RS. Scientific statement on the diagnostic criteria, epidemiology, pathophysiology, and molecular genetics of polycystic ovary syndrome. Endocr Rev. 2015;36(5):487-525.
27. Wang K, Li Y, Chen Y. Androgen excess: a hallmark of polycystic ovary syndrome. Front Endocrinol (Lausanne). 2023;14:1273542.
28. Walters KA. Polycystic ovary syndrome: is it androgen or estrogen receptor? Curr Opin Endocr Metab Res. 2020;12:1-7.
29. Wierman ME, Arlt W, Basson R, et al. Androgen therapy in women: a reappraisal: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2014;99(10):3489-510.
30. Davison SL, Bell R, Donath S, Montalto JG, Davis SR. Androgen levels in adult females: changes with age, menopause, and oophorectomy. J Clin Endocrinol Metab. 2005;90(7):3847-3853.
31. Davey DA. Androgens in women before and after the menopause and post bilateral oophorectomy: clinical effects and indications for testosterone therapy. Womens Health (Lond). 2012;8(4):437-446.
32. Mielke MM, Kapoor E, Geske JR, et al. Long-term effects of premenopausal bilateral oophorectomy with or without hysterectomy on physical aging and chronic medical conditions. Menopause. 2023;30(11):1090-1097.
33. Ding EL, Song Y, Manson JE, et al. Sex hormone-binding globulin and risk of type 2 diabetes in women and men. N Engl J Med. 2009;361(12):1152-1163.
34. Davis SR, Robinson PJ, Moufarege A, Bell RJ. The contribution of SHBG to the variation in HOMA-IR is not dependent on endogenous oestrogen or androgen levels in postmenopausal women. Clin Endocrinol (Oxf). 2012;77(4):541-547.
35. Peter A, Kantartzis K, Machann J, et al. Relationships of circulating sex hormone-binding globulin with metabolic traits in humans. Diabetes. 2010;59(12):3167-3173.

36. Lee JS, LaCroix AZ, Wu L, et al. Associations of serum sex hormone-binding globulin and sex hormone concentrations with hip fracture risk in postmenopausal women. J Clin Endocrinol Metab. 2008;93(5):1796-1803.
37. Spoletini I, Vitale C, Pelliccia F, Fossati C, Rosano GM. Androgens and cardiovascular disease in postmenopausal women: a systematic review. Climacteric. 2014;17(6):625-634.
38. Khatibi A, Agardh CD, Shakir YA, et al. Could androgens protect middle-aged women from cardiovascular events? A population-based study of Swedish women: The Women's Health in the Lund Area (WHILA) Study. Climacteric. 2007;10(5):386-392.
39. Moretti C, Lanzolla G, Moretti M, Gnessi L, Carmina E. Androgens and hypertension in men and women: a unifying view. Curr Hypertens Rep. 2017;19(5).
40. Stone T, Stachenfeld NS. Pathophysiological effects of androgens on the female vascular system. Biol Sex Differ. 2020;11(1).
41. Montalcini T, Migliaccio V, Ferro Y, Gazzaruso C, Pujia A. Androgens for postmenopausal women's health? Endocrine. 2012;42(3):514-520.
42. Iwasa T, Noguchi H, Tanano R, et al. Age-dependent changes in the effects of androgens on female metabolic and body weight regulation systems in humans and laboratory animals. Int J Mol Sci. 2023;24(23):16567.
43. Rosario ER, Chang L, Head EH, Stanczyk FZ, Pike CJ. Brain levels of sex steroid hormones in men and women during normal aging and in Alzheimer's disease. Neurobiol Aging. 2011;32(4):604-613.
44. Pike CJ, Carroll JC, Rosario ER, Barron AM. Protective actions of sex steroid hormones in Alzheimer's disease. Front Neuroendocrinol. 2009;30(2):239-258.
45. Aleman A, Bronk E, Kessels RPC, Koppeschaar HPF, Van Honk J. A single administration of testosterone improves visuospatial ability in young women. Psychoneuroendocrinology. 2004;29(5):612-617.
46. Huang G, Wharton W, Travison TG, et al. Effects of testosterone administration on cognitive function in hysterectomized women with low testosterone levels: a dose-response randomized trial. J Endocrinol Invest. 2015;38(4):455-461.
47. Abi-Ghanem C, Robison LS, Zuloaga KL. Androgens' effects on cerebrovascular function in health and disease. Biol Sex Differ. 2020;11(1):35.
48. Zhang H, Ma K, Li RM, Li JN, Gao SF, Ma LN. Association between testosterone levels and bone mineral density in females aged 40-60 years from NHANES 2011-2016. Sci Rep. 2022;12(1):16426.
49. Kousteni S, Bellido T, Plotkin LI, et al. Nongenotropic, sex-nonspecific signaling through the estrogen or androgen receptors: dissociation from transcriptional activity. Cell. 2001;104(5):719-730.
50. Vanderschueren D, Vandenput L, Boonen S, Lindberg MK, Bouillon R, Ohlsson C. Androgens and bone. Endocr Rev. 2004;25(3):389-425.
51. Slemenda C, Longcope C, Peacock M, Hui S, Johnston CC. Sex steroids, bone mass, and bone loss. A prospective study of pre-, peri-, and postmenopausal women. J Clin Invest. 1996;97(1):14-21.
52. Davis SR, McCloud P, Strauss BJ, Burger H. Testosterone enhances estradiol's effects on postmenopausal bone density and sexuality. Maturitas. 1995;21(3):227-236.
53. Chen JF, Lin PW, Tsai YR, Yang YC, Kang HY. Androgens and androgen receptor actions on bone health and disease: from androgen deficiency to androgen therapy. Cells. 2019;8(11):1318.
54. Lai JJ, Chang P, Lai KP, Chen L, Chang C. The role of androgen and androgen receptor in skin-related disorders. Arch Dermatol Res. 2012;304(7):499-510.
55. Ceruti JM, Leirós GJ, Balañá ME. Androgens and androgen receptor action in skin and hair follicles. Mol Cell Endocrinol. 2018;465:122-133.
56. Bienenfeld A, Azarchi S, Lo Sicco K, Marchbein S, Shapiro J, Nagler AR. Androgens in women: androgen-mediated skin disease and patient evaluation. J Am Acad Dermatol. 2019;80(6):1497-1506.
57. Zouboulis CC, Degitz K. Androgen action on human skin – from basic research to clinical significance. Exp Dermatol. 2004;13(s4):5-10.
58. Bermon S. Androgens and athletic performance of elite female athletes. Curr Opin Endocrinol Diabetes Obes. 2017;24(3):246-251.
59. Bermon S, Garnier PY, Hirschberg AL, et al. Serum androgen levels in elite female athletes. J Clin Endocrinol Metab. 2014;99(11):4328-4335.
60. Sheffield-Moore M, Paddon-Jones D, Casperson SL, et al. Androgen therapy induces muscle protein anabolism in older women. J Clin Endocrinol Metab. 2006;91(10):3844-3849.
61. Huang G, Basaria S, Travison TG, et al. Testosterone dose-response relationships in hysterectomized women with or without oophorectomy: effects on sexual function, body composition, muscle performance and physical function in a randomized trial. Menopause. 2014;21(6):612-623.
62. Rutkove SB, Parker RA, Nardin RA, Connolly CE, Felice KJ, Raynor EM. A pilot randomized trial of oxandrolone in inclusion body myositis. Neurology. 2002;58(7):1081-1087.
63. Traish AM, Vignozzi L, Simon JA, Goldstein I, Kim NN. Role of androgens in female genitourinary tissue structure and function: implications in the genitourinary syndrome of menopause. Sex Med Rev. 2018;6(4):558-571.
64. Scheffers CS, Armstrong S, Cantineau AEP, Farquhar C, Jordan V. Dehydroepiandrosterone for women in the peri- or postmenopausal phase. Cochrane Database of Systematic Reviews. 2015;2017(6):CD011066.
65. Cipriani S, Maseroli E, Ravelli SA, Vignozzi L. The vagina as source and target of androgens: implications for treatment of GSM/VVA, including DHEA. Climacteric. 2023;26(4):309-315.
66. Traish AM, Kim N, Min K, Munarriz R, Goldstein I. Role of androgens in female genital sexual arousal: receptor expression, structure, and function. Fertil Steril. 2002;77 Suppl 4:S11-S18.
67. Davis SR, Worsley R, Miller KK, Parish SJ, Santoro N. Androgens and female sexual function and dysfunction--findings from the fourth International Consultation of Sexual Medicine. J Sex Med. 201;13(2):168-178.
68. Maseroli E, Santangelo A, Lara-Fontes B, et al. The non-aromatizable androgen dihydrotestosterone (DHT) facilitates sexual behavior in ovariectomized female rats primed with estradiol. Psychoneuroendocrinology. 2020;115:104606.
69. Cipriani S, Maseroli E, Vignozzi L. The role of androgens in sexual health and well-being after menopause: Unmet needs and opportunities. Curr Opin Endocr Metab Res. 2022;27(4):100405.
70. Elraiyah T, Sonbol MB, Wang Z, et al. The Benefits and harms of systemic testosterone therapy in postmenopausal women with normal adrenal function: a systematic review and meta-analysis. J Clin Endocrinol Metab. 2014;99(10):3543-3550.
71. Parish SJ, Simon JA, Davis SR, et al. International Society for the Study of Women's Sexual Health Clinical Practice Guideline for the Use of Systemic Testosterone for Hypoactive Sexual Desire Disorder in Women. J Womens Health. 2021;30(4):474-491.
72. Kim Y, Jae E, Yoon M. Influence of androgen receptor expression on the survival outcomes in breast cancer: a meta-analysis. J Breast Cancer. 2015;18(2):134-142.
73. Narayanan R, Dalton JT. Androgen receptor: a complex therapeutic target for breast cancer. Cancers (Basel). 2016;8(12):108.
74. Solomon ZJ, Mirabal JR, Mazur DJ, Kohn TP, Lipshultz LI, Pastuszak AW. Selective androgen receptor modulators: current knowledge and clinical applications. Sex Med Rev. 2019;7(1):84-94.

Review

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The Importance of Exosomes in Gynecological Diseases

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ABSTRACT

Exosomes play significant roles in key functions of the female reproductive system, such as oogenesis, implantation success, embryo development, and proper fertilization. Functional connectivity between cells is essential for the viability, development, and coordination of the female reproductive system. It has been demonstrated that the information carried by exosomes is crucial for these cooperative biological mechanisms.

Exosomes are formed and encapsulate biological molecules from the cells of origin. They contribute both to the reorganization of cellular functions and to the collective functioning of cell populations. In addition the content of exosomes may be used to monitor the diagnostic and therapeutic processes of various gynecological diseases. They contain genetic and proteomic data that can be used as biomarkers or therapeutic targets in gynecological cancers and pregnancy-related disorders.

This article will review the roles of exosomes in major female reproductive disorders, including endometriosis, premature ovarian failure, polycystic ovary syndrome, Asherman syndrome, endometrial cancer, cervical cancer, ovarian cancer, and preeclampsia.

Keywords: Exosomes, infertility. women's reproductive diseases

INTRODUCTION

Exosomes are extracellular vesicles secreted by cells and possess a double-layered lipid membrane.[1-3] These vesicles typically measure between 30-150 nm in diameter.[4] Their contents include RNA molecules, proteins, lipids, and occasionally DNA.[5-7] These components regulate various cellular functions mediated by exosomes.[8] Based on size, surface markers, biogenesis, and content, three major types of extracellular vesicles are recognized: apoptotic bodies, microvesicles, and exosomes.[9,10] Exosomes are effective paracrine regulators of intercellular communication. In recent years, they have been shown to participate in biological functions including metabolic regulation, cell proliferation, apoptosis, angiogenesis, antigen presentation, inflammation, tumor pathogenesis, tissue repair, and reproduction.[11-13] Exosomes are produced through the inward budding of endosomal membranes, forming multivesicular bodies, which later fuse with the plasma membrane to release exosomes.[14] They interact with target cells via ligand-receptor binding or endocytosis.[15] Following this interaction, vesicles are internalized by phagocytosis.[2,16] Functional protein groups found in exosomes include β-actin, GPI-anchored proteins, heat shock proteins (HSP8, HSP90), tubulin, and tetraspanins such as CD9, CD63, and CD8.[17] Under both physiological and pathological conditions, exosomes reflect the molecular characteristics of their donor cells. This makes them valuable prognostic and diagnostic biomarkers.[18,19] Exosomes are be secreted from various tissues of the female reproductive system, including the fallopian tube epithelium, follicular fluid, endometrium, uterus, and placenta.[20-23] Proper reproductive function and successful pregnancy rely heavily on effective intercellular communication. Oogenesis, follicular development, implantation, fertilization, and embryo development are closely tied to maternal-embryo cellular interaction during pregnancy.[13,24,25] Studies have confirmed both direct and indirect roles of exosomes in cellular communication.[26-28]

Exosomes communicate with recipient cells through delivery of the exosomal contents.[29-31] Once exosomes are internalized, they initiate physiological processes by

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delivering bioactive molecules such as coding and non-coding RNAs, proteins, and lipids. These molecules modulate the functions of the recipient cells.[32-34] Studies in human and animal models have demonstrated that exosomes are involved in follicular development, oocyte maturation, and embryo formation. They are also known to carry microRNAs (miRNAs) involved in meiotic resumption and ovulation signaling pathways.[35] miRNAs are non-coding RNAs composed of 21-24 nucleotides and participate in various biological processes. They regulate oocyte development, follicular growth, implantation, and embryo development by targeting key genes.[36] The most common signaling pathways by miRNAs include Wnt (Wingless), neurotrophin, epidermal growth factor receptor (EGFR), and transforming growth factor-beta (TGF-β) pathways.[37-39] Research into the roles of exosomes in reproductive disorders is expanding rapidly. Due to their diagnostic and therapeutic potential, exosomes are anticipated to play an increasingly significant role in future gynecological disease management.[40] This review focuses on the implications in polycystic ovary syndrome (PCOS), premature ovarian failure (POF), Asherman syndrome, endometriosis, endometrial cancer, cervical cancer, ovarian cancer, and preeclampsia.

Polycystic Ovary Syndrome

PCOS is an endocrine disorder characterized by ovulatory dysfunction and hyperandrogenism. Affecting 6-8% of women globally, PCOS is associated with infertility, obesity, insulin resistance, dyslipidemia, type 2 diabetes, and cardiovascular diseases.[41-45] Follicular fluid analyses from PCOS patients revealed increased expression levels of miR-25-3p, miR-126-3p, miR-143-3p, miR-146a-5p, miR-193b-3p, miR-199a-5p, miR-199a-3p, miR-199b-3p, miR-629-5p, miR-4532, miR-4745-3p, and miR-6087. In addition, elevated levels of miR-10a-5p, miR-18a-3p, miR-20b-5p, miR-23b-3p, miR-98-5p, miR-106a-5p, miR-141-3p, miR-200a-3p, miR-200c-3p, miR-382-5p, miR-483-5p, miR-483-3p, and miR-3911 were observed. Changes in tRNA and piRNA expression patterns were also noted in exosomes derived from PCOS patients.[46-48] These miRNA alterations are implicated in the mitogen-activated protein kinase signaling pathway, circadian rhythm regulation, endocytosis, and overall PCOS risk.[46-48]Another study reported increased expression of S100-A9 in the exosomes of PCOS patients.[49] S100-A9, a calcium-binding protein secreted by ovarian, granulosa, and immune cells, is involved in cell cycle regulation, proliferation, and inflammation.[50,51] These exosomes were shown to activate the NF-κB signaling pathway in granulosa-like tumor cells and elevate pro-inflammatory factor expression.[49] This inflammatory mechanism may underlie reproductive dysfunction in PCOS.[41,49] In addition to their diagnostic value, exosomes may offer therapeutic benefits. For instance, exosomes derived from adipose mesenchymal stem cells (MSCs) alleviated PCOS symptoms by inhibiting apoptosis via miR-323-3p and altering PDCD4 expression.[52]

Premature Ovarian Failure

POF is an infertility disorder characterized by hypergonadotropism, amenorrhea, and estrogen deficiency due to follicular dysfunction. It affects approximately 1% of women aged 30-39 years.[53-55] While its etiology remains unclear, POF is considered a heterogeneous condition influenced by both genetic and environmental factors.[56] Recent studies have investigated the therapeutic potential of exosomes in POF. For example, exosomes derived from placenta-derived (PD)-MSCs increased the expression of antioxidant enzymes such as catalase and peroxiredoxin (PRDX1) in ovariectomized rats, improving ovarian function and reducing mitochondrial reactive oxygen species levels. Similarly, human amniotic epithelial cell-derived exosomes containing miR-1246 were found to restore ovarian function in POF mice via modulation of apoptosis- and phosphatidylinositol-related pathways.[57] Exosomes derived from various MSCs also improved follicular morphology and suppressed apoptosis through miR-664-5p, targeting p53.[58] Another study demonstrated that bone marrow-derived MSC (BMSC) exosomes containing miR-144-5p targeted PTEN, inhibited apoptosis, and improved ovarian function in POF rats.[59] Collectively, these studies suggest that exosome-based therapy could represent a promising approach for the treatment of POF.

Asherman Syndrome

Asherman syndrome is characterized by intrauterine adhesions caused by trauma, leading to hypomenorrhea and infertility.[60] These scar tissues obstruct blastocyst implantation and result in infertility. Although surgical intervention is commonly used to treat this condition, alternative therapeutic strategies are still required.[61,62] Recent studies suggest that exosomal therapy could be beneficial in Asherman syndrome. In a rat model, MSC-derived exosomes were shown to reduce fibrosis and promote proliferation and vascularization in uterine tissue. Following exosome application, gene expression levels of matrix metalloproteinases MMP-2 and MMP-9, proliferating cell nuclear antigen, CD31, and vascular EGFR were increased, while tissue inhibitor of metalloproteinase-2 levels decreased. These findings suggest that exosomes could be promising biomolecules for treating Asherman syndrome.

Endometriosis

Endometriosis is a multifactorial, estrogen-dependent disorder characterized by the presence of endometrial tissue outside the uterine cavity. The main clinical manifestations include pelvic pain and infertility.[64-66] Currently, no definitive treatment ensures the complete resolution of symptoms or long-term remission.[67,68] Exosomes have emerged as both therapeutic agents and biomarkers for understanding the pathophysiology of endometriosis. Some studies have identified novel diagnostic targets, while others suggest therapeutic roles for exosomes.

Exosomes derived from the endometrium have contributed significantly to elucidating the underlying mechanisms of endometriosis. In one study, exosomes isolated from peritoneal fluid samples of patients with endometriosis contained histone type 2-C, PRDX1, inter-α-trypsin inhibitor heavy chain H4, annexin A2, and tubulin α-chain.[69] Another study examining tissue and plasma-derived exosomes from endometriosis patients reported significant differences in miRNA and lncRNA profiles. Decreased expression was noted in lncRNAs LINC00293, LINC00929, MEG8, SNHG25, and RP5-898J17.1, while increased expression was observed in LINC00998, NEAT1, PVT1, H19, and RP4-561L24.3. These RNA molecules influence signaling pathways associated with angiogenesis and inflammation.[70] Exosomal miRNAs, including miR-130b, miR-145, miR-342, miR-365, miR-425, miR-432, miR-451a, miR-486-5p, miR-505, miR-1908, miR-4488, and miR-6508 have shown significant associations with inflammatory processes in endometriosis.[71] A recent study proposed that elevated serum levels of exosomal miR-22-3p and miR-320a could serve as diagnostic markers for endometriosis.[72] These findings highlight the potential of exosomes in improving diagnostic accuracy and developing novel treatment approaches. Another essential feature of exosomes is their therapeutic potential. Exosomes from healthy endometrial epithelial cells carry molecules important for embryo-endometrial interaction during implantation.[72] Application of these exosomes in endometriosis models has shown beneficial effects in modulating the ectopic endometrial environment.[73-76] Proteins such as focal adhesion kinase and various surface receptors have been shown to influence the adhesive and migratory capacities of trophoblast cells via exosomal signaling.[77] miRNAs, including miR-17, miR-30d, miR-106a, and miR-200c were found to play critical roles in implantation success when transferred by exosomes.[29,78,80] M2 macrophage-derived exosomes exhibit regenerative properties that may reduce endometriotic lesions. These exosomes, which are underrepresented or altered in patients with endometriosis, contribute to macrophage activation mediated by miR-223.[81,82] Wu et al.[83] demonstrated that miR-214 suppresses fibrosis and promotes lesion regression. Collectively, these studies suggest that exosomes may modulate immune escape, cell proliferation, angiogenesis, and lesion invasion in endometriosis. Exosomes derived from ectopic or shed endometrial tissue might also induce metaplasia or tissue repair in recipient cells through the miRNAs and specialized proteins they carry.[84]

Endometrial Cancers

Endometrial cancer is the fourth most common malignancy of the female reproductive system.[85] While most cases are diagnosed early due to postmenopausal bleeding, approximately 20% are identified at an advanced stage.[86,87] Surgical procedures, radiotherapy, and chemotherapy are commonly employed in treatment, but these approaches are often insufficient. Therefore, identifying new molecular targets and biomarkers is important for more effective disease management. Exosomes play important roles in the pathogenesis, progression, diagnosis, and potential treatment of endometrial cancer. Communication between endometrial fibroblasts and cancer cells via exosomes has been proposed.[88] In one study, exosomes derived from cancer-associated fibroblasts lacked miR-148b, contributing to tumor progression. Under normal conditions, miR-148b suppresses DNA (cytosine-5)-methyltransferase 1, a protein involved in metastasis by promoting epithelial-mesenchymal transition.[89] The absence of miR-148b in CAF-derived exosomes is believed to drive endometrial cancer progression through this mechanism. Furthermore, exosomes isolated from the plasma of endometrial cancer patients were shown to promote angiogenesis in human umbilical vein endothelial cells by activating the PI3K/AKT/VEGFA signaling pathway.[90] miR-320a, which targets hypoxia-inducible factor 1-alpha, normally suppresses VEGFA expression and cell proliferation. However, reduced levels of miR-320a in CAF-derived exosomes may facilitate malignancy in endometrial cancer.[91] Exosomes from the serum of PCOS patients have been reported to enhance the migration and invasion of endometrial cancer cells via upregulation of miR-27a-5p, which targets SADM4.[92] In another study, 114 dysregulated miRNAs were identified in exosomes isolated from peritoneal lavage fluid of endometrial cancer patients. Notable miRNAs included miR-10b-5p, miR-34b-3p, miR-34c-5p, miR-34c-3p, miR-449b-5p, miR-200b-3p, miR-383-5p, and miR-2110, all of which were proposed as novel biomarkers.[93] These studies suggest the potential importance of exosome-derived data in endometrial cancer research. Furthermore, they indicate that the sample type and cellular origin of exosomes may be critical for accurate diagnosis and effective therapeutic targeting. The biological source of exosomes may influence tumor behavior, highlighting the need for careful evaluation of exosome origin in both research and clinical applications.

Cervical Cancer

Cervical cancer originates from squamocolumnar junction cells of the cervix and is closely associated with human papillomavirus infection.[94,95] Early diagnosis, as in many cancers, is important for preventing disease progression and improving outcomes.[96] Consequently, identifying novel biomarkers for early detection is of great clinical significance. Exosomal miRNAs have been shown to be associated with the progression of cervical cancer. Increased expression levels of miR-21, miR-146a, miR-221-3p, miR-222, let-7d-3p, and miR-30d-5p were found in cervical lavage samples and cell lines, while plasma levels of miR-125a-5p were decreased in cervical cancer patients.[97-102] Among these, miR-221-3p has been identified as a key regulator of angiogenesis through its modulation of the *thrombospondin-2* gene.[103] These findings support the use of exosomal miRNAs and other molecules as potential diagnostic and therapeutic biomarkers for cervical cancer. Moreover, exosomes derived from cervical cancer cell lines have been found to carry high levels of miRNAs targeting Hedgehog signaling pathway components such as PTCH1, smoothened, frizzled family receptor, sonic hedgehog signaling molecule, Indian hedgehog signaling molecule. This

pathway is implicated in cervical cancer growth, metastasis, invasion, and drug resistanc.[104] Exosome-based analysis of this pathway may help identify novel therapeutic agents that can inhibit Hedgehog signaling and halt disease progression. Ongoing research has also demonstrated the potential of exosomes as therapeutic agents. For example, exosomes enriched with miR-22 have shown a positive effect on radiotherapy efficacy by downregulating c-Myc binding protein (MYCBP) and human telomerase reverse transcriptase (hTERT) gene expression.[105] Given the molecular cargo carried by exosomes, they may hold potential for contributing to both diagnosis and therapy in the management of cervical cancer.

Ovarian Cancer

Ovarian cancer is the most lethal gynecological malignancy and ranks among the most prevalent cancers affecting the female reproductive system.[106] More than 50% of patients are diagnosed at an advanced stage, contributing to a five-year survival rate of less than 50%.[85,107] The poor prognosis and quality of life among ovarian cancer patients are partly attributed to the absence of effective early diagnostic tools. Thus, the development of novel diagnostic and therapeutic strategies will be beneficial for identifying cases earlier and improving outcomes.[108] Exosomes secreted by ovarian cancer cells have been shown to play significant roles in tumor progression, metastasis, and invasion. Exosomal proteins such as TSG101, CD9, CD24, CD44, and CD63 contribute to the development of ovarian cancer by facilitating intercellular communication. Molecules like HSP27, HSP70, and HSP90 are highly expressed in ovarian cancer patients and are involved in disease pathogenesis.[109-113] Enzymes, such as aldehyde reductase, phosphoglycerate isomerase, fatty acid synthase, and PRDX1, together with major histocompatibility complex class I and II have also been associated with tumor development and metastasis[108,114]. In addition to their roles in tumor biology, exosomal proteins are involved in drug resistance. Elevated levels of annexin A3 in exosomes has been linked to increased platinum resistance in ovarian cancer cells.[115] Exosomal miRNAs including miR-106a, miR-130a, miR-221, miR-222, miR-433, and miR-591 have also been associated with drug resistance mechanisms.[116-120] Exosome-associated miRNAs miR-21, miR-184, miR-193b, miR-200a, miR-200b, miR-200c, miR-203, miR-214, and miR-215 have shown potential as diagnostic biomarkers for ovarian cancer.[112,114,121-123] Additional miRNAs including miR-25, miR-29b, miR-100, miR-105, miR-150, miR-187, miR-221, and miR-335 are implicated in the development of malignant ovarian tumors.[112,114,124] Notably, miR-21 has emerged as a critical player in oncogenesis and metastasis in serous ovarian carcinoma, functioning as an oncomiR.[125] Moreover, exosome-delivered molecules have therapeutic potential. For instance, miR-29c, miR-101, miR-128, miR-182, miR-506, and miR-520d-3p are under investigation as possible treatment targets for ovarian cancer.[126] Together, these studies suggest that non-coding RNAs and proteins delivered via exosomes may play important roles in the biology, diagnosis, and treatment of ovarian cancer. Gaining a better understanding of the mechanisms through which exosomes influence ovarian cancer progression could potentially contribute to the development of more effective therapies and improved disease management.

Preeclampsia

Preeclampsia is a hypertensive disorder of pregnancy responsible for 10-15% of all fetal deaths. It is associated with significant maternal and fetal morbidity and typically occurs after the 20th week of gestation. The condition is often characterized by placental hypoxia.[127-130] Despite considerable research, the molecular mechanisms underlying preeclampsia remain unclea.[131-133] Recent evidence suggests that exosomes released by placental trophoblasts into maternal circulation may contribute to the pathogenesis of preeclampsia.[134] Hypoxic conditions in the placenta are known to increase the release of exosomes from the syncytiotrophoblast layer.[135,136] Therefore, analyzing the contents of PD exosomes may help to understand disease pathogenesis and improve diagnostic capabilities. Increased levels of syncytin a protein involved in the differentiation of syncytiotrophoblasts from villous trophoblasts, have been found in the exosomes of preeclamptic patients. These trophoblasts play a key role in remodeling maternal spiral arteries and differentiating vascular endothelial and smooth muscle cells.[137-139] Exosomal profiling in preeclamptic patients revealed that miR-23a-3p, miR-125b-2-3p, miR-144-3p, miR-192-5p, miR-205-5p, miR-208a-3p, miR-335-5p, miR-451a, miR-518a-3p, and miR-542-3p were downregulated. In contrast, miR-7a-5p, miR-17-5p, miR-26a-5p, miR-30c-3p, miR-141-3p, miR-199a-3p, miR-221-3p, miR-584-5p, miR-744-5p, and miR-6724-5p were upregulated.[140-143]

Safety of Exosomes

Exosomes have been shown to distribute into all body compartments bypassing blood-brain barrier, blood testis barrier and blood follicle barriers.[144-146] This suggests a novel use for exosomes as drug delivery agents. This is in addition to their potential as diagnostic agents in different diseases and direct use as therapeutic agents. Specific tissue cell culture exosomes were classified as enhanced exosomes while natural *in vivo* produced exosomes from stem cells can be classified as naive exosomes.[147] Naive exosomes are mostly obtained from human umblical cord stem cells, human umblical cord blood stem cells, human and human induced pluripotent stem cell derived MSCs.[148] Exosomes were not reported to cause immunologic reactions and can be applied to the area of inflamation.[149] Exosomes were not shown to form teratomas in contrast to some stem cell therapie.[150]

Advences in technology are driving progress in the diagnostic and therapeutic strategies for gynecological diseases. However, new approaches are still needed to address the complexities of these conditions. Research suggests that exosomes offer promising potential in gynecological disorders by providing new mechanisms for diagnosis, treatment, and disease monitoring. Exosome-based studies are expected to make substantial

contributions to understanding and managing gynecological diseases, particularly through the identification of novel diagnostic markers and therapeutic targets, and improved patient monitoring strategies.

Footnote

Authorship Contributions

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REFERENCES

1. Cocucci E, Racchetti G, Meldolesi J. Shedding microvesicles: artefacts no more. Trends Cell Biol. 2009;19(2):43-51.
2. EL Andaloussi S, Mäger I, Breakefield XO, Wood MJ. Extracellular vesicles: biology and emerging therapeutic opportunities. Nat Rev Drug Discov. 2013;12(5):347-357.
3. Tannetta D, Dragovic R, Alyahyaei Z, Southcombe J. Extracellular vesicles and reproduction-promotion of successful pregnancy. Cell Mol Immunol. 2014;11(6):548-563.
4. György B, Szabó TG, Pásztói M, et al. Membrane vesicles, current state-of-the-art: emerging role of extracellular vesicles. Cell Mol Life Sci. 2011;68(16):2667-2688.
5. Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol. 2007;9(6):654-659.
6. Simpson RJ, Jensen SS, Lim JW. Proteomic profiling of exosomes: current perspectives. Proteomics. 2008;8(19):4083-4099.
7. Subra C, Laulagnier K, Perret B, Record M. Exosome lipidomics unravels lipid sorting at the level of multivesicular bodies. Biochimie. 2007;89(2):205-212.
8. Ding S, Zhang J, Dai Q, et al. Cardioprotective effects of exosomes and their potential therapeutic use. Adv Exp Med Biol. 2017;998:163-177.
9. Lai CP, Breakefield XO. Role of exosomes/microvesicles in the nervous system and use in emerging therapies. Front Physiol. 2012;3:228.
10. Neven KY, Nawrot TS, Bollati V. Extracellular vesicles: how the external and internal environment can shape cell-to-cell communication. Curr Environ Health Rep. 2017;4(1):30-37.
11. Rezaie J, Ajezi S, Avci ÇB, et al. Exosomes and their application in biomedical field: difficulties and advantages. Mol Neurobiol. 2018;55(4):3372-3393.
12. Kalluri R. The biology and function of exosomes in cancer. J Clin Invest. 2016;126(4):1208-1215.
13. Machtinger R, Laurent LC, Baccarelli AA. Extracellular vesicles: roles in gamete maturation, fertilization and embryo implantation. Hum Reprod Update. 2016;22(2):182-193.
14. Aghabozorgi AS, Ahangari N, Eftekhaari TE, et al. Circulating exosomal miRNAs in cardiovascular disease pathogenesis: New emerging hopes. J Cell Physiol. 2019;234(12):21796-21809.
15. Urbanelli L, Magini A, Buratta S, et al. Signaling pathways in exosomes biogenesis, secretion and fate. Genes (Basel). 2013;4(2):152-170.
16. Mathivanan S, Ji H, Simpson RJ. Exosomes: extracellular organelles important in intercellular communication. J Proteomics. 2010;73(10):1907-1920.
17. Lee Y, El Andaloussi S, Wood MJ. Exosomes and microvesicles: extracellular vesicles for genetic information transfer and gene therapy. Hum Mol Genet. 2012;21(R1):R125-R134.
18. Gould SJ, Raposo G. As we wait: coping with an imperfect nomenclature for extracellular vesicles. J Extracell Vesicles. 2013;2.
19. Lötvall J, Hill AF, Hochberg F, et al. Minimal experimental requirements for definition of extracellular vesicles and their functions: a position statement from the International Society for Extracellular Vesicles. J Extracell Vesicles. 2014;3:26913.
20. Almiñana C, Corbin E, Tsikis G, Soleilhavoup C, Galio L, Sandra O. 108 characterization of bovine oviductal exosomes from in vivo and in vitro origin. Reprod Fertil Dev 2015;27(1):147.
21. Kropp J, Salih SM, Khatib H. Expression of microRNAs in bovine and human pre-implantation embryo culture media. Front Genet. 2014;5:91.
22. Kropp J, Khatib H. Characterization of microRNA in bovine in vitro culture media associated with embryo quality and development. J Dairy Sci. 2015;98(9):6552-6563.
23. Kropp J, Khatib H. mRNA fragments in in vitro culture media are associated with bovine preimplantation embryonic development. Front Genet. 2015;6:273.
24. Ng YH, Rome S, Jalabert A, et al. Endometrial exosomes/microvesicles in the uterine microenvironment: a new paradigm for embryo-endometrial cross talk at implantation. PLoS One. 2013;8(3):e58502.
25. Valipour J, Nashtaei MS, Khosravizadeh Z, et al. Effect of sulforaphane on apoptosis, reactive oxygen species and lipids peroxidation of human sperm during cryopreservation. Cryobiology. 2021;99:122-130.
26. da Silveira JC, Veeramachaneni DN, Winger QA, Carnevale EM, Bouma GJ. Cell-secreted vesicles in equine ovarian follicular fluid contain miRNAs and proteins: a possible new form of cell communication within the ovarian follicle. Biol Reprod. 2012;86(3):71.
27. Al-Dossary AA, Strehler EE, Martin-Deleon PA. Expression and secretion of plasma membrane Ca2+-ATPase 4a (PMCA4a) during murine estrus: association with oviductal exosomes and uptake in sperm. PLoS One. 2013;8(11):e80181.
28. Ruiz-González I, Xu J, Wang X, Burghardt RC, Dunlap KA, Bazer FW. Exosomes, endogenous retroviruses and toll-like receptors: pregnancy recognition in ewes. Reproduction. 2015;149(3):281-291.
29. Zhao H, Achreja A, Iessi E, Logozzi M, Mizzoni D, Di Raimo R, Nagrath D, Fais S. The key role of extracellular vesicles in the metastatic process. Biochim Biophys Acta Rev Cancer. 2018;1869(1):64-77.
30. Parolini I, Federici C, Raggi C, et al. Microenvironmental pH is a key factor for exosome traffic in tumor cells. J Biol Chem. 2009;284(49):34211-34222.
31. Andreola G, Rivoltini L, Castelli C, et al. Induction of lymphocyte apoptosis by tumor cell secretion of FasL-bearing microvesicles. J Exp Med. 2002;195(10):1303-1316.
32. Yáñez-Mó M, Siljander PR, Andreu Z, et al. Biological properties of extracellular vesicles and their physiological functions. J Extracell Vesicles. 2015;4:27066.
33. Ahmed KA, Xiang J. Mechanisms of cellular communication through intercellular protein transfer. J Cell Mol Med. 2011;15(7):1458-1473.
34. Smyth T, Kullberg M, Malik N, Smith-Jones P, Graner MW, Anchordoquy TJ. Biodistribution and delivery efficiency of unmodified tumor-derived exosomes. J Control Release. 2015;199:145-155.
35. Ebrahimi R, Bahiraee A, Niazpour F, Emamgholipour S, Meshkani R. The role of microRNAs in the regulation of insulin signaling

pathway with respect to metabolic and mitogenic cascades: a review. J Cell Biochem. 2019;120(12):19290-19309.

36. Pasquariello R, Manzoni EFM, Fiandanese N, et al. Implications of miRNA expression pattern in bovine oocytes and follicular fluids for developmental competence. Theriogenology. 2020;145:77-85.
37. Boyer A, Goff AK, Boerboom D. WNT signaling in ovarian follicle biology and tumorigenesis. Trends Endocrinol Metab. 2010;21(1):25-32.
38. Dissen GA, Garcia-Rudaz C, Ojeda SR. Role of neurotrophic factors in early ovarian development. Semin Reprod Med. 2009;27(1):24-31.
39. Ali M, Esfandyari S, Al-Hendy A. Evolving role of microRNAs in uterine fibroid pathogenesis: filling the gap! Fertil Steril. 2020;113(6):1167-1168.
40. Conlan RS, Pisano S, Oliveira MI, Ferrari M, Mendes Pinto I. Exosomes as reconfigurable therapeutic systems. Trends Mol Med. 2017;23(7):636-650.
41. Rocha AL, Oliveira FR, Azevedo RC, et al. Recent advances in the understanding and management of polycystic ovary syndrome. F1000Res. 2019;8:F1000 Faculty Rev-565.
42. Ndefo UA, Eaton A, Green MR. Polycystic ovary syndrome: a review of treatment options with a focus on pharmacological approaches. P T. 2013;38(6):336-355.
43. Chugh RM, Park HS, Esfandyari S, Elsharoud A, Ulin M, Al-Hendy A. Mesenchymal stem cell-conditioned media regulate steroidogenesis and inhibit androgen secretion in a PCOS cell model via BMP-2. Int J Mol Sci. 2021;22(17):9184.
44. Qu J, Wang Y, Wu X, Gao L, Hou L, Erkkola R. Insulin resistance directly contributes to androgenic potential within ovarian theca cells. Fertil Steril. 2009;91(5 Suppl):1990-1997.
45. Rashidi Z, Khosravizadeh Z, Talebi A, Khodamoradi K, Ebrahimi R, Amidi F. Overview of biological effects of Quercetin on ovary. Phytother Res. 2021;35(1):33-49.
46. Hu J, Tang T, Zeng Z, Wu J, Tan X, Yan J. The expression of small RNAs in exosomes of follicular fluid altered in human polycystic ovarian syndrome. PeerJ. 2020;8:e8640.
47. Thomson T, Lin H. The biogenesis and function of PIWI proteins and piRNAs: progress and prospect. Annu Rev Cell Dev Biol. 2009;25:355-376.
48. Jiang X, Li J, Zhang B, et al. Differential expression profile of plasma exosomal microRNAs in women with polycystic ovary syndrome. Fertil Steril. 2021;115(3):782-792.
49. Li H, Huang X, Chang X, et al. S100-A9 protein in exosomes derived from follicular fluid promotes inflammation via activation of NF-κB pathway in polycystic ovary syndrome. J Cell Mol Med. 2020;24(1):114-125.
50. Adams J, Liu Z, Ren YA, et al. Enhanced inflammatory transcriptome in the granulosa cells of women with polycystic ovarian syndrome. J Clin Endocrinol Metab. 2016;101(9):3459-3468.
51. Heizmann CW, Fritz G, Schäfer BW. S100 proteins: structure, functions and pathology. Front Biosci. 2002;7:d1356-d1368.
52. Zhao Y, Tao M, Wei M, Du S, Wang H, Wang X. Mesenchymal stem cells derived exosomal miR-323-3p promotes proliferation and inhibits apoptosis of cumulus cells in polycystic ovary syndrome (PCOS). Artif Cells Nanomed Biotechnol. 2019;47(1):3804-3813.
53. Zhang C. The roles of different stem cells in premature ovarian failure. Curr Stem Cell Res Ther. 2020;15(6):473-481.
54. Esfandyari S. miRNA-144 Increases estrogen-producing genes expression and suppress apoptosis in human granulosa cell line. Fertil. Steril. 2020, 114, e438.
55. Chen L, Guo S, Wei C, Li H, Wang H, Xu Y. Effect of stem cell transplantation of premature ovarian failure in animal models and patients: a meta-analysis and case report. Exp Ther Med. 2018;15(5):4105-4118.
56. Hoek A, Schoemaker J, Drexhage HA. Premature ovarian failure and ovarian autoimmunity. Endocr Rev. 1997;18(1):107-134.
57. Zhang Q, Sun J, Huang Y, et al. Human amniotic epithelial cell-derived exosomes restore ovarian function by transferring micrornas against apoptosis. Mol Ther Nucleic Acids. 2019;16:407-418.
58. Sun B, Ma Y, Wang F, Hu L, Sun Y. miR-644-5p carried by bone mesenchymal stem cell-derived exosomes targets regulation of p53 to inhibit ovarian granulosa cell apoptosis. Stem Cell Res Ther. 2019;10(1):360.
59. Yang M, Lin L, Sha C, et al. Bone marrow mesenchymal stem cell-derived exosomal miR-144-5p improves rat ovarian function after chemotherapy-induced ovarian failure by targeting PTEN. Lab Invest. 2020;100(3):342-352.
60. Esfandyari S, Chugh RM, Park HS, Hobeika E, Ulin M, Al-Hendy A. Mesenchymal stem cells as a bio organ for treatment of female infertility. Cells. 2020;9(10):2253.
61. Dreisler E, Kjer JJ. Asherman's syndrome: current perspectives on diagnosis and management. Int J Womens Health. 2019;11:191-198.
62. March CM. Asherman's syndrome. Semin Reprod Med. 2011;29(2):83-94.
63. Saribas GS, Ozogul C, Tiryaki M, Alpaslan Pinarli F, Hamdemir Kilic S. Effects of uterus derived mesenchymal stem cells and their exosomes on asherman's syndrome. Acta Histochem. 2020;122(1):151465.
64. Sasson IE, Taylor HS. Stem cells and the pathogenesis of endometriosis. Ann N Y Acad Sci. 2008;1127:106-115.
65. Ceccaroni M, Roviglione G, Rosenberg P, et al. Pericardial, pleural and diaphragmatic endometriosis in association with pelvic peritoneal and bowel endometriosis: a case report and review of the literature. Wideochir Inne Tech Maloinwazyjne. 2012;7(2):122-131.
66. Khodarahmian M, Amidi F, Moini A, et al. A randomized exploratory trial to assess the effects of resveratrol on VEGF and TNF-α 2 expression in endometriosis women. J Reprod Immunol. 2021;143:103248.
67. Maybin JA, Critchley HO. Menstrual physiology: implications for endometrial pathology and beyond. Hum Reprod Update. 2015;21(6):748-761.
68. Karamian A, Paktinat S, Esfandyari S, Nazarian H, Ziai SA, Zarnani AH, et al. Pyrvinium pamoate induces in-vitro suppression of IL-6 and IL-8 produced by human endometriotic stromal cells. Hum. Exp. Toxicol. 2020.
69. Nazri HM, Imran M, Fischer R, et al. Characterization of exosomes in peritoneal fluid of endometriosis patients. Fertil Steril. 2020;113(2):364-373.
70. Khalaj K, Miller JE, Lingegowda H, et al. Extracellular vesicles from endometriosis patients are characterized by a unique miRNA-lncRNA signature. JCI Insight. 2019;4(18):e128846.
71. Chen Y, Wang K, Xu Y, et al. Alteration of myeloid-derived suppressor cells, chronic inflammatory cytokines, and exosomal mirna contribute to the peritoneal immune disorder of patients with endometriosis. Reprod Sci. 2019;26(8):1130-1138.
72. Zhang L, Li H, Yuan M, Li D, Sun C, Wang G. Serum exosomal microRNAs as potential circulating biomarkers for endometriosis. Dis Markers. 2020;2020:2456340.
73. Sharpe-Timms KL. Endometrial anomalies in women with endometriosis. Ann N Y Acad Sci. 2001;943:131-147.
74. Pabona JM, Simmen FA, Nikiforov MA, et al. Krüppel-like factor 9 and progesterone receptor coregulation of decidualizing endometrial stromal cells: implications for the pathogenesis of endometriosis. J Clin Endocrinol Metab. 2012;97(3):E376-E392.

75. Zelenko Z, Aghajanova L, Irwin JC, Giudice LC. Nuclear receptor, coregulator signaling, and chromatin remodeling pathways suggest involvement of the epigenome in the steroid hormone response of endometrium and abnormalities in endometriosis. Reprod Sci. 2012;19(2):152-162.
76. Aghajanova L, Giudice LC. Molecular evidence for differences in endometrium in severe versus mild endometriosis. Reprod Sci. 2011;18(3):229-251.
77. Greening DW, Nguyen HP, Elgass K, Simpson RJ, Salamonsen LA. Human endometrial exosomes contain hormone-specific cargo modulating trophoblast adhesive capacity: insights into endometrial-embryo interactions. Biol Reprod. 2016;94(2):38.
78. Homer H, Rice GE, Salomon C. Review: embryo- and endometrium-derived exosomes and their potential role in assisted reproductive treatments-liquid biopsies for endometrial receptivity. Placenta. 2017;54:89-94.
79. Shomali N, Hemmatzadeh M, Yousefzadeh Y, et al. Exosomes: emerging biomarkers and targets in folliculogenesis and endometriosis. J Reprod Immunol. 2020;142:103181.
80. Vilella F, Moreno-Moya JM, Balaguer N, et al. Hsa-miR-30d, secreted by the human endometrium, is taken up by the pre-implantation embryo and might modify its transcriptome. Development. 2015;142(18):3210-3221.
81. Ismail N, Wang Y, Dakhlallah D, et al. Macrophage microvesicles induce macrophage differentiation and miR-223 transfer. Blood. 2013;121(6):984-995.
82. Schjenken JE, Panir K, Robertson SA, Hull ML. Exosome-mediated intracellular signalling impacts the development of endometriosis-new avenues for endometriosis research. Mol Hum Reprod. 2019;25(1):2-4.
83. Wu D, Lu P, Mi X, Miao J. Exosomal miR-214 from endometrial stromal cells inhibits endometriosis fibrosis. Mol Hum Reprod. 2018;24(7):357-365.
84. Klemmt PAB, Starzinski-Powitz A. Molecular and cellular pathogenesis of endometriosis. Curr Womens Health Rev. 2018;14(2):106-116.
85. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin. 2019;69(1):7-34.
86. Leskela S, Pérez-Mies B, Rosa-Rosa JM, et al. Molecular basis of tumor heterogeneity in endometrial carcinosarcoma. Cancers (Basel). 2019;11(7):964.
87. Carvalho MJ, Laranjo M, Abrantes AM, Torgal I, Botelho MF, Oliveira CF. Clinical translation for endometrial cancer stem cells hypothesis. Cancer Metastasis Rev. 2015;34(3):401-416.
88. Maida Y, Takakura M, Nishiuchi T, Yoshimoto T, Kyo S. Exosomal transfer of functional small RNAs mediates cancer-stroma communication in human endometrium. Cancer Med. 2016;5(2):304-314.
89. Li BL, Lu W, Qu JJ, Ye L, Du GQ, Wan XP. Loss of exosomal miR-148b from cancer-associated fibroblasts promotes endometrial cancer cell invasion and cancer metastasis. J Cell Physiol. 2019;234(3):2943-2953.
90. Song Y, Wang M, Tong H, et al. Plasma exosomes from endometrial cancer patients contain LGALS3BP to promote endometrial cancer progression. Oncogene. 2021;40(3):633-646.
91. Zhang N, Wang Y, Liu H, Shen W. Extracellular vesicle encapsulated microRNA-320a inhibits endometrial cancer by suppression of the HIF1α/VEGFA axis. Exp Cell Res. 2020;394(2):112113.
92. Che X, Jian F, Chen C, Liu C, Liu G, Feng W. PCOS serum-derived exosomal miR-27a-5p stimulates endometrial cancer cells migration and invasion. J Mol Endocrinol. 2020;64(1):1-12.
93. Roman-Canal B, Moiola CP, Gatius S, et al. EV-Associated miRNAs from peritoneal lavage are a source of biomarkers in endometrial cancer. Cancers (Basel). 2019;11(6):839.
94. Ault KA. Epidemiology and natural history of human papillomavirus infections in the female genital tract. Infect Dis Obstet Gynecol. 2006;2006 Suppl:40470.
95. Barchuk A, Bespalov A, Huhtala H, et al. Breast and cervical cancer incidence and mortality trends in Russia 1980-2013. Cancer Epidemiol. 2018;55:73-80.
96. Kori M, Yalcin Arga K. Potential biomarkers and therapeutic targets in cervical cancer: Insights from the meta-analysis of transcriptomics data within network biomedicine perspective. PLoS One. 2018;13(7):e0200717.
97. Liu J, Sun H, Wang X, et al. Increased exosomal microRNA-21 and microRNA-146a levels in the cervicovaginal lavage specimens of patients with cervical cancer. Int J Mol Sci. 2014;15(1):758-773.
98. Zheng M, Hou L, Ma Y, et al. Exosomal let-7d-3p and miR-30d-5p as diagnostic biomarkers for non-invasive screening of cervical cancer and its precursors. Mol Cancer. 2019;18(1):76.
99. Lv A, Tu Z, Huang Y, Lu W, Xie B. Circulating exosomal miR-125a-5p as a novel biomarker for cervical cancer. Oncol Lett. 2021;21(1):54.
100. Pan ZX, Zhang XY, Chen SR, Li CZ. Upregulated exosomal miR-221/222 promotes cervical cancer via repressing methyl-CpG-binding domain protein 2. Eur Rev Med Pharmacol Sci. 2019;23(9):3645-3653.
101. Wu XG, Zhou CF, Zhang YM, et al. Cancer-derived exosomal miR-221-3p promotes angiogenesis by targeting THBS2 in cervical squamous cell carcinoma. Angiogenesis. 2019;22(3):397-410.
102. Zhang L, Li H, Yuan M, Li M, Zhang S. Cervical cancer cells-secreted exosomal microRNA-221-3p promotes invasion, migration and angiogenesis of microvascular endothelial cells in cervical cancer by down-regulating MAPK10 expression. Cancer Manag Res. 2019;11:10307-10319.
103. Nahand JS, Vandchali NR, Darabi H, et al. Exosomal microRNAs: novel players in cervical cancer. Epigenomics. 2020;12(18):1651-1660.
104. Bhat A, Sharma A, Bharti AC. Upstream hedgehog signaling components are exported in exosomes of cervical cancer cell lines. Nanomedicine (Lond). 2018;13(17):2127-2138.
105. Konishi H, Hayashi M, Taniguchi K, et al. The therapeutic potential of exosomal miR-22 for cervical cancer radiotherapy. Cancer Biol Ther. 2020;21(12):1128-1135.
106. Szajnik M, Czystowska-Kuźmicz M, Elishaev E, Whiteside TL. Biological markers of prognosis, response to therapy and outcome in ovarian carcinoma. Expert Rev Mol Diagn. 2016;16(8):811-826.
107. Dorayappan KDP, Wallbillich JJ, Cohn DE, Selvendiran K. The biological significance and clinical applications of exosomes in ovarian cancer. Gynecol Oncol. 2016;142(1):199-205.
108. Cheng L, Wu S, Zhang K, Qing Y, Xu T. A comprehensive overview of exosomes in ovarian cancer: emerging biomarkers and therapeutic strategies. J Ovarian Res. 2017;10(1):73.
109. Runz S, Keller S, Rupp C, et al. Malignant ascites-derived exosomes of ovarian carcinoma patients contain CD24 and EpCAM. Gynecol Oncol. 2007;107(3):563-571.
110. Nakamura K, Sawada K, Kinose Y, et al. Exosomes promote ovarian cancer cell invasion through transfer of CD44 to peritoneal mesothelial cells. Mol Cancer Res. 2017;15(1):78-92.
111. Li QL, Bu N, Yu YC, Hua W, Xin XY. Exvivo experiments of human ovarian cancer ascites-derived exosomes presented by dendritic cells derived from umbilical cord blood for immunotherapy treatment. Clin Med Oncol. 2008;2:461-467.
112. Kobayashi M, Salomon C, Tapia J, Illanes SE, Mitchell MD, Rice GE. Ovarian cancer cell invasiveness is associated with discordant exosomal sequestration of Let-7 miRNA and miR-200. J Transl Med. 2014;12:4.

113. Wyciszkiewicz A, Kalinowska-Łyszczarz A, Nowakowski B, Kaźmierczak K, Osztynowicz K, Michalak S. Expression of small heat shock proteins in exosomes from patients with gynecologic cancers. Sci Rep. 2019;9(1):9817.
114. Liang B, Peng P, Chen S, et al. Characterization and proteomic analysis of ovarian cancer-derived exosomes. J Proteomics. 2013;80:171-182.
115. Yin J, Yan X, Yao X, et al. Secretion of annexin A3 from ovarian cancer cells and its association with platinum resistance in ovarian cancer patients. J Cell Mol Med. 2012;16(2):337-348.
116. Liang T, Guo Q, Li L, Cheng Y, Ren C, Zhang G. MicroRNA-433 inhibits migration and invasion of ovarian cancer cells via targeting Notch1. Neoplasma. 2016;63(5):696-704.
117. Wurz K, Garcia RL, Goff BA, Mitchell PS, Lee JH, Tewari M, Swisher EM. MiR-221 and MiR-222 alterations in sporadic ovarian carcinoma: relationship to CDKN1B, CDKNIC and overall survival. Genes Chromosomes Cancer. 2010;49(7):577-584.
118. Huh JH, Kim TH, Kim K, et al. Dysregulation of miR-106a and miR-591 confers paclitaxel resistance to ovarian cancer. Br J Cancer. 2013;109(2):452-461.
119. Sorrentino A, Liu CG, Addario A, Peschle C, Scambia G, Ferlini C. Role of microRNAs in drug-resistant ovarian cancer cells. Gynecol Oncol. 2008;111(3):478-486.
120. Azmi AS, Bao B, Sarkar FH. Exosomes in cancer development, metastasis, and drug resistance: a comprehensive review. Cancer Metastasis Rev. 2013;32(3-4):623-642.
121. Taylor DD, Gercel-Taylor C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. Gynecol Oncol. 2008;110(1):13-21.
122. Li SD, Zhang JR, Wang YQ, Wan XP. The role of microRNAs in ovarian cancer initiation and progression. J Cell Mol Med. 2010;14(9):2240-2249.
123. Cappellesso R, Tinazzi A, Giurici T, et al. Programmed cell death 4 and microRNA 21 inverse expression is maintained in cells and exosomes from ovarian serous carcinoma effusions. Cancer Cytopathol. 2014;122(9):685-693.
124. Vaksman O, Tropé C, Davidson B, Reich R. Exosome-derived miRNAs and ovarian carcinoma progression. Carcinogenesis. 2014;35(9):2113-2120.
125. Mahmoud EH, Fawzy A, A Elshimy RA. Serum MicroRNA-21 negatively relates to expression of programmed cell death-4 in patients with epithelial ovarian cancer. Asian Pac J Cancer Prev. 2018;19(1):33-38.
126. Kinose Y, Sawada K, Nakamura K, Kimura T. The role of microRNAs in ovarian cancer. Biomed Res Int. 2014;2014:249393.
127. Rana S, Lemoine E, Granger JP, Karumanchi SA. Preeclampsia: pathophysiology, challenges, and perspectives. Circ Res. 2019;124(7):1094-1112.
128. Uzan J, Carbonnel M, Piconne O, Asmar R, Ayoubi JM. Pre-eclampsia: pathophysiology, diagnosis, and management. Vasc Health Risk Manag. 2011;7:467-474.
129. Phipps E, Prasanna D, Brima W, Jim B. Preeclampsia: updates in pathogenesis, definitions, and guidelines. Clin J Am Soc Nephrol. 2016;11(6):1102-1113.
130. Berzan E, Doyle R, Brown CM. Treatment of preeclampsia: current approach and future perspectives. Curr Hypertens Rep. 2014;16(9):473.
131. Guibourdenche J, Leguy MC, Tsatsaris V. Biologie des marqueurs de la pré-éclampsie [Biology and markers of preeclampsia]. Ann Biol Clin (Paris). 2013;71:79-87.
132. Pillay P, Moodley K, Moodley J, Mackraj I. Placenta-derived exosomes: potential biomarkers of preeclampsia. Int J Nanomedicine. 2017;12:8009-8023.
133. Gilani SI, Weissgerber TL, Garovic VD, Jayachandran M. Preeclampsia and extracellular vesicles. Curr Hypertens Rep. 2016;18(9):68.
134. Salomon C, Rice GE. Role of exosomes in placental homeostasis and pregnancy disorders. Prog Mol Biol Transl Sci. 2017;145:163-179.
135. Knight M, Redman CW, Linton EA, Sargent IL. Shedding of syncytiotrophoblast microvilli into the maternal circulation in pre-eclamptic pregnancies. Br J Obstet Gynaecol. 1998;105(6):632-640.
136. Redman CW, Sargent IL. Placental debris, oxidative stress and pre-eclampsia. Placenta. 2000;21(7):597-602.
137. Record M. Intercellular communication by exosomes in placenta: a possible role in cell fusion? Placenta. 2014;35(5):297-302.
138. Vargas A, Zhou S, Éthier-Chiasson M, et al. Syncytin proteins incorporated in placenta exosomes are important for cell uptake and show variation in abundance in serum exosomes from patients with preeclampsia. FASEB J. 2014;28(8):3703-3719.
139. Pillay P, Maharaj N, Moodley J, Mackraj I. Placental exosomes and pre-eclampsia: maternal circulating levels in normal pregnancies and, early and late onset pre-eclamptic pregnancies. Placenta. 2016;46:18-25.
140. Cronqvist T, Saljé K, Familari M, et al. Syncytiotrophoblast vesicles show altered micro-RNA and haemoglobin content after ex-vivo perfusion of placentas with haemoglobin to mimic preeclampsia. PLoS One. 2014;9(2):e90020.
141. Sandrim VC, Luizon MR, Palei AC, Tanus-Santos JE, Cavalli RC. Circulating microRNA expression profiles in pre-eclampsia: evidence of increased miR-885-5p levels. BJOG. 2016;123(13):2120-2128.
142. Ospina-Prieto S, Chaiwangyen W, Herrmann J, et al. MicroRNA-141 is upregulated in preeclamptic placentae and regulates trophoblast invasion and intercellular communication. Transl Res. 2016;172:61-72.
143. Truong G, Guanzon D, Kinhal V, et al. Oxygen tension regulates the miRNA profile and bioactivity of exosomes released from extravillous trophoblast cells - Liquid biopsies for monitoring complications of pregnancy. PLoS One. 2017;12(3):e0174514.
144. Felker J, Agnihotri S. Hurdling over the blood-brain barrier with exosome technology. Neuro Oncol. 2022;24(11):1884-1885.
145. Ma Y, Ma QW, Sun Y, Chen XF. The emerging role of extracellular vesicles in the testis. Hum Reprod. 2023;38(3):334-351.
146. Kenigsberg S, Wyse BA, Librach CL, da Silveira JC. Protocol for exosome isolation from small volume of ovarian follicular fluid: evaluation of ultracentrifugation and commercial kits. Methods Mol Biol. 2017;1660:321-341.
147. Mousaei Ghasroldasht M, Liakath Ali F, Park HS, et al. A comparative analysis of na.ve exosomes and enhanced exosomes with a focus on the treatment potential in ovarian disorders. J Pers Med. 2024;14(5):482.
148. Chen LY, Kao TW, Chen CC, et al. Frontier review of the molecular mechanisms and current approaches of stem cell-derived exosomes. Cells. 2023;12(7):1018.
149. Konala VB, Mamidi MK, Bhonde R, Das AK, Pochampally R, Pal R. The current landscape of the mesenchymal stromal cell secretome: a new paradigm for cell-free regeneration. Cytotherapy. 2016;18(1):13-24.
150. Lu M, Peng L, Ming X, Wang X, et al. Enhanced wound healing promotion by immune response-free monkey autologous iPSCs and exosomes vs. their allogeneic counterparts. EBioMedicine. 2019;42:443-457.

Original Article

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Randomized Controlled Trial Comparing Single Embryo Culture with Group Culture in a Micro-Well Dish: Impact on Embryo Development and Clinical Outcomes

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ABSTRACT

Purpose: The optimal culture environment for embryos *in vitro* remains a topic of ongoing debate. Group embryo culture using a micro-well dish has been suggested to enhance embryo development by facilitating autocrine and paracrine signaling, but its effect on clinical outcomes in human in vitro fertilization (IVF) remains unclear. The aim of this study was to compare embryo development and clinical outcomes between single embryo culture and group embryo culture in micro-well dishes in human IVF cycles.

Methods: In this prospective, randomized controlled trial, patients undergoing IVF at the Eurofertil IVF Center were allocated to either the single embryo culture group or the group culture (GC) arm. Patients under 40 years old with a minimum of five normally fertilized oocytes were included. Primary outcomes were blastocyst formation rates, while secondary outcomes included embryo development, clinical pregnancy rates, implantation rates, and live birth rates.

Results: A total of 160 patients participated, split equally between the two study arms. GC significantly increased the rate of top-quality blastocysts compared to single culture ($p<0.05$), with no significant differences in clinical pregnancy and live birth rates between the two groups ($p>0.05$). Fresh embryo transfer was performed in all cycles, and no preimplantation genetic testing was applied to the embryos.

Conclusion: GC using a micro-well dish led to a higher yield of top-quality blastocysts, although it did not significantly improve clinical outcomes. These results suggest that GC may be advantageous for cycles requiring cryopreservation or biopsy of multiple blastocysts.

Keywords: Embryo development, embryo culture, micro-well dish, pregnancy rate

INTRODUCTION

The quest to establish optimal culture conditions for human embryos *in vitro* remains a central focus in assisted reproductive technologies (ART). Different methods, such as single embryo culture and group culture (GC), have been extensively studied, yet no consensus has been reached regarding which technique yields the best clinical outcomes (Dai et al.[1], Ieda et al.[2]). Recent advances have suggested that GC, particularly when using micro-well dishes, may enhance embryo development due to autocrine and paracrine signaling effects, which may positively impact embryo quality (Contramaestre et al.[3]). Studies in animal models have provided significant evidence supporting these mechanisms, though data in humans remain inconsistent (Hoelker et al.[4]). Despite encouraging results from these various animal studies, there is limited and conflicting evidence regarding the impact of GC on clinical outcomes in human ART, particularly regarding blastocyst formation and live birth rates (Herreros et al.[5]). In addition, recent innovations like the well-of-the-well dish have

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shown promise, but their application to human embryos has yet to be thoroughly explored (Rebollar-Lazaro and Matson[6]). The aim of this study was to attempt to address the gap in the literature by comparing single and group embryo culture in a micro-well dish system, focusing on blastocyst development, clinical pregnancy, and live birth rates in human in vitro fertilization (IVF) cycles.

METHODS

Study Design

This study was a prospective, randomized controlled trial conducted at Eurofertil IVF Center between May 2013 and April 2014. Patients were randomly assigned to either the single embryo culture group or the group embryo culture; all culturing was carried out in micro-well dishes. Randomization was performed using a computer-generated list, with allocation occurring after the fertilization check.

The ethics approval for the study was obtained from Kocaeli University Faculty of Medicine Ethics Committee (approval number: KOU KAEK 2013179, date: 19.09.2023).

Participants

A total of 215 patients were initially screened, and those who met the inclusion criteria were invited to participate. Inclusion criteria were: female age <40 years, male age <60 years, and a minimum of five normally fertilized oocytes at the time of fertilization check. Patients with failed fertilization, use of surgically retrieved sperm, or those undergoing preimplantation genetic testing (PGT) were excluded. The study population consisted exclusively of antagonist protocol IVF cycles with high fertilization rates. The participants were allocated to the GC arm or the single culture (SC) arm, randomly.

Data Collection

Embryo development was assessed on days 2, 3, and 5 post-fertilization, following the ESHRE/alpha consensus timeline. Blastocysts were graded using Gardner's criteria (Zou et al.[7]). Fresh embryo transfer was performed on day 5, and all embryo transfers were performed by the same clinician using the same type of catheter.

Statistical Analysis

Statistical analyses were performed using SPSS, version 20.0 (IBM Inc., Armonk, NY, USA). Continuous variables were tested for normality using the Kolmogorov-Smirnov test and compared using the Student's t-test, while categorical variables were analyzed using the chi-square test. A p value of <0.05 was considered statistically significant. Adjustments were made for multiple comparisons using the Bonferroni correction method where applicable.

RESULTS

A total of 160 patients were included in the study, with 80 patients allocated to the SC arm and 80 to the GC arm (Figure 1). The review of study as shown on CONSORT diagram (Diagram 1). Baseline characteristics, such as age, body mass index, and duration of infertility were similar between the two groups ($p>0.05$), ensuring comparability (Table 1). The total blastocyst development rate was significantly higher in the GC arm compared to the SC arm (GC: 62.5% vs. SC: 51.8%, $p=0.04$). Furthermore, the number of top-quality blastocysts was significantly greater in the GC arm (GC: 40.2% vs. SC: 27.5%, $p<0.05$). Furthermore, a significantly higher number of cryopreserved blastocysts were obtained from the GC arm (GC: 15.3% vs. SC: 8.6%, $p=0.04$) (Table 2). In terms of clinical outcomes, clinical pregnancy rates were higher in the GC arm, although this difference was not significant (GC: 48.1% vs. SC: 44.7%, $p>0.05$). Similarly, no

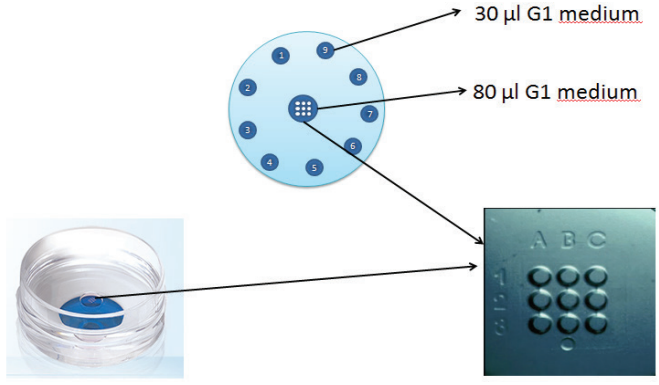

Figure 1. Preparation of WOW dish
WOW: Well-of-the-well

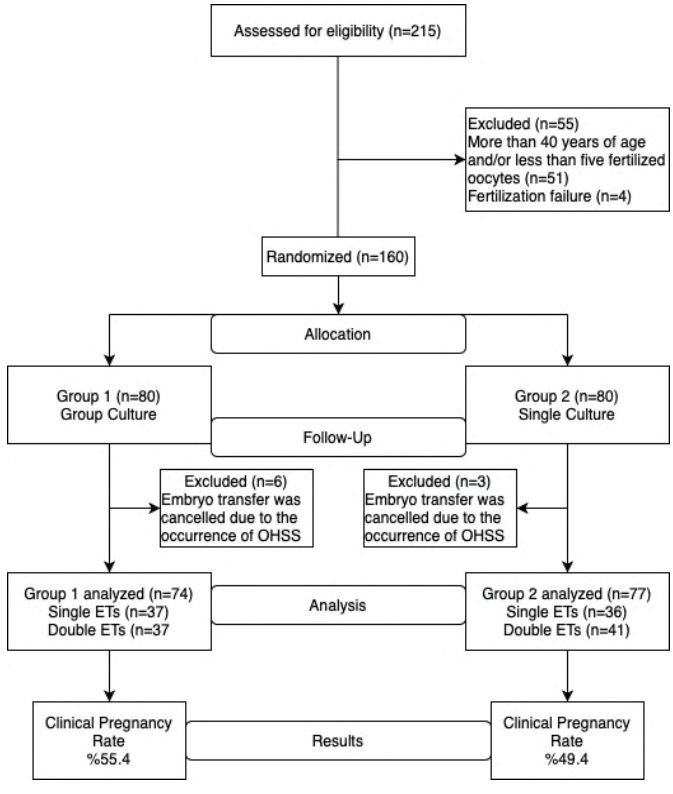

Diagram 1. The CONSORT 2010 flowchart
ETs: OHSS: Ovarian hyperstimulation syndrome

significant differences were found in implantation rates or live birth rates between the two groups ($p>0.05$) (Table 3). Of note, all embryo transfers in this study were fresh, with no frozen embryo transfers performed during the study period. Moreover, none of the embryos underwent PGT.

Table 1. Demographic characteristics of the patients in both groups

Variable	Group 1, (n=80)	Group 2, (n=80)	*p* value
Female age (y)	29±4.8	29.3±4.4	0.7
Male age (y)	33±4.7	33.2±4.8	0.7
BMI (kg/m^2)	25.3±4.2	25.2±4.9	0.9
Duration of infertility (y)	4.86±3.2	4.94±3.5	0.88

BMI: Body mass index, y: Years

Table 2. Cycle characteristics of the groups

Variable	Group 1, (n=80)	Group 2, (n=80)	*p* value
Number of oocytes retrieved (n)	14.1±6.7	14.4±6.2	0.76
Number of 2PN (n)	8.9±4.2	8.7±3.6	0.79
Fertilization rate (%)	81.6±14.5	79±14.5	0.26
Number of cleaved embryos (n)	8.7±4.2	8.5±3.6	0.79
Cleavage rate (%)	97.5±5.7	97.9±6.04	0.58
Day 2 good quality embryos (n)	5.84±3.3	5.35±2.9	0.32
Rate of day 2 good quality embryos (%)	66.3±22.2	62.1±23.1	0.25
Day 3 good quality embryos (n)	5.35±3.5	4.72±3.15	0.23
Rate of day 3 good quality embryos (%)	59.6±23.9	53.9±25.8	0.15
Total blastocysts (n)	6.47±3.7	5.85±3.1	0.24
Rate of total blastocysts (%)	72.2±19.8	67.4±21.0	0.02*
Top quality blasts (n)	3.6±2.9	2.6±2.1	0.005*
Rate of top quality blasts (%)	38.5±22.2	31.1±23.3	0.04*
Cryopreserved blasts (n)	3.16±2.9	2.43±2.3	

*$p<0.05$ statistically significant; 95% confidence interval

Table 3. Clinical and cumulative outcomes of both groups

Clinical outcomes (all transfers)	GC group, (n=74)	SC group, (n=77)	*p* value
Single ETs (n/%)	37/46	36/45	0.56
Double ETs (n/%)	37/46	41/51	0.72
Clinical pregnancy rate (%)	55.4	49.4	0.46
Implantation rate (%)	36.9	32.2	0.5
Live birth rate (%)	45.3	40.3	0.52
Cumulative live birth rate (%) (including fresh and all thaw cycles)	53.2	47.5	0.68

ETs: GC: Group culture, SC: Single culture

These results suggest that while GC improves blastocyst quality and the number of cryopreserved embryos, it does not significantly impact clinical pregnancy or live birth rates.

DISCUSSION

The findings from the present study support previous research, indicating that group embryo culture enhances blastocyst development and improves the yield of top-quality blastocysts (Herreros et al.[5]). Our results also align with those of Herreros et al.[5], who found that micro-well GC systems promote better embryo development, likely due to enhanced autocrine and paracrine signaling within the microenvironment. Despite these promising findings, we did not observe significant differences in clinical pregnancy or live birth rates between the SC and GC groups. This is again consistent with earlier human studies (Hoelker et al.[4], Tao et al.[8]), which also failed to find significant improvements in clinical outcomes, despite enhanced blastocyst quality. One potential explanation for the lack of significant differences in clinical outcomes may be related to the patient population. All patients in this study were under 40 years old and had favorable prognostic factors, which may have minimized the potential impact of culture conditions on clinical outcomes. Future studies should investigate the effects of GC in a broader population, including older patients and those with poorer prognosis. Moreover, the present study was limited to fresh embryo transfers, and the potential impact of GC on frozen-thawed embryo transfers remains unexplored. Further research should focus on examining whether the benefits observed in blastocyst development translate to improved outcomes in frozen-thawed cycles.

Study Limitations

One limitation of this study was the exclusion of patients with poor prognosis or male factor infertility, which limits the generalizability of the findings. In addition, the exclusive use of fresh embryo transfers may have influenced the results, as frozen embryo transfers could yield different outcomes. The study also lacks long-term follow-up data on live birth outcomes.

CONCLUSION

The use of a micro-well dish for GC provides a practical method to improve blastocyst quality without additional costs, making it a viable option for IVF laboratories aiming to maximize embryo yield. However, the lack of significant clinical outcome differences suggests that more research is needed to determine whether these improvements in blastocyst quality can consistently lead to better pregnancy outcomes, particularly in specific patient subgroups.

Ethics

Ethics Committee Approval: The ethics approval for the study was obtained from Kocaeli University Faculty of Medicine Ethics Committee (approval number: KOU KAEK 2013179, date: 19.09.2023).

Informed Consent: Patient approval has not been obtained as it is performed on animals.

Authorship Contributions

Surgical and Medical Practices: E.E., A.İ.T., Z.Ö., E.Y.K., H.M.Ö., Concept: E.E., A.İ.T., Z.Ö., E.Y.K., H.M.Ö., Design: E.E., A.İ.T., Z.Ö., E.Y.K., H.M.Ö., Data Collection or Processing: E.E., A.İ.T., Z.Ö., E.Y.K., H.M.Ö., Analysis or Interpretation: E.E., A.İ.T., Z.Ö., E.Y.K., H.M.Ö., Literature Search: E.E., A.İ.T., Z.Ö., E.Y.K., H.M.Ö., Writing: E.E., A.İ.T., Z.Ö., E.Y.K., H.M.Ö.

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REFERENCES

1. Dai SJ, Xu CL, Wang J, Sun YP, Chian RC. Effect of culture medium volume and embryo density on early mouse embryonic development: tracking the development of the individual embryo. J Assist Reprod Genet. 2012;29(7):617-623.
2. Ieda S, Akai T, Sakaguchi Y, et al. A microwell culture system that allows group culture and is compatible with human single media. J Assist Reprod Genet. 2018;35(10):1869-1880.
3. Contramaestre AP, Sifontes F, Marín R, Camejo MI. Secretion of stem cell factor and granulocyte-macrophage colony-stimulating factor by mouse embryos in culture: influence of group culture. Zygote. 2008;16(4):297-301.
4. Hoelker M, Rings F, Lund Q, et al. Effect of the microenvironment and embryo density on developmental characteristics and gene expression profile of bovine preimplantative embryos cultured in vitro. Reproduction. 2009;137(3):415-425.
5. Herreros M, Martí L, Díaz N, et al. Impact of group vs individual embryo culture strategies on blastocyst and clinical outcomes. Reprod Sci. 2024;31(6):1705-1711.
6. Rebollar-Lazaro I, Matson P. The culture of human cleavage stage embryos alone or in groups: effect upon blastocyst utilization rates and implantation. Reprod Biol. 2010;10(3):227-234.
7. Zou H, Kemper JM, Hammond ER, et al. Blastocyst quality and reproductive and perinatal outcomes: a multinational multicentre observational study. Hum Reprod. 2023 4;38(12):2391-2399.
8. Tao T, Robichaud A, Mercier J, Ouellette R. Influence of group embryo culture strategies on the blastocyst development and pregnancy outcome. J Assist Reprod Genet. 2013;30(1):63-68.

Original Article

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A Novel Alternative to Sequential Protocol in Clomiphene Citrate-Resistant PCOS Patients with a Combined Regimen of Clomiphene Citrate and Recombinant Gonadotropin

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ABSTRACT

Purpose: The aim of this study was to compare treatment outcomes of different ovulation induction (OI) protocols with combination of clomiphene citrate (CC) and recombinant gonadotropin in infertile women with polycystic ovary syndrome (PCOS) undergoing an intrauterine insemination (IUI) cycle.

Methods: In this retrospective cohort study, 75 patients with PCOS undergoing OI and IUI cycles with a combination of CC and recombinant gonadotropins were evaluated. Participants were divided into two groups according to their (OI) protocol: group 1 [sequential protocol (SP): OI with CC plus gonadotropin started on the fourth or fifth day of CC treatment; n=37], group 2 [modified sequential protocol (MSP): OI with CC was started on days 2-5 of the cycle, and gonadotropin was added once the dominant follicle size reached over 10 mm; n=38].The two groups were compared for cycle cancellation, absence of follicular development, multifollicular development, ovulation rate (OR), implantation, clinical pregnancy, ongoing pregnancy and abortion rates.

Results: Demographic features were distributed homogenously between the groups. The number of days of gonadotropin use was also significantly higher in group 1 (6.37±2.58 days) than in group 2 (3.15±1.74 days) ($p<0.0001$). Similarly, the total gonadotropin dose was significantly higher in group 1 (477.36±316.19 IU) compared to group 2 (188.15±168.83 IU) ($p<0.0001$). Multifollicular development in group 1 was significantly higher than in group 2 ($p=0.01$). The OR was 84.8% in group 1 and 89.5% in group 2. The ongoing pregnancy rate was 14.3% for group 1 and 11.8% for group 2.

Conclusion: MSP may help reduce multifollicular development, potentially lowering costs and improving patient compliance by decreasing the duration and doses of gonadotropin treatment compared to SP in PCOS patients resistant to CC. Furthermore, when compared to SP, MSP can provide similar ongoing pregnancy rates.

Keywords: Ovulation induction, clomiphene citrate, sequential protocol, modified sequential protocol, polycystic ovary syndrome.

INTRODUCTION

Polycystic ovary syndrome (PCOS), with a prevalence of 5-10%, is a common endocrinological disorder in reproductive-aged women.[1] Women with PCOS who fail to ovulate or conceive with clomiphene citrate (CC) are recommended to undergo ovulation induction with gonadotropin.[2] Recently, a combination of CC plus gonadotropin treatment has been tested as a method for ovulation induction in women resistant to CC.[3-6] This protocol, termed a "sequential protocol (SP)," was first described by Kistner[7] and performed with a combination of CC and human menopausal gonadotropin (hMG). In SP, a low-dose gonadotropin treatment is initiated on the day following the fourth or fifth day of CC administration.

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Acceptable fecundity rates have been reported in CC-resistant patients with SP when compared to ovulation induction with gonadotropin.[8] SP has been demonstrated to decrease the gonadotropin dose required for optimum induction when compared to gonadotropin-only induction in women who fail to respond to CC.[9] Previous studies have shown that CC + gonadotropin combinations reduce costs and provide effective ovulation induction in infertile cases resistant to CC [10].

We have developed a "modified sequential protocol (MSP)" to initiate gonadotropin therapy in CC-resistant patients with PCOS. In this protocol, gonadotropin treatment is initiated in conjunction with CC when a leading follicle reaches >10 mm, as determined by ultrasonography (USG).

The main aim of this study was to achieve successful inductions with less multifollicular development and lower gonadotropin doses as a potential alternative to SP. We compared ovulation induction cycles conducted with SP and MSP protocols. The treatment outcomes were evaluated by comparing pregnancy results, cycle cancellation rates, and the doses of medications used.

METHODS

This retrospective cohort study was performed in the infertility unit of tertiary health center between 2012 and 2016. The study was approved by the Marmara University Ethics Committee (approval number: 09.2024.169, date: 09/02/2024). In our clinic, informed consent for the use of personal data is obtained from all patients at the start of treatment. The data is anonymized when used.

Inclusion criteria were a diagnosis of infertility due to PCOS, having at least one patent fallopian tube on hysterosalpingography and having normozoospermic male partners, as per WHO guidelines.[11] PCOS diagnosis was based on the revised Rotterdam criteria.[12] Women who failed to ovulate or conceive with CC despite doses of 150 mg/day or six cycles and were considered CC-resistant cases and were included in this study.

Women with bilateral tubal occlusion confirmed with hysterosalpingography or with hypo/hypethyroidism, hyperprolactinemia, or other endocrinopathies (such as adrenal hyperplasia, insulin resistance, or diabetes) that might affect ovulatory response were excluded from the study.

Demographic features, maternal habits (smoking, alcohol consumption), cycle characteristics, and presence of hirsutism (evaluated at a minimum of eight points by the Ferriman-Gallwey scores) were recorded.[13] Body mass index was calculated by the standard formula: wseight (kg) divided by the height squared (m^2). Measurements included tubal patency, day 2 basal hormone levels and levels of thyroid stimulation hormone and prolactin on a random day, as well as the progressive motile sperm count of the male partner.

The participants were randomly selected in a 1:1 ratio. This selection was made based on patient file numbers, using arithmetically increasing numbers as a reference.

Group 1 (Sequential Protocol)

Group 1 received CC (50-150 mg daily) (Klomen® Koçak Farma, İstanbul, Turkey) from day 2-5 of the menstrual cycle for 5 days. On the day following the fourth or fifth day of CC administration, gonadotropin (GONAL-f®; Merck KGaA, Darmstadt, Germany) therapy was initiated at a low dose (37.5-75 IU) until human chorionic gonadotropin (hCG; Ovitrelle® Merck Serono, Geneva, Switzerland) administration. The gonadotropin dose was adjusted according to the follicular response.

Group 2 (Modified Sequential Protocol)

Group 2 received CC (50-150 mg daily) from day 2-5 of the menstrual cycle for 5 days. Following CC induction, low-dose (37.5-75 IU) gonadotropin treatment was started when a dominant follicle (≥10 mm) was observed on the transvaginal (TV)-USG examination. Daily gonadotropin injections were continued until the day of hCG administration. The subsequent gonadotropin dose was adjusted according to the follicular response.

The protocol used for ovulation induction continued until one or two follicles reached a size of 17 mm or more. The adjusted gonadotropin dose was adjusted by the clinician based on follicle diameter and estradiol levels.

Cycle Monitoring

Follicular development was monitored by TV-USG. The first ultrasound examinations were repeated every 2 days until the hCG day.

The hCG injection was administered as a single dose (10.000 IU) to trigger ovulation when one or two leading follicles had reached at least 17 mm in diameter. IUI was performed 36 h after the administration of hCG following a standard swim-up procedure under transabdominal guidance, as previously described.[14] A daily intravaginal progesterone administration (400 mg) was also initiated for luteal support. Vaginal progesterone used for luteal phase support was continued until the 8th week of pregnancy. Serum β-hCG concentrations were determined at 14 days following IUI.

Cycles with an absence of follicular development or with multifollicular development were cancelled. An absence of follicular development was defined as no follicular development observed or failure of a follicle to achieve a diameter large enough for ovulation after selection of a dominant follicle. Multifollicular development was defined as the development of three or more than three follicles.

Outcome Measures

Ovulation was accepted as observation of shrinkage of follicle(s) >17 mm in diameter on TV-USG following hCG administration. Implantation was defined as a positive β-hCG reading 14 days after IUI. Clinical pregnancy was defined as the presence of a gestational sac with a fetal heart beat detected by TV-USG at 7 weeks of gestation. Ongoing pregnancy was defined as a positive heartbeat at or beyond 12 weeks of gestation. Abortion was defined as the termination of a gestation before 20 weeks.

The primary outcome measures were cycle cancellation, absence of follicular development, multifollicular development, ovulation rate (OR), implantation rate, clinical pregnancy rate (CPR), ongoing pregnancy rate (OPR) and abortion rate (AR).

Statistical Analysis

A post hoc power analysis was conducted to evaluate the statistical power of the study after data collection. Data were expressed as mean ± standard deviation for continuous data or frequencies (n) with percentages (%) for categorical data. Kolmogorov-Smirnov test was used to test normality of data. For variables with normal distribution, Student's t-test was used, whereas for non-normally distributed variables, the Mann-Whitney U test was applied. Chi-square test and Fisher's exact test were used for comparisons of categorical data. A p value less than 0.05 was considered as statistically significant in all analyses.

RESULTS

In this study 75 women who were infertile due to PCOS and needed IUI cycle treatment were retrospectively reviewed. They were divided into the, SP (group 1, n=37) and MSP groups (group 2, n=38) according to the OI protocol used.

Evaluation of Basal Characteristic Features

Comparison of the demographic and basal characteristic features of the participants revealed no significant differences between the two groups (Table 1).

Evaluation of Ovulation Induction Cycle Characteristics

There was no significant difference between the groups in terms of the start of CC treatment and the total dose of CC (p=0.5 and; p=0.9 respectively). The number of days of gonadotropin used was also significantly higher in group 1 than group 2 (p<0.0001). The total dose of gonadotropin used was also significantly higher in group 1 than in group 2 (p<0.0001). Groups 1 and 2 did not differ significantly in terms of the number of follicles obtained (p=0.2) (Table 2). In terms of CC treatment, 12 participants received 50 mg/day, 42 participants received 100 mg/day, 18 participants received 150 mg/day, and 3 participants received 200 mg/day of CC treatment.

Evaluation of Treatment Results

The cycle cancellation rate did not differ significantly between groups 1 and 2 (p=0.1). Follicular development failed in 13.5% of the cases in group 1 and in 10.5% of the cases in group 2. The absence of follicular development did not differ significantly in groups 1 and 2 (p=0.7). Multifollicular development was observed in 10.8% of the cases in group 1 but was not noted in any patient in group 2. Multifollicular development was therefore significantly higher in group 1 than in group 2 (p=0.01) (Table 3).

The OR was 84.8% in group 1 and 89.5% in group 2. The OR did not differ between groups 1 and 2 (p=0.7).

In group 1, β-hCG positivity and clinical pregnancy were achieved in 5 (17.9%) of 28 cases who had ovulation, but abortion was observed in 1 case (1/5, 20%) in the later period

Table 1. Demographic features and baseline characteristics of two groups

	Group 1 (n=37)	Group 2 (n=38)	p
Age (years)	26 (21-36)	27 (21-39)	0.2
BMI (kg/m²)	29.01±4.45	28.65±4.51	0.732
Duration of infertility (years)	4 (2-17)	4 (1-17)	0.57
Infertility type (n, %) **Primary** **Secondary**	 31/37 (83.8%) 6/37 (16.2%)	 31/38 (81.6%) 7/38 (18.4%)	 0.801
Habits **Smoking (n, %)** **Alcohol (n, %)**	 1/37 (2.7%) 0	 0 0	 0.5 0.999
Hirsutism (n, %)	34/37 (91.9%)	36/38 (94.7%)	0.621
Cycle length (days)	49.76±27.6	58.61±30.37	0.3
Tubal patency **One tubal occlusion (n, %)**	 1/37 (2.7%)	 2/38 (5.3%)	 0.4
Day 2 basal hormone levels **FSH (mIU/mL)** **LH (mIU/mL)** **FSH/LH** **E2 (pg/mL)**	 6±1.26 7.6 (2.5-12.6) 0.78 (0.46-2.36) 38 (13-94)	 6.23±1.38 6.1 (1.2-33.0) 1.03 (0.26-3.08) 36.5 (13-117)	 0.447 0.084 0.049 0.736
TSH (μU/mL)	2.00 (1-5.3)	2.00 (1-5.2)	0.183
PRL (ng/mL)	12 (4-23)	12 (4-25)	0.531
PMSC (x10⁶)	35.18±19.3	45.89±40.72	0.2

BMI: Body mass index, FSH: Follicle stimulating hormone, LH: Luteinizing hormone, E2: Estradiol, TSH: Thyroid stimulating hormone, PMSC: Progressive motile sperm count, PRL: Prolactin

Table 2. Ovulation induction treatment characteristics of two groups			
	Group 1 (n=37)	**Group 2 (n=38)**	***p***
Start day of CC **Day 2 (n, %)** **Day 3 (n, %)** **Day 4 (n, %)** **Day 5 (n, %)**	3 (2-5) 9/37 (24.3%) 20/37 (54.1%) 5/37 (13.5%) 3/37 (8.1%)	3 (2-5) 9/38 (23.7%) 24/38 (63.2%) 4/38 (10.5%) 1/38 (2.6%)	0.507
CC total dose (mg)	500 (250-1000)	500 (250-1000)	0.943
Start day of gonadotropin	7 (5-8)	10 (6-14)	**<0.0001**
Total day of gonadotropin use	7 (2-11)	2.5 (2-11)	**<0.0001**
Gonadotropin total dose (IU)	375 (100-1200)	150 (50-1050)	**<0.0001**
Dominant follicle count (n)	11 (0-18)	12 (0-20)	0.203
Dominant follicle diameter (cm)	15.5 (0-19)	16.75 (0-21)	0.413
hCG day	11 (0-18)	12 (0-20)	0.548
IUI day	13 (0-20)	14 (0-22)	0.710
CC: Clomiphene citrate, hCG: Human chorionic gonadotrophine, IUI: Intrauterine insemination			

Table 3. The comparison of the outcome of ovulation induction and intrauterin insemination cycle between two groups			
	Group 1	**Group 2**	***p***
Cycle cancellation (n, %)	9/37 (24.3%)	4/38 (10.5%)	0.1
Absence of follicular development	5/37 (13.5%)	4/38 (10.5%)	0.7
Multifollicular (≥3) development	4/37 (10.8%)	0	**0.01**
Ovulation rate (n, %)	28/33 (84.8%)	34/38 (89.5%)	0.7
Implantation rate (n, %)	5/28 (17.9%)	7/34 (20.6%)	0.7
Clinical pregnancy rate (n, %)	5/28 (17.9%)	6/34 (17.6%)	0.8
Ongoing pregnancy rate (n, %)	4/28 (14.3%)	4/34 (11.8%)	0.9
Abortion (n, %)	1/5 (20%)	3/7 (42.9%)	0.2

of pregnancy, giving an OPR of 14.3% (4/28). In group 2, β-hCG positivity was observed in 7 (20.6%) of 34 patients who underwent IUI, while 6 (17.6%) clinical pregnancies were detected, 4 (11.8%) had ongoing pregnancy, and 3/7 cases of pregnancy had abortion (42.9%).

The power analysis based on multifollicular development data revealed that, with alpha set at 0.05 and beta at 80%, 66 participants were required in each group. Conversely, to detect at least a 50% reduction in gonadotropin dose with alpha set at 0.05 and beta at 80%, 20 participants per group were found to be sufficient.

DISCUSSION

The present study showed that MSP may reduce the risk of multifollicular development and there was lower gonadotropin doses and a shorter duration of gonadotropin administration compared to SP in CC resistance PCOS patient. Furthermore, when compared to SP, MSP can provide similar ORs, and comparable clinical and ongoing pregnancy rates but there was twice the rate of abortion in the MSP group. Once again, because of the small group sizes, it would be necessary to confirm this finding in larger groups.

The development of this protocol is based on two factors. Firstly, the role of decreased FSH secretion in monoovulatory cycles is well-known.[14] In the SP protocol, the endogenous FSH effect provided by CC is continuously augmented with exogenous FSH stimulation. As a result, multiple follicle development emerges as a complication. Therefore, it is anticipated that initiating exogenous FSH administration after the selection of the dominant follicle will help restrict the formation of multiple follicles. It is generally considered that follicle selection is completed when the follicle diameter is above 8-9 mm.[15,16] Therefore, we preferred to continue with exogenous FSH support after observing a 10 mm follicle. Secondly, it has been reported that continuing to support the cycle with exogenous FSH during the gonadotropin-dependent phase of follicular development is important.[17] In patients with PCOS, follicular development may be halted due to the local androgenic environment, leading to ovulation disorders.[15] After the selection of a dominant follicle through MSP, exogenous FSH support can ensure the continuation of follicular development, potentially increasing success rates in IUI cycles. Considering that this study was conducted on CC-resistant patients, success rates may be higher in PCOS patients who are not resistant to CC. However, further evidence from studies conducted on different patient populations is needed to clarify the efficacy of these combinations.

Nowadays, studies on combinations of agents used for ovulation induction are increasingly being conducted.

The goal is to achieve an effective treatment option while minimizing side effects and complication rates. Recent studies have shown that in CC-resistant PCOS cases, CC is being combined with other ovulation-enhancing agents such as letrozole and coenzyme Q10 have had a beneficial effect.[16,17] Moreover, successful cycles have been reported using combinations of gonadotropins with other ovulation induction agents.[18] Recent studies on the combination of letrozole and gonadotropins can be found in the literature[19] The current study demonstrated that the combination of CC and gonadotropins can be used more effectively in ovulation induction by reducing side effects and costs. However, we would like to emphasize that while achieving this, the cycle cancellation rates increased due to the absence of follicular development. Nevertheless, in both groups, whether due to multifollicular development or the absence of follicular growth, the similarity in overall cycle cancellation rates makes MSP a safer option in terms of side effects.

The total gonadotropin dose required was lower and the total days of gonadotropin administration were shorter with MSP than with SP in the present study. Researchers have previously compared ovulation induction cycles with gonadotropin and SP, and they found SP to be more cost-effective than gonadotropin induction cycles but gave similar pregnancy rates.[20,21] This study demonstrated that the combination of CC with gonadotropins offers a new alternative in the application protocol, achieving OI success rates similar to the SP protocol while providing lower multifollicular development and reduced costs. Although cost-effectiveness was not directly assessed in this study, MSP may offer practical advantages. Considering the shorter duration and lower total dose of gonadotropin use, MSP achieves similar ovulation, clinical pregnancy, and OPRs compared to SP, suggesting it could be a viable alternative for selected patients

Ovulation induction cycles can be cancelled due to the absence of follicular development and ovulation or due to multifollicular development. Ovulation can fail in 20-25% of patients with PCOS, despite recurrent CC induction.[22,23] Some publications have reported ORs of 51% with CC and 95.7% with SP.[24,25] However, no similar data are available related to MSP. In the present study, the cycle cancellation rates (24.3% in SP and 10.5% in MSP) were lower in the MSP than in the SP group. However, no statistically significant difference was detected, probably due to the low number of participants in the study groups. In addition, MSP exhibited an apparent lower rate of multifollicular development compared to SP in our study. However, our study was underpowered to draw firm conclusions about the rate of multifollicular development and so larger, powered studies are required to confirm the trend we noted. MSP decreased cycle cancellation rates due to a decrease in multifollicular development when compared to SP. MSP may be an ovulation induction protocol that results in less multifollicular development and also similar absense of follicle development compared to SP.

None of our cases developed ovarian hyperstimulation syndrome (OHSS) or multiple pregnancy, although multifollicular development increases the risk of both of these conditions and both are accepted as complications of ovulation induction.[23,26] Therefore, MSP can be viewed as better than SP in light of the lower rate of multifollicular development and the subsequently decreased risk of multiple pregnancy and OHSS. Previous research has confirmed lower rates of multiple pregnancy and OHSS with SP than with ovulation induction with hMG[7]; for example, the OHSS risk was 0.2% in SP that used a CC and hMG combination[8] and 16% in a gonadotropin induction in patients with PCOS.[27] Our study group did not include patients induced with only gonadotropin; however, a higher dose of gonadotropin is required in SP than in MSP due to the earlier addition of gonadotropin to CC. The lower gonadotropin dose used in MSP than in SP decreases the risk of multifollicular development; therefore, MSP seems to be more a rational approach than gonadotropin protocols for reducing OHSS.

Pregnancy rates are higher with SP than with CC induction in patients with PCOS and the fecundity rates are acceptable with SP compared to OI with gonadotropin in CC-resistant patients.[8] Also, we found similar implantation, clinical pregnancy, ongoing pregnancy and ARs were found in our study between the SP and MSP groups and the participants in our SP and MSP groups consisted of CC-resistant cases. Of note, the abortion rate in the MSP group was more than twice that of the SP group (43% vs. 20%), but this may have been due to small numbers of pregnancies and abortions (3 vs. 1). It has been shown that pregnancy rates are similar in PCOS patients regardless of the ovulation induction protocol used. In additionally, the number of previous unsuccessful cycles does not significantly reduce pregnancy rates following IUI.[28] This rate can vary between 13% and 24%.[29,30] A pregnancy rate of 18% was reported by Hock et al.[31] with SP (day 2-7, 50-100 mg CC; day 9, hMG injection), consistent with our results. In the present study, the clinical pregnancy rates were 17.9% and 17.6%, respectively, falling within acceptable limits.

If follicular development is stimulated with a combination of CC plus gonadotropin in all PCOS cases, not only the CC-resistant cases, higher pregnancy rates would be probably achieved, in agreement with previous studies. For example, one previous study reported a CPR of 14.2% and a live birth rate of 12.5% in 416 cycles with SP using a CC and hMG combination.[8] In this study, OPRs were determined as 14.3% and 11.8%, respectively. However, it should be noted that these rates may have been lower due to the study population consisting exclusively of CC-resistant patients.

Among the limitations of the study is the emergence of letrozole therapy as a prominent ovulation induction protocol for PCOS patients in recent years. CC used to be the first-line treatment for ovulation induction, but in recent years, letrozole has replaced it.[32] Additionally, combination therapies with ovulation-inducing agents have gained importance A recent study has reported successful ovulation induction cycles using a combination of letrozole and gonadotropins.[19] However, gonadotropins and CC remain widely used ovulation induction agents and continue to maintain their significance. Although the post hoc analysis demonstrated that the sample size used for evaluating the agents in the IUI protocol was sufficient, the sample size for demonstrating multifollicular development fell below the expected level. This is because multifollicular development was a relatively rare complication.

CONCLUSION

In CC-resistant infertile PCOS patients, MSP may help reduce multifollicular development, potentially lowering costs and improving patient compliance by decreasing the duration and doses of gonadotropin treatment compared to SP. Further studies with larger sample sizes are needed to more thoroughly evaluate the potential benefits of MSP in this patient group.

Ethics

Ethics Committee Approval: The study was approved by the Marmara University Ethics Committee (approval number: 09.2024.169, date: 09/02/2024).

Informed Consent: In our clinic, informed consent for the use of personal data is obtained from all patients at the start of treatment.

Authorship Contributions

Surgical and Medical Practices: Ü.K.T., E.N.T., T.P., Concept: Ü.K.T., T.P., Design: Ü.K.T., T.P., Data Collection or Processing: Ü.K.T., T.P., Analysis or Interpretation: E.N.T., Ü.K.T., Literature Search: Ü.K.T., E.N.T., Writing: Ü.K.T., E.N.T.

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Lauritsen MP, Bentzen JG, Pinborg A, et al. The prevalence of polycystic ovary syndrome in a normal population according to the Rotterdam criteria versus revised criteria including anti-Mullerian hormone. Hum Reprod. 2014;29(4):791-801.
2. Eijkemans MJ, Polinder S, Mulders AG, Laven JS, Habbema JD, Fauser BC. Individualized cost-effective conventional ovulation induction treatment in normogonadotrophic anovulatory infertility (WHO group 2). Hum Reprod. 2005;20(10):2830-2837.
3. Thabet M, Abdelhafez MS, Elshamy MR, et al. Competence of combined low dose of human chorionic gonadotropin (HCG) and clomiphene citrate (CC) versus continued CC during ovulation induction in women with CC-resistant polycystic ovarian syndrome: a randomized controlled trial. Medicina (Kaunas). 2024;60(8):1300.
4. Kemmann E, Jones JR. Sequential clomiphene citrate-menotropin therapy for induction or enhancement of ovulation. Fertil Steril. 1983;39(6):772-779.
5. Dickey RP, Olar TT, Taylor SN, Curole DN, Rye PH. Sequential clomiphene citrate and human menopausal gonadotrophin for ovulation induction: comparison to clomiphene citrate alone and human menopausal gonadotrophin alone. Hum Reprod. 1993;8(1):56-59.
6. Lu PY, Chen AL, Atkinson EJ, Lee SH, Erickson LD, Ory SJ. Minimal stimulation achieves pregnancy rates comparable to human menopausal gonadotropins in the treatment of infertility. Fertil Steril. 1996;65(3):583-587.
7. Kistner RW. Sequential use of clomiphene citrate and human menopausal gonadotropin in ovulation induction. Fertil Steril. 1976;27(1):72-82.
8. Brzechffa PR, Daneshmand S, Buyalos RP. Sequential clomiphene citrate and human menopausal gonadotrophin with intrauterine insemination: the effect of patient age on clinical outcome. Hum Reprod. 1998;13(8):2110-2114.
9. Mitwally MF, Casper RF. Aromatase inhibition reduces gonadotrophin dose required for controlled ovarian stimulation in women with unexplained infertility. Hum Reprod. 2003;18(8):1588-1597.
10. Zafarjanovna KM, Niyetbayevna BG, Atabayevna DZ. Optimization of ovulation induction in clomifene resistant patient with infertility. Biomed J Sci Tech Res. 2021;39(2):31257-31259.
11. Lamb D. World Health Organization laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 4th ed. J Androl; 2000.
12. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril. 2004;81(1):19-25.
13. Goodman NF, Bledsoe MB, Cobin RH, et al. American Association of Clinical Endocrinologists medical guidelines for the clinical practice for the diagnosis and treatment of hyperandrogenic disorders. Endocr Pract. 2001;7(2):120-134.
14. Dodson WC, Whitesides DB, Hughes CL Jr, Easley HA 3rd, Haney AF. Superovulation with intrauterine insemination in the treatment of infertility: a possible alternative to gamete intrafallopian transfer and in vitro fertilization. Fertil Steril. 1987;48(3):441-445.
15. Gervásio CG, Bernuci MP, Silva-de-Sá MF, Rosa-E-Silva AC. The role of androgen hormones in early follicular development. ISRN Obstet Gynecol. 2014;2014:818010.
16. Ashkar A, Jadoon B, Ali Baig MM, Irfan SA, El-Gayar M, Siddiqui FZ. Is combined letrozole and clomiphene superior to either as monotherapy: a systemic review and meta-analysis based on clinical trials. Gynecol Endocrinol. 2024;40(1):2405114.
17. Izhar R, Husain S, Tahir MA, Husain S. Effect of administrating coenzyme Q10 with clomiphene citrate on ovulation induction in polycystic ovary syndrome cases with resistance to clomiphene citrate: a randomized controlled trial. J Reprod Infertil. 2022;23(3):177-183.
18. Xia TT, Zeng KF, Peng QM. Comparison of hree ovulation induction therapies for patients with polycystic ovary syndrome and infertility. J Clin Pharmacol. 2023;63(12):1371-1376.
19. Baradwan S, Al-Shalhoub F, Alshahrani MS, et al. Effects of ietrozole alone or in combination with gonadotropins on ovulation induction and clinical pregnancy in women with polycystic ovarian syndrome: a systematic review and meta-analysis of randomized controlled trials. Hormones (Athens). 2024;23(3):497-508.
20. Abdelazim IA, Makhlouf HH. Sequential clomiphene citrate/hMG versus hMG for ovulation induction in clomiphene citrate-resistant women. Arch Gynecol Obstet. 2013;287(3):591-597.
21. Altay M, Gelisen O, Koçak M, Demir B, Beydilli G, Haberal A. Comparison of a novel minimal stimulation protocol with clomiphene citrate plus recombinant follicle-stimulating hormone to recombinant follicle-stimulating hormone alone for ovulation induction: a prospective study. J Turk Ger Gynecol Assoc. 2007;8(4):397-401.
22. Elnashar A, Fouad H, Eldosoky M, Saeid N. Letrozole induction of ovulation in women with clomiphene citrate-resistant polycystic ovary syndrome may not depend on the period of infertility, the body mass index, or the luteinizing hormone/follicle-stimulating hormone ratio. Fertil Steril. 2006;85(2):511-513.
23. Melo AS, Ferriani RA, Navarro PA. Treatment of infertility in women with polycystic ovary syndrome: approach to clinical practice. Clinics (Sao Paulo). 2015;70(11):765-769.
24. Tanbo T, Mellembakken J, Bjercke S, Ring E, Åbyholm T, Fedorcsak P. Ovulation induction in polycystic ovary syndrome. Acta Obstet Gynecol Scand. 2018;97(10):1162-1167.
25. Jarrell J, McInnes R, Cooke R, Arronet G. Observations on the combination of clomiphene citrate-human menopausal gonadotropin-human chorionic gonadotropin in the management of anovulation. Fertil Steril. 1981;35(6):634-637.
26. Kligman I. Polycystic ovarian syndrome (PCOS). In: Problem-focused reproductive endocrinology and infertility. Springer; 2023:63-69.

27. Fischer D, Reisenbüchler C, Rösner S, Haussmann J, Wimberger P, Goeckenjan M. Avoiding OHSS: controlled ovarian low-dose stimulation in women with PCOS. Geburtshilfe Frauenheilkd. 2016;76(6):718-726.
28. Gao Y, Jiang S, Chen L, et al. The pregnancy outcomes of infertile women with polycystic ovary syndrome undergoing intrauterine insemination with different attempts of previous ovulation induction. Front Endocrinol (Lausanne). 2022;13:922605.
29. Najafi PZ, Noghabi SP, Afzali N, Mohammadzadeh S. Comparing the effect of clomiphene citrate and letrozole on ovulation induction in infertile women with polycystic ovary syndrome. J Pak Med Assoc. 2020;70(2):268-271.
30. Amirian M, Shariat Moghani S, Jafarian F, et al. Combination of pioglitazone and clomiphene citrate versus clomiphene citrate alone for infertile women with the polycystic ovarian syndrome. BMC Womens Health. 2021;21(1):302.
31. Hock DL, Bohrer MK, Ananth CV, Kemmann E. Sonographic assessment of endometrial pattern and thickness in patients treated with clomiphene citrate, human menopausal gonadotropins, and intrauterine insemination. Fertil Steril. 1997;68(2):242-245.
32. Tsiami AP, Goulis DG, Sotiriadis AI, Kolibianakis EM. Higher ovulation rate with letrozole as compared with clomiphene citrate in infertile women with polycystic ovary syndrome: a systematic review and meta-analysis. Hormones (Athens). 2021;20(3):449-461.

Original Article

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A Double Blind Randomised Controlled Trial Comparing Two Panty Linerswith Different Surfaces with Respect to Microbial Colony per Square Centimeter

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ABSTRACT

Purpose: To compare microbial colonies per square centimeter on two different panty liner pads after 4-6 hour of vulvar contact. The secondary objective was to assess whether daily pad use induces dermatological changes in the vulva and to investigate its impact on the vulva in women with or without vaginal discharge or bacterial vaginosis.

Methods: A total of 250 healthy women aged 20-43 years participated in this study. Baseline vulvar and vaginal conditions were assessed through physical examinations, culture samples, and laboratory analyses. Participants were randomly assigned toone of the panty liner groups through internet-based random number generation. Even numbers were assigned to pad group 102 and odd numbers were assigned pad group 103. The panty liners were identical in appearance and neither the patients, nor the clinicians and microbiologist were aware of the technology until the study finished. After 4-6 hours of use, microbial cultures were obtained from the pads to determine colony counts, while dermatological evaluations of the vulva were conducted to assess any skin irritation or changes.

Results: The frequency of bacterial vaginosis, and percent of cases with pathogenic microbial species isolation were similar in the two groups at the time of randomization. The vulva and panty liner contact time was similar in zinc coated and non-coated groups respectively (280±65 vs 275±72 minutes $p<0.58$). The-zinc coated group, coded as 102, had 60 (53.7%) patients without microbial growth, significantly lower than group 103 with non-coated regular panty liners (n=44, 37.6%, $p=0.02$). Number of colonies per square centimeter on zinc coated panty liners was significantly lower than the non-coated group (9324±24046 vs 56663±99618 colonies $p<0.001$). Dermatological assessment of the vulva showed no notable difference between group and within group frequencies of vulvar erythema, and excoriation in either panty liner group.

Conclusion: The study confirmed that zinc-coated panty liners bear significantly less microbial colonies with 4-6 hours of use compared to non-coated panty liners. The use of panty liners lead to a non-significant decrease in vulvar erythema and excoriation after short term of use which should be re-evaluated for longer and repeated use.

Keywords: Daily pads, panty liner, vulvar irritation, bacterial vaginosis, hygiene products

INTRODUCTION

Daily pads, also known as panty liners, are thinner and narrower than standard menstrual pads, specifically designed for use during non-menstrual periods. They serve to absorb vaginal discharge, light vaginal bleeding, and, in some cases, small amounts of urine in women with urinary incontinence. Despite their widespread use, daily pads have been associated with potential health concerns due to their tendency to trap heat and moisture against the skin, creating conditions that may predispose users to complications such as microbial overgrowth and skin irritation.[1] These risks are further heightened by the unique vulnerability of the vaginal mucosa, which lacks the robust barrier function of the skin,

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allowing pathogens to penetrate more easily, potentially leading to systemic exposure and adverse effects in the anogenital region.[2]

Research into the impact of panty liner use on vulvar and vaginal health has yielded varying findings. A study investigating the microbial flora of the labia revealed that it differs from the vaginal flora. Yet, no significant increase in clinically important microbial species was observed after six months of continuous use.[3] Regarding dermatological effects, studies across different populations suggest that daily pads are generally well-tolerated. For instance, a 2011 study from China reported high vulvar skin tolerance to daily pads, consistent with findings from earlier studies in diverse populations.[4]

Zinc has started to be used in biological materials due to its antibacterial properties.[5] The antibacterial properties of zinc also help biomaterial biocompatibility.[6] The aim of the current study was to research any differences in microbial colony growth on zinc coated versus regular non-coated panty liners from the same company after 4-6 hours of vulvar skin and vaginal contact.

METHODS

Healthy, sexually active, consecutive women aged 20 to 43 years who visited Okan University Hospital *In Vitro* Fertilization Center between January and June 2021 were included in this study. The İstanbul Okan University Ethics Committee approval was obtained for the study (approval number: 128, date: 11.11.2020). All costs of the study was covered by the panty liner producer Hayat Chemistry Company, Turkey, and all participants were given one pack of free of charge panty liner at the end of the study. Participants were provided with both verbal and written information prior to their inclusion in the study, and their informed consent was obtained. Inclusion criteria were all consecutive women who started controlled ovarian hyperstimulation for in vitro fertilization (IVF) treatment, had no diagnosis of recurrent *in vitro* fertilization failure, and were free of immunosuppression. As exclusion criteria, patients with active vaginal bleeding and patients with zinc allergy were not included in the study.

The primary outcome of the study was difference in mean number of colonies between the two panty liners per square centimeter (cm^2). Secondary outcome measures were number of women with vulvar erythema or excoriation.

This was a double blindstudy with blocked randomization. The block size was determined to be the same for the number of people and there was no stratification based on any variables (e.g., age, body mass index). The two identical looking panty liner pads were prepared by the company with code 102 and 103 labeled on the pack without any additional information about the product. At the time of first visit on day 2-4 of the menstrual period patients were informed about the study and were assigned to each group with blocked randomization. Pre-prepared 250 code written closed envelopes were put in a closed box and mixed. Code 102 was written in 125 envelopes and 103 was written in another 125 sealed envelopes. The envelopes were taken from the box at the time of randomization and the panty liner pack was given according to the code inside the envelope until the 250th patient was recruited. The flow chart of the study is presented in Figure 1.

The panty liners were outwardly identical and neither the patients, nor the clinicians or microbiologist were aware of the technology until the study was finished. The second visit of the patients for controlled ovarian hyperstimulation was done at the follicular phase of the cycle without any menstrual bleeding after 5-6 days of their first visit with planned weekends.The patients were instructed not to use panty liners or any sanitary products until the second visit when comprehensive clinical evaluation, including a physical examination of the vulva and a speculum-based assessment of the vagina was done. The evaluation focused on identifying dermatological conditions such as vulvar erythema, excoriation, and discharge. Biological samples were obtained from specific anatomical sites: cultures were collected from the interlabial space (between the labia minora and labia majora), while cervicovaginal samples and bacterial vaginosis specimens were retrieved from the upper lateral vaginal wall. These assessments aimed to establish baseline microbial profiles for participants prior to pad use. Patients were instructed to avoid sexual activity, douching, or other potential confounders before sample collection. Optimal conditions for processing swab samples (e.g., transport conditions, temperature control) were provided.

Swab samples were collected from the lateral wall of the vagina for Gram staining and applied to slides. These were evaluated for bacterial vaginosis using Nugent scoring at 100x magnification. In addition, a second swab was collected to assess for vaginal candidiasis. The samples were cultured on sabouraud dextrose agar (SDA) and incubated for 48 hours at 37 °C. When growth was observed, colonies were stained, and *Candida* was diagnosed upon detection of gram-positive blastospores. Species identification and antifungal susceptibility testing were performed using the VITEK 2 (BioMerieux) system with YST and AST-YS07 cards. Pathogenic isolates of vagina and vulva was defined by isolation of aerobic bacteria like *E. coli, Klebsiella* spp., *Streptococcus agalactiae*, *Enterococcus* spp. and *Candida* spp.

The patients were provided with coded panty liners and instructed to use the panty liner for 2-3 days between the second and third visit. On the day of third visit they used the last panty liner 4 to 6 hours prior to their ultrasound evaluation. This pad was evaluated for colony counts. At this third visit all patients continue to do their daily routines and after 4-6 hours of panty liner use microbial cultures were obtained from the pads to determine colony counts, while dermatological evaluations of the vulva were conducted to assess any skin irritation or changes. When the pads were retrieved a 1 cm^2 section from the area exhibiting the highest level of discharge or moisture was excised from the center of each pad using a sterile scalpel and placed in a Sabouraud broth tube. All samples were promptly transported to the laboratory for microbial analysis. After homogenization with a vortex mixer, 0.1 mL of the sample was cultured on SDA and incubated at 37 °C for 48 hours. Upon observing growth, colonies were stained, and *Candida* was diagnosed by detecting gram-positive blastospores. Colony counting was performed, and species identification and antifungal susceptibility testing were

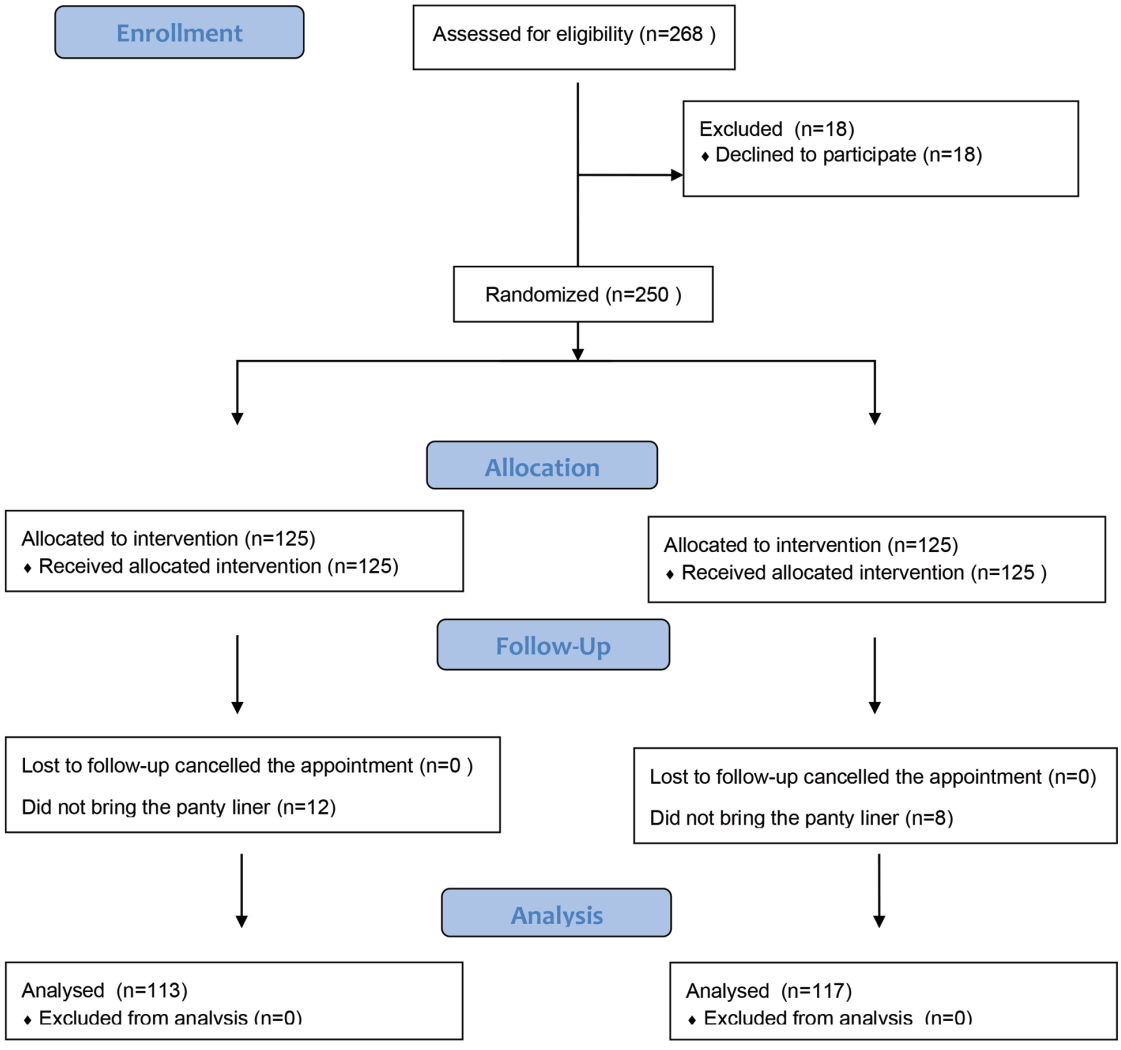

Figure 1. Consort flow diagram

carried out using the VITEK 2 (BioMerieux) system with YST and AST-YS07 cards. Whenever microbial cultures of all cases were evaluated and reported, the codes of the panty liner 102 was revealed to be zinc coated group and 103 was revealed to be non-coated regular panty liner group.

Statistical Analysis

The statistical analysis was done using SPSS, version 21 (IBM Inc., Armonk, NY, USA). Continuous variables are presented as means ± standard deviation and categorical data are presented as counts and percentages. Comparison of the categorical data frequency between the two groups was done using chi square test. Comparison of categorical data frequency within the group prior to and after the use of panty liner was done using McNemar test. The comparison of continuous data between the groups was done using independent samples t test. A probability (p) less than 0.05 was considered to be statistically significant for all comparisons.

RESULTS

A total of 268 women were eligible for the study. Women who refused to use the pad (n=18) were excluded from the study leaving 250 cases for allocation. After allocation, 12 patients with code 102 did not bring their panty liners or did not comply with the use and were excluded from the analysis while eight patients with code 103 were excluded due to the same reasons. As a result 113 patients with code 102 and 117 patients with code 103 were analyzed.

The demographical data are given in Table 1. The frequency of vaginal discharge, foul odor in the perineum, vulvar itching, erythema on the vulva, and excoriation on the vulva were similar in the Zinc coated panty liner group (n=113) vs. non-coated group (n=117). The frequency of bacterial vaginosis in the Zinc coated group was 15.9% (n=13) which was not statistically different in non-coated group 17.9% (n=21, p=0.6). The percent of cases with pathogenic microbial species isolation in the vagina (n=23, 28%) and vulva (n=42, 51.2%) of the coated group was again not statistically different from the

Table 1. Demographic data		
Demographic data	**Zinc-coated group (n=113) n (%)**	**Non-coated group (n=117) n (%)**
Age (mean)	33.1	32.3
Marital status	Never married: 25 (22.1) Currently married: 30 (26.5) Other: 58 (51.3)	Never married: 26 (22.2) Currently married: 31 (26.4) Other: 60 (51.2)
Cigarettes per day	0: 58 (51.3) 1-9: 30 (26.5) 10+: 25 (22.1)	0: 60 (51.2) 1-9: 31 (26.4) 10+: 26 (22.2)
Frequency of alcohol drinking	None: 31 (27.4) Less than weekly: 35 (30) Weekly or more: 47 (41.5)	None: 32 (27.3) Less than weekly: 36 (30.7) Weekly or more: 49 (41.8)
Hormonal contraception	Yes: 20 (17.6) No: 93 (82.3)	Yes: 22 (18.8) No: 95 (81.1)

Table 2. Baseline symptomatology and genital findings			
Syptoms-findings	**Zinc-coated group (n=113) n (%)**	**Non-coated group (n=117) n (%)**	***p****
Vaginaldis charge	15 (18.3)	23 (19.7)	0.8
Foulodor in the perineum	18 (22)	25 (21.4)	0.9
Vulvarit ching	7 (8.5)	10 (8.5)	0.9
Erythemaon the vulva	8 (9.8)	12 (10.3)	0.9
Excoriationon the vulva	8 (9.8)	12 (10.3)	0.9
Bacterial vaginosis	13 (15.9)	21 (17.9)	0.6
Pathogen in the vagina	23 (28)	43 (36.8)	0.1
Pathogen in the vulva	42 (51.2)	60 (51.3)	0.9
*chi-square test, not significant, $p>0.05$			

vagina (n=43, 36.8%, p=0.1) and vulva (n=60, 51.3%, $p=0.9$) of the non-coated group. The baseline symptomatology and genital findings are given in Table 2.

The vulva and panty liner contact time was similar in zinc coated and non-coated groups respectively (280±65 vs 275±72 minutes, $p<0.58$). In the zinc-coated group coded as (102) there were 60 (53.7%) patients without microbial growth. This proportion was significantly higher than in group 103 with non-coated regular panty liners (n=44, 37.6%, $p=0.02$). Furthermore, the number of colonies per cm^2 of the zinc-coated panty liners was significantly lower than in the non-coated group (m=9324±24046 vs 56663±99618, $p<0.001$). The microbial proliferation in the two types of panty liners in given in Table 3.

The number of cases with vulvar erythema and excoriation was similar in the two groups before and after panty liner use. Furthermore, within group change in the frequency of vulvar erythema and excoriation was not significant. Vulvar skin findings before and after panty liner use is shown in Table 4. There were no adverse events or side effect in either group.

DISCUSSION

These results show that zinc-coated panty liners harbored less microbial colonies compared to the non-coated regular panty liners. While most studies[2] focus on isolating and analyzing individual pathogen species through separate cultures, the present study prioritized assessing the total number of cultured microorganisms. Although this approach represents a limitation when compared to studies that provide detailed pathogen-level data, a key strength of our study lies in its dual focus on microbial counts and vulvar symptomatology and findings within a single research setting.

When daily pads were examined symptomatically in terms of side effect profiles such as edema, erythema, burning, stinging, and itching, in a study conducted by Xuemin et al.[3] in Chinese women, comparing two pads with non-woven and perforated surfaces, no significant difference was observed between the two groups. Similarly, in the present study, there was no significant difference between and within the groups with and without a zinc-coated surface in terms of erythema, excoriation, and itching.

Basit et al.[7] discussed traditional beliefs about hygienic products and economic problems in their study conducted in Bangladesh during the flood period. We did not mention financial access to the product in our study, but only 18 (0.6%) of the 268 women in the clinic refused to use pads.

Runeman et al.[8] demonstrated that breathable pads maintained vulvar microclimate stability better than traditional pads. Our

Table 3. Microbial proliferation in the two types of panty liners

Finding	Zinc-coated group (n=113)	Non-coated group (n=117)	*p*
Vulva pantyliner contact time	280±65	275±72	0.58*
Non-microbialgrowth	60 (53.7%)	44 (37.6%)	0.02**
Colonycount	9324±24046	56663±99618	<0.001***

*independent samples t-test
**chi-square test
***Independent samples t-test

Table 4. vulvar skin findings before and after panty liner use

Finding	Zinc-coated group (n=113)	Non-coated group (n=117)	*p**
Pre-existing vulvar erythema	11 (9.8%)	12 (10.3%)	0.9
Post-use vulvar erythema	7 (6.1%)	9 (7.7%)	0.6
Within group comparison (p)	0.12**	0.25**	
Pre-existing vulvar excoriation	11 (9.8%)	12 (10.3%)	0.9
Post-use vulvar excoriation	7 (6.1%)	9 (7.7%)	0.6
Within group comparison (p)	0.12**	0.25**	

*chi-square test
**McNemar's test

double-blind study design addressed vaginal microclimate, vulvar findings and symptomatology and panty liner microbial colony forming unit (CFU) count. This rigorous approach lends greater reliability to our findings when compared to similar studies.

Farage et al.[2] conducted a study comparing daily pads with deodorant and a control group without deodorant in terms of aerobic bacteria cultures. They reported no statistically significant difference in positive cultures of undesirable microorganisms such as *Candida albicans*, non-*Candida* yeasts, Candida spp, *Gardnerella vaginalis*, *Staphylococcus aureus*, *coliforms*, *proteus*, *pseudomonas*, *streptococci Groups* A,B, D and *Streptococcus viridans* before and after six months of panty liner use. In the present study, although no significant difference was observed in incidence of bacterial vaginosis between the two pads tested, the total colony count of cultured bacteria from the panty liners was significantly lower for the zinc-coated group. This suggests that zinc has an inhibiting effect on the number of colonies formed by the undesirable bacteria in the vaginal flora compared with non-zinc coated regular panty liners.

Zinc can be classified as a form of immunotherapy and has effects on macrophage and neutrophil functions, natural killer cell/phagocytic activity, and various inflammatory cytokines.[9] Zinc also directly modulates the interaction between host cells and viral components.[10] Although the exact mechanism remains unclear, there are promising reports in the literature of zinc being used effectively in various topical and oral forms and concentrations for the treatment of cutaneous viral warts.[11]

The zinc ion was found to have a more profound antibacterial effect on gram-positive bacteria such as *S. aureus* and *Staphylococcus epidermidis* compared to gram-negative bacteria.[12,13] The proposed mechanism of action includebinding of zinc to the membranes of microorganisms and increasing the lag time and new microbial cell generation time, such as in C. albicans.[14] Moreover, zinc has been shown to cause direct bacterial cell membrane disruption and indirectly through mediating the induction of reactive oxygen species (ROS).[15,16] However, extended exposure to zinc oxide was suspected to play a reversible role in aminoglycoside resistance and ampicillin and other b-lactam resistance in *Escherichia coli* by modifying cell drug efflux systems, switching the bacteria to anaerobic respiration state and increasing ribosomal protein production.[17,18] Antibiotic resistance of bacteria as panty liner use was short lasting. Further studies are needed to investigate if zinc coated panty liners may lead to an increased number of antibiotic resistant organisms.

Giraldo et al.[4] examined the effects of breathable versus conventional daily pads and found no significant differences in vulvovaginal irritation or bacterial vaginosis. Their study included colposcopic examinations, while our study relied on speculum-assisted visualization of the vagina and cervix. In addition, the present study only considered a single-day use of 4-6 hours, whereas the study of Giraldo et al.[4] extended to a 75-day period.

Kim et al.[19] investigated the presence of volatile organic compounds in pads used in Korea and found that these pads have no cancer or non-cancer risk. Again, given the short term nature of the present study, this aspect was not investigated.

In a study conducted by Yadav et al.[20] in Nepal, high awareness and self-efficacy in menstrual hygiene management among female adolescents were noted. In our study, population women showed high compliance with the terms of use, although they were although they were in this respect.

The strength of our study is that it was double-blind. The weakness of our study is that microbiological cultures were not identified and therefore pathogen levels were unavailable and there was also no testing of antibiotic resistance.

CONCLUSION

Research into the effects of daily panty liners used during non-menstrual periods, such as for vaginal discharge, spotting, and mild urinary incontinence, has become important with increasing use and will be important for enhancing patient comfort and quality of life. Studies examining the impact of panty liners with different formulations and constructions on vulvar irritation, vaginal pH, microbiological outcomes, and potential triggers for discharge or itching will be important for safe product development. Designing commercial products based on such findings can significantly improve user comfort and deliver broader health benefits.

Ethics

Ethics Committee Approval: The İstanbul Okan University Ethics Committee approval was obtained for the study (approval number: 128, date: 11.11.2020).

Informed Consent: Participants were provided with both verbal and written information prior to their inclusion in the study, and their informed consent was obtained.

Authorship Contributions

Surgical and Medical Practices: Ş.Ç., C.Ö., B.A., Concept: Ş.Ç., Design: Ş.Ç., Data Collection or Processing: Ş.Ç., Ö.D.S., Analysis or Interpretation: Ş.Ç., Ö.D.S., Literature Search: Ö.D.S., Writing: Ş.Ç., Ö.D.S.

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Farage M, Bramante M, Otaka Y, Sobel J. Do panty liners promote vulvovaginal candidiasis or urinary tract infections? A review of the scientific evidence. Eur J Obstet Gynecol Reprod Biol. 2007;132(1):8-19.
2. Farage MA, Enane NA, Baldwin S, Berg RW. Labial and vaginal microbiology: effects of extended panty liner use. Infect Dis Obstet Gynecol. 1997;5(3):252-258.
3. Xuemin W, Sato N, Chao Y, et al. Cutaneous and sensory effects of two types of sanitary pads with different surfaces in the Shanghai, Chinese population. Cutan Ocul Toxicol. 2011;30(3):212-216.
4. Giraldo PC, Amaral RL, Juliato C, Eleutério J Jr, Brolazo E, Gonçalves AK. The effect of "breathable" panty liners on the female lower genital tract. Int J Gynaecol Obstet. 2011;115(1):61-64.
5. Su Y, Cockerill I, Wang Y, et al. Zinc-based biomaterials for regeneration and therapy. Trends Biotechnol. 2019;37(4):428-441.
6. Su Y, Wang K, Gao J, et al. Enhanced cytocompatibility and antibacterial property of zinc phosphate coating on biodegradable zinc materials. Acta Biomater. 2019;98:174-185.
7. Basit A, Antu OS, Mithun M, Islam MS. Navigating the currents: understanding awareness, attitudes, and menstrual hygiene management challenges in Bangladesh's Haor Region. J Biosoc Sci. 2025;57(1):128-145.
8. Runeman B, Rybo G, Larkö O, Faergemann J. The vulva skin microclimate: influence of panty liners on temperature, humidity and pH. Acta Derm Venereol. 2003;83(2):88-92.
9. Gupta M, Mahajan VK, Mehta KS, Chauhan PS. Zinc therapy in dermatology: a review. Dermatol Res Pract. 2014;2014:709152.
10. Lazarczyk M, Favre M. Role of Zn2+ ions in host-virus interactions. J Virol. 2008;82(23):11486-11494.
11. Songsantiphap C, Asawanonda P. Topical 15% zinc oxide ointment significantly reduces the size of common warts after four weeks: a randomized, triple-blinded, placebo-controlled trial. J Clin Aesthet Dermatol. 2019;12(9):26-31.
12. Atmaca S, Gül K, Çiçek R. The effect of zinc on microbial growth. Turk J Med Science. 1998;28(6):595-597.
13. Söderberg TA, Sunzel B, Holm S, Elmros T, Hallmans G, Sjöberg S. Antibacterial effect of zinc oxide in vitro. Scand J Plast Reconstr Surg Hand Surg. 1990;24(3):193-197.
14. Radke LL, Hahn BL, Wagner DK, Sohnle PG. Effect of abscess fluid supernatants on the kinetics of Candida albicans growth. Clin Immunol Immunopathol. 1994;73(3):344-349.
15. Brayner R, Ferrari-Iliou R, Brivois N, Djediat S, Benedetti MF, Fiévet F. Toxicological impact studies based on Escherichia coli bacteria in ultrafine ZnO nanoparticles colloidal medium. Nano Lett. 2006;6(4):866-870.
16. Jones N, Ray B, Ranjit KT, Manna AC. Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms. FEMS Microbiol Lett. 2008;279(1):71-76.
17. Ibacache-Quiroga C, Oliveros JC, Couce A, Blázquez J. Parallel evolution of high-level aminoglycoside resistance in Escherichia coli under low and high mutation supply rates. Front Microbiol. 2018;9:427.
18. Rihacek M, Kosaristanova L, Fialova T, et al. Zinc effects on bacteria: insights from Escherichia coli by multi-omics approach. mSystems. 2023;8(6):e0073323.
19. Kim HY, Lee JD, Kim JY, et al. Risk assessment of volatile organic compounds (VOCs) detected in sanitary pads. J Toxicol Environ Health A. 2019;82(11):678-695.
20. Yadav RN, Joshi S, Park JS. Awareness of menstrual hygiene management rights and perception of risks, self-efficacy, and behavior. J Nepal Health Res Counc. 2024;22(1):66-72.

Original Article

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A Case-control Study of Female Genital Measurements in Polycystic Ovary Syndrome and Their Relation to Sexual Function and Genital Perception

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ABSTRACT

Purpose: To analyze analyze differences in external genital measurements between women with polycystic ovary syndrome (PCOS) and healthy controls, and to evaluate how these anatomical differences relate to sexual function and genital self-image.

Methods: A prospective multicenter case-control study was conducted between January and December 2024. The study included 155 women with PCOS and a control group of women with mild male factor infertility or unexplained infertility, all 18-45 years old and evaluated at an infertility clinic. PCOS was diagnosed according to the Rotterdam criteria. Participants with a history of gynecological surgery, hormonal medication use, or endocrine disorders were excluded. External genital measurements, including clitoral size, labial dimensions, vaginal length, and anogenital distance (AGD), were recorded. The female sexual function index (FSFI) and the female genital self-image scale (FGSIS) were administered to assess sexual function and genital self-image.

Results: There were 155 women with PCOS and 155 women in the control group. Women with PCOS had a significantly higher body mass index (BMI) (26.5±6.2 vs. 24.6±4.7 kg/m^2, $p=0.003$) and later menarche (12.7±1.3 vs. 11.6±1.1 years, $p<0.001$) compared to controls. Genital measurements revealed that women with PCOS had a shorter clitoral prepuce length (18.3±7.02 vs. 20.7±4.9 mm, $p=0.001$), shorter AGD (21.2±13.3 vs. 26.1±7.7 mm, $p<0.001$), and reduced labia majora length (62.7±19.8 vs. 74.2±8.7 mm, $p<0.001$). In contrast, clitoral glans width was significantly larger in the PCOS group (6.5±2.8 vs. 5.8±1.5 mm, $p=0.008$). Women with PCOS also had higher FSFI subscale scores for arousal (3.8±1.6 vs. 3.3± 1.2, $p=0.002$) and orgasm (4.1±1.2 vs. 3.6±1.8, $p=0.003$) but lower FGSIS scores (18.2±3.8 vs. 22.8±4.1, $p<0.001$), indicating less positive genital self-image.

Conclusion: This study demonstrated marked differences in external genital measurements between women with PCOS and healthy controls, including shorter clitoral prepuce length, shorter AGD, and smaller labia majora in the PCOS group. These results imply that hormonal imbalances in PCOS may lead to distinct genital morphological changes that could influence sexual function and self-image. Despite higher arousal and orgasm scores, women with PCOS reported a lower genital self-image, highlighting a complex relationship between PCOS, body image, and sexual health. These findings provide new insights into the physical and psychological dimensions of PCOS and should inform future research and clinical strategies addressing sexual health in this population.

Keywords: Polycystic ovary syndrome, genital measurements, female genital anatomy, sexual function, genital self-image

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in women of reproductive age, with an estimated prevalence of about 7-12% of the female population worldwide.[1] It is characterized by clinical or biochemical hyperandrogenism, chronic anovulation, and polycystic ovarian morphology.[2] Beyond its metabolic and reproductive implications, PCOS is associated with significant psychosocial and sexual health challenges, including sexual dysfunction and altered body image.[3,4]

Sexual dysfunction in women with PCOS has been widely studied, with multiple reports indicating higher rates of sexual dissatisfaction compared to women without the syndrome.[5] Women with PCOS frequently report reduced sexual desire, arousal, lubrication, and overall satisfaction.[6,7] Common symptoms of PCOS such as hirsutism, acne, and menstrual irregularities are often linked to body image concerns, which may negatively affect sexual self-esteem and genital perception.[4,8] However, the anatomical factors contributing to these issues remain poorly understood. Relatively few studies have examined how PCOS affects genital morphology and how these anatomical variations might relate to sexual function and genital self-image.

Emerging evidence suggests that hormonal imbalances in PCOS may lead to subtle anatomical variations in the genitalia that could influence sexual function.[9] Recent research has highlighted certain genital measurements, such as clitoral size and anogenital distance (AGD), as potential biomarkers of androgen exposure in women.[10,11] However, no comprehensive study has evaluated a broad range of external genital measurements in PCOS patients, nor the relationship between these anatomical features and measures of sexual function and genital self-image.

The aim of this study was to compare a wide array of external genital measurements between women with PCOS and healthy controls. In addition, we sought to evaluate how any anatomical differences correlate with sexual function and genital self-image in these two groups.

METHODS

A prospective case-control study was conducted as a multicenter study between January and December 2024.

The study included equal numbers of women with PCOS and women with mild male factor infertility or unexplained infertility, all between 18 and 45 years of age and attending an infertility clinic. Control cases were enrolled after being evaluated by an infertility specialist and determined to have either unexplained infertility or only a mild male factor contributing to infertility.

The Rotterdam criteria were used to diagnose PCOS[3]. Patients were identified as having PCOS if they had at least two of the following: 1) oligo- and/or anovulation, 2) clinical and/or biochemical signs of hyperandrogenism (e.g., hirsutism, acne, or alopecia) and (3) polycystic ovarian morphology on ultrasound, characterized by ≥12 follicles measuring 2-9 mm in diameter and/or an ovarian volume >10 cm^3. All ovarian ultrasound evaluations were performed transvaginally using a high-frequency transducer to assess for polycystic ovarian morphology. Of note, we primarily relied on clinical indicators of hyperandrogenism for PCOS diagnosis; serum androgen levels were not routinely measured.

The diagnosis of unexplained infertility was made by excluding other potential causes in couples who had not conceived after 12 months of regular, unprotected intercourse. This evaluation ruled out male factor infertility, oligo/anovulatory infertility, and anatomical issues such as bilateral fallopian tube occlusion (confirmed by hysterosalpingography), endometriosis, fibroids, uterine cavity abnormalities, or cervical/vaginal obstructions.[12] Mild male factor infertility was defined as being present when male factors were the sole cause of infertility and only one semen parameter was below the World Health Organization reference range for normal semen analysis.[13]

Exclusion criteria for both groups included: history of vaginal delivery or obstetric perineal trauma (e.g., episiotomy), pelvic organ prolapse surgery, or cosmetic gynecologic procedures (such as labiaplasty or clitoral hoodoplasty); presence of endocrine disorders such as Cushing's syndrome, congenital adrenal hyperplasia, androgen-secreting tumors, hyperprolactinemia, hyperthyroidism, or hypothyroidism; and use of hormonal medications (including contraceptives) or antidepressants within the six months prior to enrollment.

Detailed clinical histories were obtained, and physical examinations were performed for all participants. Recorded information included age, body mass index [(BMI) calculated as weight in kilograms divided by height in meters squared], parity, history of tobacco use, age at menarche, years of sexual activity, and menstrual regularity (regular menses defined as cycles lasting 25-35 days with bleeding for 4-6 days per cycle).

Signs of hyperandrogenism were assessed clinically. Hirsutism was evaluated using the modified Ferriman-Gallwey score (mFG).[14] Acne severity was assessed using the Investigator Global Assessment scale for acne vulgaris, which ranges from 0 (clear) to 4 (severe).[15] Alopecia (scalp hair thinning or hair loss) was evaluated on a scale from 0 (no alopecia) to 4 (severe alopecia), reflecting increasing degrees of androgenic alopecia.

External genital measurements were obtained with participants in the lithotomy position. To minimize inter-observer variability, two gynecology specialists conducted all measurements, and the examiners were blinded to each participant's group. For consistency, the two examiners underwent joint training and a calibration session for the measurement techniques prior to the study, although no formal inter-rater reliability analysis was performed. Measurements were taken using a digital stainless-steel Vernier caliper; vaginal depth measurements were obtained using a hysterometer. The clitoral glans length was measured by gently retracting the clitoral prepuce. The lengths and widths of the labia minora and labia majora were measured bilaterally. Additionally, the AGD [the distance from the anus to the posterior fourchette (AGD_{AF})] was measured with the caliper to represent the perineal body length. The measurement protocol followed a standardized template

(Figure 1) as described in a previous study.[16] Each distance was measured three times, and the average of the three values was used for analysis.

After the physical examination, all participants completed validated Turkish versions of the female sexual function index (FSFI)[17] and the female genital self-image scale (FGSIS)[18] in a private setting, with a research nurse available for assistance if needed. The FSFI is a 19-item questionnaire assessing sexual function over the prior four weeks, evaluating domains of desire, arousal, lubrication, orgasm, satisfaction, and pain.[19] Female sexual dysfunction was defined as a total FSFI score ≤26.55.[20] The FGSIS is a 7-item questionnaire that assesses a woman's feelings and perceptions about her genitalia; higher scores indicate a more positive genital self-image, with a maximum score of 28.[21]

This study was approved by the Non-Interventional Research Ethics Committee of the University of Health Sciences Turkey, Şehit Prof. Dr. İlhan Varank Sancaktepe Training and Research Hospital (approval number: 178, date: 12.06.2024). Written informed consent was obtained from all participants. The research was conducted in accordance with the principles of the Declaration of Helsinki.

Statistical Analysis

The data were analyzed using IBM SPSS Statistics, version 25 (IBM Corporation, Armonk, NY, USA). Categorical data are presented as numbers and percentages, and continuous variables as mean ± standard deviation. For comparisons between the PCOS and control groups, the chi-square test (or Fisher's exact test when appropriate) was used for categorical variables. Continuous variables were compared using an independent samples *t*-test for normally distributed data, or the Mann-Whitney U test for non-parametric data. A <0.05 was considered statistically significant.

RESULTS

The study included 155 women with PCOS and 155 controls. The mean age of the participants was 30±6.3 years for the PCOS group and 31.2±6.4 years for the control group ($p=0.1$). The mean BMI was significantly higher in the PCOS group (26.5±6.2 kg/m²) compared to the control group (24.6±4.7 kg/m²) ($p=0.003$). No statistically significant difference was found between the groups in the comparison made in terms of characteristics such as parity, sexually active years, and tobacco use.

However, the PCOS group experienced delayed menarche compared to the control group, with a mean age of 12.7±1.3 years versus 11.6±1.1 years, respectively ($p<0.001$). In addition, only 45.8% of women in the PCOS group reported regular menstruation, compared to 95.5% in the control group, indicating a higher prevalence of menstrual irregularities in the PCOS group, as expected ($p<0.001$).

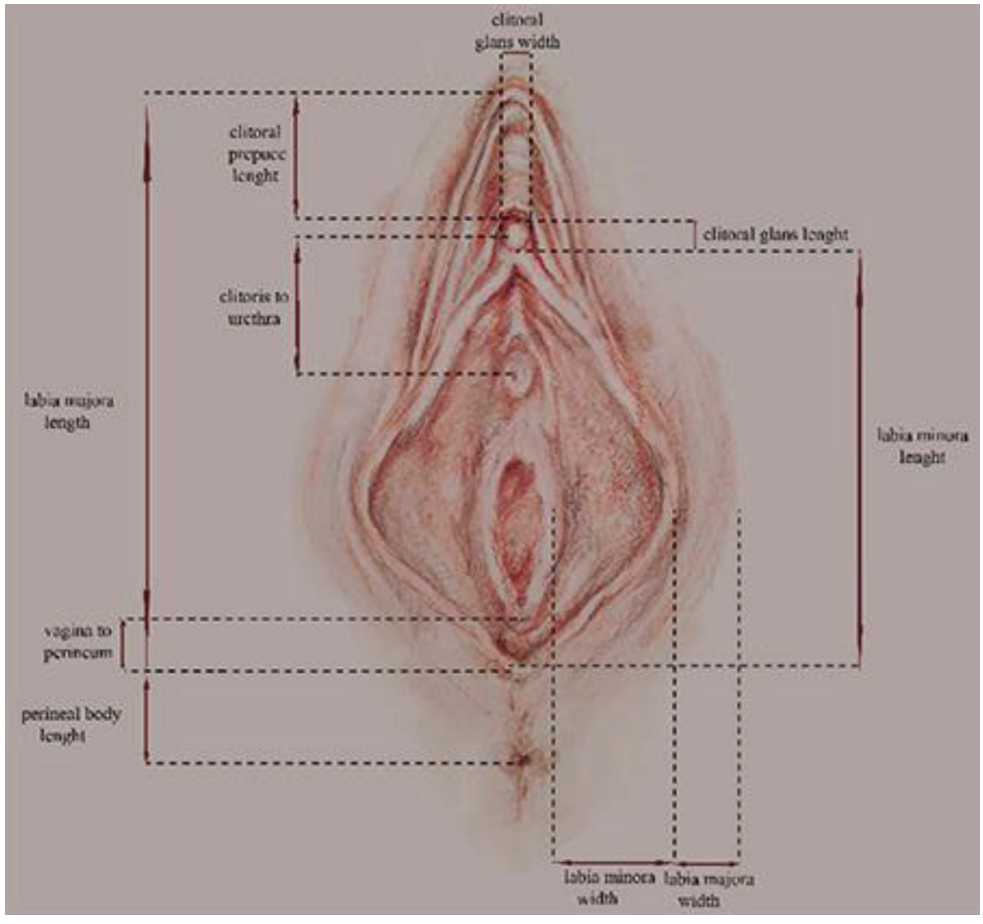

Figure 1. A schematic drawing of a part of the female external genitalia measurements

The distribution of mFG scores, acne scores, and alopecia scores between the groups is shown in Table 1. In the PCOS group, the average mFG score was 13.6±7.8, with values ranging from 1 to 30. Conversely, the control group had an average score of 3.5±4.1, ranging from 1 to 9, showing a markedly higher level of hirsutism in the PCOS group, with a statistically significant difference ($p<0.001$).

Furthermore, the PCOS group had significantly higher acne scores compared to the control group, with a mean of 1.2±1.1 (range 0-4) versus 0.2±0.3 (range 0-4) ($p<0.001$). Similarly, alopecia scores were elevated in the PCOS group, with a mean of 2.2±0.9 (range 0-4), whereas the control group had a mean score of 0.4±0.5 (range 0-2) ($p<0.001$).

The genital measurements of the subjects are displayed in Table 2. The PCOS group had a significantly shorter clitoris prepuce length (18.3±7.02 mm) in comparison to the control group (20.7±4.9 mm) ($p=0.001$). The clitoral length tended to be slightly longer in the PCOS group (18.5±7.1 mm) compared to the control group (17.1±6.7 mm), ($p=0.07$). The clitoris glans width was significantly larger in the PCOS group (6.5±2.8 mm) compared to the control group (5.8±1.5 mm) ($p=0.008$).

The PCOS group exhibited a shorter distance between the clitoris and the urethra (20.8±7.5 mm, 22.4±5.7 mm respectively) compared to the control group ($p=0.03$). Similarly, the AGD was significantly shorter in the group with PCOS (21.2±13.3 mm) compared to the control group (26.1±7.7 mm) ($p<0.001$). There was also a significant decrease in vaginal length in the PCOS group (83.5±17.2 mm) compared to the control group (92.7±8.1 mm) ($p<0.001$).

Comparison in terms of the measurements of the labia majora and minora between the groups is summarized as follows.

Table 1. Demographic variables in women with polycystic ovary syndrome and controls

Variable	Polycystic ovary syndrome cases (n=155)	Control group (n=155)	*p*
Age (years)	30±6.3	31.2±6.4	0.1
Body mass index (kg/m^2)	26.5±6.2	24.6±4.7	0.003*
Parity	0.3±0.7	0.4±1	0.3
Tobacco use Current user Past user	 29 (18.7%) 12 (7.7%)	 32 (20.6%) 20 (12.9%)	0.1
Age at menarche (year)	12.7±1.3	11.6±1.1	<0.001*
Sexually active (years)	4.9±4.3	5.1±5.2	0.7
Ferriman-gallway score	13.6±7.8 (1-30)	3.5±4.1 (1-9)	<0.001*
Regular menstruation	71 (45.8%)	148 (95.5%)	<0.001**
Acne score	1.2±1.1 (0-4)	0.2±0.3 (0-4)	<0.001*

Statistically significant, independent samples t-test, $p<0.05$
Statistically significant, chi-square test, $p<0.05$

Table 2. Genital measurements polycystic ovary syndrome and controls

Variable	Polycystic ovary syndrome cases (n=155)	Control group (n=155)	*p*
Clitoris prepuce length (mm)	18.3±7.02	20.7±4.9	0.001*
Clitoris length (mm)	18.5±7.1	17.1±6.7	0.07
Clitoris glans width (mm)	6.5±2.8	5.8±1.5	0.008*
Clitoris to urethra (mm)	20.8±7.5	22.4±5.7	0.03*
AGD/perineal body length (mm)	21.2±13.3	26.1±7.7	<0.001*
Vaginal Length (mm)	83.5±17.2	92.7±8.1	<0.001*
Labia minora width (mm)			
Left	17.4±10.3	22.5±9.6	<0.001*
Right	16±9.4	21.5±7.9	<0.001*
Labia minora length (mm)			
Left	36.5±12.6	37.9±12.9	0.28
Right	34.4±12.6	35.9±12	0.26
Labia majora length (mm)	62.7±19.8	74.2±8.7	<0.001*
Labia majora width (mm)	29.6±8.4	29.6±6.3	0.9

*Statistically significant, independent samples t-test, $p<0.05$

In the PCOS group, the widths of both the left and right labia minora were significantly smaller (left: 17.4±10.3 mm, right: 16±9.4 mm) compared to the control group (left: 22.5±9.6 mm, right: 21.5±7.9 mm) ($p<0.001$ for both). However, there was no substantial difference in the labia minora lengths among the groups ($p=0.28$, $p=0.26$, respectively). The labia majora in the PCOS group had a significantly reduced length (62.7±19.8 mm) compared to the control group (74.2±8.7 mm) ($p<0.001$). In contrast, there were no noticeable differences in the width 29.6±8.4 mm for PCOS compared to 29.6±6.3 mm for the control group ($p=0.9$).

The comparison of FSFI and FGSIS measurements between the PCOS and control groups is presented in Table 3. The groups did not show any statistically significant differences in terms of desire, lubrication, satisfaction, or pain subdomain scores ($p=0.2$, $p=0.4$, $p=0.1$ and $p=0.3$, respectively) (3.8±1.6 vs 3.3±1.2, $p=0.002$). However, the PCOS group had significantly higher arousal and orgasm scores (4.1±1.2 vs 3.6±1.8, p=0.003) compared to the control group (3.3±1.2 and 3.6±1.8, respectively) ($p=0.002$ and $p=0.003$, respectively). Consequently, the overall FSFI score was significantly higher in the PCOS group (24.4±6.6) compared to the control group (21.9±9) ($p=0.005$). In contrast, the FGSIS score exhibited a substantial decrease in the PCOS group (18.2±3.8) compared to the control group (22.8±4.1) ($p<0.001$). The study showed that 55.5% of the individuals in the PCOS group (86 out of 155) and 58.7% of the individuals in the control group (91 out of 155) had FSFI scores below 26.55

DISCUSSION

This study provides new insights into the anatomical differences in external genital measurements between women with PCOS and healthy controls, and discusses the implications for sexual function and genital self-image. The results suggested that women with PCOS exhibit notable differences in genital morphology, particularly in clitoral size, labial dimensions, and vaginal and perineal body length. Moreover, women with PCOS exhibited higher levels of sexual arousal and orgasm but reported lower genital self-image satisfaction as reflected by the FGSIS. This duality in sexual function and self-image highlights a complex interaction between anatomical and psychological factors in PCOS. While several studies have examined genital morphology and sexual function in PCOS patients, our study distinguishes itself with a larger sample size and a more comprehensive range of genital measurements, providing more robust results concerning the physical and psychosexual dimensions of PCOS.

Several studies have highlighted the role of prenatal androgen exposure in shaping the reproductive phenotype of females, including the development of PCOS in adulthood.[22] This association suggests that early hormonal imbalances may set the stage for later development of androgen-related disorders, including the distinct morphological changes observed in external genitalia among women with PCOS. AGD, a sexually dimorphic trait, has been used as a biomarker for intrauterine androgen exposure, with longer AGD in females indicating higher prenatal androgen levels.[23-25] Interestingly, our study observed a shorter perineal body length in PCOS women, measured using the same method employed in previous studies to assess AGD from the upper verge of the AGD_{AF}. This finding is in contrast with some earlier research that suggested a longer AGD could be a potential marker for PCOS.[11] Variations in study populations, ethnic backgrounds, or measurement techniques may influence this discrepancy. Moreover, factors such as amount of intrauterine androgen exposure and the timing of hormonal imbalances during development could play pivotal roles in determining AGD outcomes.

Despite the findings on AGD, the other morphological findings in the present study align with previous research indicating that even moderate elevations in androgen levels, as observed in women with PCOS, can result in subclinical changes in external genital morphology. Specifically, we found that women with PCOS exhibited a shorter clitoral prepuce length, and a larger clitoris glans width compared to the control group. These differences, though subtle, mirror the findings of Köşüş et al.[9], who reported significant increases in clitoral and labial dimensions in PCOS patients. Importantly, their study highlighted a strong correlation between clitoral size and hyperandrogenism, reinforcing the notion that genital measurements may serve as valuable markers of androgen excess in PCOS.

Table 3. Mean total and sub-dimension FSFI scores and FGSIS scores in polycystic ovary syndrome and controls

Variable (mean ± SD)	Polycystic ovary syndrome cases (n=155)	Control group (n=155)	*p*
Desire	3.7±1.4	3.5±1.3	0.2
Arousal	3.8±1.6	3.3±1.2	0.002*
Lubrication	4.1±1.2	4±1.3	0.4
Orgasm	4.1±1.2	3.6±1.8	0.003*
Satisfaction	3.9±1.4	3.7±1.1	0.1
Pain	4.3±1.6	4.1±1.8	0.3
Total score	24.4±6.6	21.9±9	0.005*
FSFI <26.55, (%)	86 (55.5%)	91 (58.7%)	0.3
FGSIS score	18.2±3.8	22.8±4.1	<0.001*

Statistically significant, independent samples t-test, $p<0.05$
SD: Standard deviation, FSFI: Female sexual function index, FGSIS: Female genital self-image scale

However, our study builds on this previous work by providing a more comprehensive analysis of genital morphology, as we evaluated a broader range of measurements beyond clitoral dimensions. We assessed labia minora and majora widths and lengths, along with vaginal length, offering a more detailed picture of the morphological changes associated with PCOS. Notably, we found that the labia minora were significantly smaller in width, and the labia majora were shorter in length in women with PCOS compared to controls-findings that have not been previously reported in such detail in the literature. This expanded dataset provides further evidence that moderate hyperandrogenism may impact various external genital structures, not just the clitoral area, suggesting that these changes may contribute to the broader phenotypic spectrum of PCOS.

Despite the PCOS group demonstrated higher levels of hirsutism, worse acne, higher BMI, and differences in genital appearance, which contributed to lower FGSIS scores and greater dissatisfaction with genital appearance, these factors did not result in overall impairment in sexual function. Indeed, in the present study, women with PCOS reported significantly higher scores in arousal and orgasm domains, which contrasts with the widely held belief that PCOS leads to generalized sexual dysfunction. Most studies, including a meta-analysis by Pastoor et al.[5], have reported lower sexual function scores in PCOS patients across domains such as arousal, lubrication, and orgasm.[7]

The paradox of enhanced sexual response despite a negative genital self-image raises intriguing questions. One possible explanation is that elevated androgen levels contribute to this heightened sexual response, particularly in terms of arousal and orgasm, as suggested by previous studies on androgenic effects on libido.[7,9] Elevated androgens are known to sensitize the brain and genitals to sexual stimuli, potentially amplifying arousal and orgasmic responses.[26] Additionally, we believe that although Ellibes Kaya et al.[18] reported no significant relationship between genital measurements and sexual function, shorter clitoral prepuce length and wider clitoral width could still be considered contributing factors to the heightened sexual response that we observed in women with PCOS.

Our study, while providing valuable insights, has several limitations. First, the genital measurements-particularly those involving soft tissues like skin and subcutaneous fat-may be subject to measurement variability. Despite standardized techniques and experienced examiners, reproducibility remains a concern due to inherent anatomical diversity. Second, the cross-sectional design limits our ability to infer causality or the progression of genital changes in PCOS. A longitudinal study could better reveal how these anatomical features evolve over time or with treatment. Third, the control group consisted of women with infertility (unexplained or mild male factor) rather than exclusively healthy fertile women. This selection could introduce bias, as infertility itself might affect genital anatomy or sexual function, potentially confounding the comparisons. Fourth, we did not adjust for potential confounders such as BMI or past long-term use of hormonal medications. The PCOS group had a higher BMI on average, which might influence both anatomical measurements and sexual function scores. Although we excluded recent use of hormones or antidepressants, we did not account for prior usage beyond six months, which could have lingering effects on androgen levels or sexual health. Future studies with multivariate analyses could clarify the independent effects of PCOS on outcomes when controlling for such factors. Fifth, although our study population was relatively homogenous in terms of ethnicity and clinical setting, the results may not be widely generalizable to other populations due to genetic and environmental differences. Lastly, we relied on self-reported questionnaires for sexual function and genital self-image; such measures are subjective and may be influenced by personal or cultural factors, which could introduce response bias.

CONCLUSION

This study found significant anatomical differences in genital morphology in women with PCOS, which may be linked to hormonal influences. Despite these anatomical differences, women with PCOS in our sample experienced higher arousal and orgasm scores, even as they reported a more negative genital self-image. These findings underscore the multifaceted nature of sexual health in PCOS, involving both biological and psychological components. Future research should include diverse populations to explore genetic and environmental contributions to these findings. In addition, studies examining different phenotypes of PCOS or the impact of treatments (such as androgen-lowering therapy) on genital anatomy and sexual function would further elucidate the connections observed here. Addressing body image concerns through counseling or therapy may be a useful component of holistic PCOS management, potentially improving quality of life and sexual satisfaction for these patients.

Ethics

Ethics Committee Approval: This study was approved by the Non-Interventional Research Ethics Committee of the Health Sciences University Turkey, Şehit Prof. Dr. İlhan Varank Sancaktepe Training and Research Hospital (approval number: 178, date: 12.06.2024).

Informed Consent: Written informed consent was obtained from all participants.

Authorship Contributions

Surgical and Medical Practices: Ö.D.S., E.K., Concept: E.A., Design: E.A., Data Collection or Processing: Ö.D.S., E.K., Analysis or Interpretation: E.A., Ö.D.S., E.K., Literature Search: E.A., Writing: E.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Salari N, Nankali A, Ghanbari A, et al. Global prevalence of polycystic ovary syndrome in women worldwide: a comprehensive systematic review and meta-analysis. Arch Gynecol Obstet. 2024;310(3):1303-1314.

2. Azziz R, Carmina E, Dewailly D, et al. The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. Fertil Steril. 2009;91(2):456-488.
3. Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod. 2004;19(1):41-47.
4. Glowinska A, Duleba AJ, Zielona-Jenek M, Siakowska M, Pawelczyk L, Banaszewska B. Disparate relationship of sexual satisfaction, self-esteem, anxiety, and depression with endocrine profiles of women with or without PCOS. Reprod Sci. 2020;27(1):432-442.
5. Pastoor H, Mousa A, Bolt H, et al. Sexual function in women with polycystic ovary syndrome: a systematic review and meta-analysis. Hum Reprod Update. 2024;30(3):323-340.
6. Stovall DW, Scriver JL, Clayton AH, Williams CD, Pastore LM. Sexual function in women with polycystic ovary syndrome. J Sex Med. 2012;9(1):224-230.
7. Eftekhar T, Sohrabvand F, Zabandan N, Shariat M, Haghollahi F, Ghahghaei-Nezamabadi A. Sexual dysfunction in patients with polycystic ovary syndrome and its affected domains. Iran J Reprod Med. 2014;12(8):539-546.
8. Elsenbruch S, Hahn S, Kowalsky D, et al. Quality of life, psychosocial well-being, and sexual satisfaction in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2003;88(12):5801-5807.
9. Köşüş A, Kamalak Z, Köşüş N, Hizli D, Eser A. Clitoral and labial sizes in women with PCOS. J Obstet Gynaecol. 2016;36(1):97-101.
10. Cattrall FR, Vollenhoven BJ, Weston GC. Anatomical evidence for in utero androgen exposure in women with polycystic ovary syndrome. Fertil Steril. 2005;84(6):1689-1692.
11. Simsir C, Pekcan MK, Aksoy RT, et al. The ratio of anterior anogenital distance to posterior anogenital distance: a novel-biomarker for polycystic ovary syndrome. J Chin Med Assoc. 2019;82(10):782-786.
12. Aboulghar MA, Mansour RT, Serour GI, Al-Inany HG. Diagnosis and management of unexplained infertility: an update. Arch Gynecol Obstet. 2003;267(4):177-188.
13. WHO laboratory manual for the examination and processing of human semen, sixth edition. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO.
14. Ferriman D, Gallwey JD. Clinical assessment of body hair growth in women. J Clin Endocrinol Metab. 1961;21:1440-1447.
15. U.S. Food and Drug Administration. (2018). Acne vulgaris: Establishing effectiveness of drugs intended for treatment guidance for industry. U.S. Department of Health and Human Services. https://www.fda.gov/media/71152/download.
16. Ellibeş Kaya A, Doğan O, Yassa M, Başbuğ A, Özcan C, Çalışkan E. Do external female genital measurements affect genital perception and sexual function and orgasm? Turk J Obstet Gynecol. 2020;17(3):175-181.
17. Oksuz E, Malhan S. Reliability and validity of the female sexual function index in Turkish population. SENDROM. 2005;17:54-60.
18. Ellibes Kaya A, Yassa M, Dogan O, Basbug A, Pulatoglu C, Caliskan E. The female genital self-image scale (FGSIS): cross-cultural adaptation and validation of psychometric properties within a Turkish population. Int Urogynecol J. 2019;30(1):89-99.
19. Rosen R, Brown C, Heiman J, et al. The female sexual function index (FSFI): a multidimensional self-report instrument for the assessment of female sexual function. J Sex Marital Ther. 2000;26(2):191-208.
20. Wiegel M, Meston C, Rosen R. The female sexual function index (FSFI): cross-validation and development of clinical cutoff scores. J Sex Marital Ther. 2005;31(1):1-20.
21. Herbenick D, Reece M. Development and validation of the female genital self-image scale. J Sex Med. 2010;7(5):1822-1830.
22. Filippou P, Homburg R. Is foetal hyperexposure to androgens a cause of PCOS? Hum Reprod Update. 2017;23(4):421-432.
23. Barrett ES, Hoeger KM, Sathyanarayana S, et al. Anogenital distance in newborn daughters of women with polycystic ovary syndrome indicates fetal testosterone exposure. J Dev Orig Health Dis. 2018;9(3):307-314.
24. Wu Y, Zhong G, Chen S, Zheng C, Liao D, Xie M. Polycystic ovary syndrome is associated with anogenital distance, a marker of prenatal androgen exposure. Hum Reprod. 2017;32(4):937-943.
25. Sánchez-Ferrer ML, Mendiola J, Hernández-Peñalver AI, et al. Presence of polycystic ovary syndrome is associated with longer anogenital distance in adult Mediterranean women. Hum Reprod. 2017;32(11):2315-2323.
26. Pastoor H, Both S, Timman R, Laan ETM, Laven JSE. Sexual function in women with polycystic ovary syndrome: design of an observational prospective multicenter case control study. Sex Med. 2020;8(4):718-729.

Original Article

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Emergency Blood Transfusion in Gynecology Cases: A Multicenter Analysis

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ABSTRACT

Purpose: The aim of this study was to determine the indications, usage rates, and complications of emergency and elective blood transfusions in gynecological cases in Türkiye.

Methods: A retrospective hospital database analysis was performed on blood transfusion records from eight hospitals. A total of 12,176 gynecological surgery patient files were reviewed, and 399 blood transfusions were identified. All patient files were reviewed retrospectively for the demographic variables of the patients, indications and timing of surgeries, surgical outcomes, type and timing of blood transfusion, and any complications of surgeries or blood transfusions.

Results: A total of 12,176 gynecological operations were conducted, and 399 (3.27%) blood transfusions were performed. In patients with anemia (hemoglobin <12 g/dL), the number of emergency blood transfusions was 25 (24.2%), and the number of elective blood transfusions was 149 (50.3%). Anemia was significantly more common in the elective blood transfusion group compared to the emergency group ($p<0.05$). The incidence of thrombocytopenia, massive transfusion, and fresh frozen plasma use were significantly higher in the emergency transfusion group compared to the elective group ($p\leq0.05$).

Conclusion: In this study, emergency and elective blood transfusion practices in gynecological surgery were examined. Our findings contribute to the existing literature by providing data on blood transfusion frequencies, indications, and complications. In particular, emergency cases showed a higher incidence of thrombocytopenia and massive transfusion, highlighting the importance of blood product management and planning in emergency settings.

Keywords: Blood transfusion, emergency transfusion, gynecology bleeding

INTRODUCTION

The first blood transfusion was performed in the 17th century. However, since the importance of matching blood groups was undiscovered mortality rates were high. Modern blood transfusion began with the discovery of blood groups in 1901 and the development of compatibility testing using the blood agglutination technique in 1907. Blood transfusion medicine evolved further with the separation and storage of blood components.[1]

Different components such as packed red blood cells (PRBCs), individual factor concentrates, fresh frozen plasma (FFP), platelet concentrates, and cryoprecipitate began to be used separately. A single PRBC unit is approximately 350 mL and contains approximately 250 mg of iron.[2] One unit of PRBCs typically increases the Hb value by 1 g/dL and the hematocrit by 3%.

Platelet transfusion is useful in cases of platelet deficiency or dysfunction. FFP transfusion is effective in managing coagulation factor deficiencies in bleeding patients. Cryoprecipitate is administered in cases of dysfibrinogenemia or fibrinogen deficiency, particularly during bleeding episodes or acute disseminated intravascular coagulation.[3] Anemia is defined as a Hb value of less than 12 g/dL in women. It is important to consider symptoms in cases where the patient is actively bleeding or has an acute onset of hemorrhage. Cardiac and vasoactive changes due to acute blood loss begin after

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approximately 20% of the blood volume is lost. Transfusion is indicated for bleeding patients with Hb levels less than 8 g/dL, accompanied by symptoms such as tachycardia, weakness, and dyspnea on exertion.[4] The first treatment step in acute blood loss is fluid replacement to restore blood volume. However, infusions and fluid resuscitation can cause a sudden decrease in Hb levels. As Hb declines, reversible organ damage can occur. If blood loss continues, irreversible symptoms may develop. Severe bleeding can result in shock, defined as the inability to deliver adequate oxygen to tissues for cellular metabolism. The most critical step in managing hemorrhagic shock is the replenishment of red blood cell mass.[3] According to the American Association of Blood Banks, the most common blood transfusion complication is febrile reactions. Other complications include infections, (such as hepatitis C virus infection, hepatitis B virus infection, and human immunodeficiency virus infection, hemolytic reactions, allergic reactions, transfusion-related acute lung injury (TRALI), transfusion-associated circulatory overload (TACO), and electrolyte imbalances.[5]

METHODS

The study was conducted in eight hospitals from January 1, 2023, to January 1, 2024. Ethical approval for this study was obtained from the Bolu Abant İzzet Baysal University Ethics Committee (approval number: 2024/49, date: 02.04.2024). After obtaining ethical committee approval, written permission for the study was granted by all participating hospitals. A retrospective hospital database analysis was conducted using the blood transfusion registry data from the hospitals. All hospitals have a dedicated blood transfusion unit and obtain blood from the Turkish Red Crescent Blood Bank. In Turkey, only the Turkish Red Crescent Blood Bank is authorized to collect, process, and distribute blood and blood products. A district-wide organization provides an online registration system for available blood products by blood type across all hospitals to ensure effective use of blood products and reduce waste. All hospitals are required to maintain a minimum stock of blood products for emergency cases. If blood products are unavailable, hospitals are supplied within 2 to 4 hours, depending on the availability from the Turkish Red Crescent Blood Bank. In emergency situations, hospitals are permitted to exchange blood products but must report this to the Turkish Red Crescent. Despite this well-organized system, emergency blood transfusions in unplanned or unexpected settings still pose risks to patients due to the untimely supply of blood products, either due to insufficient quantities or the rarity of the patient's blood type. For all patients receiving blood products, a blood request form must be filled out with the indication for transfusion, and reporting any adverse events is mandatory. The study included all patients over the age of 18 who received blood or blood products before, during, or after gynecological surgery. All patient records were reviewed retrospectively for demographic variables, indications and timing of surgeries, surgical outcomes, types and timing of blood transfusions, and any complications related to surgery or blood transfusion. The transfusions were classified as "elective" if the blood transfusion was planned due to known chronic anemia or ongoing blood loss, with a foreseen need for blood transfusion and sufficient time to prepare donor blood. The transfusions were classified as "emergency" if the need for transfusion was unplanned, such as in cases of emergency surgery in an anemic patient, intraoperative blood loss due to bleeding, or postoperative transfusion due to continuous blood loss following ineffective surgery or new-onset coagulopathy. Anemia was defined as a Hb value of less than 12 g/dL.[6]

Statistical Analysis

Statistical analysis was conducted using SPSS version 22.0 (IBM Corporation, Armonk, NY, USA). The normality of distribution was tested using the Kolmogorov-Smirnov test. For continuous variables with normal distribution, descriptive statistics are presented as mean ± standard deviation (SD). Fisher's exact test or the chi-squared test was used to compare categorical variables. The independent samples t-test was used to compare continuous variables that were normally distributed. A *p*-value of less than 0.05 was considered statistically significant for all tests.

RESULTS

A total of 12,176 gynecological operations were performed, and 399 blood transfusions (3.27%) were conducted. Among these transfusions, 80 (20.1%) were performed in General State Hospitals, 180 (45.1%) in Research and Education Hospitals, and 139 (34.8%) in Private Hospitals. The perioperative blood transfusion rate was 2.9% (139/4,679) in private hospitals, 4% (180/4,344) in training and research hospital, and 2.5% (80/3,153) in general state hospitals. Among the blood transfusions, emergency blood transfusions constituted 35% (36/139) in private hospitals, 47.6% (49/180) in research and education hospitals, and 17.5% (18/80) in general state hospitals ($p=0.7$). Demographic data of patients, categorized by emergency vs. elective blood transfusions, are presented in Table 1. There was no significant difference between the two groups in terms of age, gravidity, parity, education, employment status, or comorbidities ($p>0.05$).

The clinical characteristics of patients who underwent emergency and elective blood transfusions are presented in Table 2. No significant differences were found between the two groups regarding dysfunctional uterine bleeding, preoperative iron use, presence of gynecologic infection, gynecologic malignancy, type of operation, intraoperative blood loss, and type of anesthesia ($p>0.05$). In patients with anemia (Hb <12), the number of emergency blood transfusions was 25 (24.2%), while the number of elective blood transfusions was 149 (50.3%). Anemia was significantly more prevalent in the elective blood transfusion group compared to the emergency blood transfusion group. Hematological parameters between the two groups are shown in Table 3. No significant difference was found between the groups in terms of pretransfusion Hb, severe anemia, and erythrocyte suspension use. However, the incidence of thrombocytopenia, massive transfusion, and FFP use was significantly higher in the emergency blood transfusion group compared to the elective blood transfusion group. There was no significant difference in transfusion reactions, the need for re-operation, or the duration of hospitalization between patients who received emergency vs. elective blood

transfusions (Table 4). Postoperative adverse events included one case of disseminated intravascular coagulation and adult respiratory distress syndrome in the emergency blood transfusion group. Acute renal failure was observed in one case in the emergency blood transfusion group. Hypovolemic shock was observed in one case in the emergency group and two cases in the elective group. Pulmonary edema was observed in one case in the elective blood transfusion group.

DISCUSSION

The number of clinical practice guidelines regarding blood transfusions has increased recently, reflecting the growing interest of professional societies and healthcare institutions worldwide. Blood transfusion is a critical component of healthcare, essential for sustaining vital functions. Its primary goal is to maximize patient benefit by accurately determining

Table 1. Demographic variables of patients receiving emergency blood transfusion and controls

Variable	Emergency blood transfusion (n=103)	Elective blood transfusion (n=296)	*p* value
Age	45.6±12.1	44.8±10.2	-
Gravida	2.5±1.8	2.6±1.7	-
Parity	2.4±2.5	2.3±1.3	-
Tobacco use	58 (56%)	165 (55%)	0.9
Education status			0.4
Illiterate	-	11 (3.7%)	-
Primary school	31 (30%)	115 (38.8%)	-
Secondary school	19 (18.4%)	79 (26.6%)	-
High school	24 (23.3%)	68 (22.9%)	-
University	29 (28.1%)	23 (7.7%)	-
Working status			0.6
Housewife/unemployed	59 (57.2%)	161 (54.3%)	-
Hypertension	19 (18.4%)	44 (14.8%)	0.4
Diabetes	9 (8.7%)	18 (6%)	0.3
Heart disease	6 (5.8%)	14 (4.7%)	0.6
COPD	5 (4.8%)	13 (4.3%)	0.8

Note: Data are presented as mean ± standard deviation or n (%), where appropriate
a. Independent samples t-test, b. Fisher's exact test, c. Pearson chi-square test
COPD: Chronic obstructive pulmonary disease

Table 2. Clinical characteristics of patients receiving emergency blood transfusion and controls

Variable	Emergency blood transfusion (n=103)	Elective blood transfusion (n=296)	*p* value
Dysfunctional uterine bleeding	49 (47.5%)	162 (54.7%)	0.2
Anemia <12 g/dL	25 (24.2%)	149 (50.3%)	<0.001
Preoperative oral iron treatment	8 (7.7%)	48 (16.2%)	0.03
Preoperative IV iron treatment	1 (0.9%)	9 (3%)	0.2
Gynecologic infections	3 (2.9%)	6 (2%)	0.6
Gynecologic malignancy	6 (5.8%)	20 (6.7%)	0.7
Access route of operation			0.7
Laparoscopy	18 (17.4%)	77 (26%)	-
Hysteroscopy	8 (7.7%)	36 (12.1%)	-
Vaginal	7 (6.7%)	26 (8.7%)	-
Laparotomy	70 (67.9%)	157 (53%)	-
Transfusion for operative blood loss	37 (35.9%)	116 (39.1%)	0.5
Anesthesia			0.4
Spinal	17 (16.5%)	58 (19.5%)	-
General	86 (83.4%)	238 (80.4%)	-

Data are presented as mean ± standard deviation or n (%), where appropriate
a. Independent samples t-test, b. Fisher's exact test, c. Pearson chi-square.

Table 3. Hematological parameters and blood products used in patients receiving emergency blood transfusion and controls

Variable	Emergency blood transfusion (n=103)	Elective blood transfusion (n=296)	*p* value
Pretransfusion hemoglobin (g/dL)	8.1±1.3	8.4±4.2	0.6
Severe anemia (Hb <9 g/dL)	30 (29.1%)	107 (36.1%)	0.2
Thrombocytopenia (<150x10^9/L)	7 (6.8%)	6 (2%)	0.01
Erythrocyte suspension	2.3±1.3	2±1	0.08
Fresh frozen plasma	0.6±0.8	0.38±0.68	0.005
Massive transfusion (6 packs or more)	5 (4.9%)	4 (1.4%)	0.03

Data are presented as mean ± standard deviation or n (%), where appropriate
a. Independent samples t-test, b. Fisher's exact test, c. Pearson chi-square test
Hb: Hemoglobin

Table 4. Outcomes of patients receiving emergency blood transfusion and controls

Variable	Emergency blood transfusion (n=103)	Elective blood transfusion (n=296)	*p* value
Transfusion reaction	2 (1.9%)	2 (0.6%)	0.2
Re-operation	3 (2.9%)	2 (0.6%)	0.07
Duration of hospitalization (days)	3.1±2	3.3±2.1	0.5

Data are presented as mean ± standard deviation or n (%), where appropriate
a. Independent samples t-test, b. Fisher's exact test, c. Pearson chi-square test

indications, ensuring appropriateness, and anticipating potential risks. Although Hb levels are a key marker, relying solely on this parameter is considered controversial. Contemporary guidelines recommend that Hb levels be evaluated alongside other clinical parameters, such as comorbidities, acute blood loss, and the overall clinical condition. This multidimensional approach ensures that transfusions are administered appropriately, optimizing outcomes while minimizing unnecessary risks. There is a broad consensus that in acute clinical scenarios, transfusion should be considered when Hb levels fall below 6-7 g/dL to prevent life-threatening complications and improve prognosis.[7] Demographic factors play a significant role in influencing the need for both emergency and elective blood transfusions. Stanhier et al.[8] reported an age-related increase in transfusion requirements, while Smith et al.[9] highlighted that elderly women are particularly vulnerable to hemorrhagic complications. However, in the present study, no significant difference in mean age was observed between emergency and elective transfusion groups likely due to our younger patient cohort. Smoking is another risk factor, known to exacerbate bleeding risks through its association with cardiovascular and chronic diseases. Demir et al.[10] proposed that smoking may reduce Hb levels and indirectly increase transfusion needs. In the present study, smoking rates were similar between elective (55%) and emergency (56%) groups, suggesting no direct association but a possible indirect contribution to bleeding risk. Furthermore, we observed no significant differences in the prevalence of hypertension or diabetes mellitus between the two groups. However, it is well established that metabolic diseases may predispose individuals to anemia and heightened bleeding tendencies. More detailed examination of demographic variables may have aid in developing targeted transfusion management strategies, especially for patients requiring emergency interventions. Anemia remains a critical determinants of transfusion necessity. Zhou et al.[11] found that anemia significantly increased transfusion needs prior to surgical interventions. In the present study, anemia was significantly more prevalent in the elective transfusion group (50.3%) than in the emergency group (24.2%) ($p<0.001$). These findings suggest that preoperative anemia management may be insufficient, particularly in emergency settings. Supporting this notion, Caldwell et al.[12] demonstrated that preoperative iron therapy reduce transfusion rates among patients with iron deficiency. In gynecologic surgery, transfusion rates vary according to surgical complexity. Stanhiser et al.[13] reported that transfusions occurred in 2% of cases, with higher rates in hysterectomies, myomectomies, and malignancy surgeries. In line with these findings, 3.2% of patients required transfusions for gynecological indications, with more than twice this transfusion rate (6.5%) among those with gynecologic malignancies. This is likely attributable to the invasive nature and increased bleeding risk associated with oncologic surgery. Notably, no significant difference was found between emergency and elective transfusion rates. Thrombocytopenia was more frequently observed among patients undergoing emergency surgery, in line with previous studies. Schatz et al. (2016) also demonstrated a higher prevalence of thrombocytopenia in emergency surgical cases, contributing to greater transfusion requirements.[14] FFP use was higher among emergency transfusion cases, consistent with Sanghani et al.[15] who reported increased FFP requirement in major surgeries and cases of significant blood loss. Moreover, Hickok et al.[16] reported that massive transfusion needs were significantly higher during emergency and complex surgeries, which was corroborated by our findings. Regarding transfusion-related complications, allergic reactions were observed in 1% of our cohort, comparable to rates reported by Ferraris et al.[17] (1.8%) and Efe et al.[18] (0.95%). Urticaria, pruritus, and skin rashes were the most commonly reported adverse effects.

Serious complications such as hemolytic reactions, transfusion-associated circulatory overload (TACO), and TRALI were rare. No TACO cases were identified in our cohort, although it is frequently reported among elderly patients. TRALI remains a serious concern, especially following emergency transfusions, necessitating vigilant clinical monitoring. Reoperation rates were higher among patients who underwent emergency transfusions compared to elective cases; however, no significant difference was observed regarding the length of hospital stay ($p=0.5$). This may be attributed to the more complex clinical trajectories of emergency cases.

Study Limitations

This study offers valuable data comparing emergency and elective blood transfusions in gynecological surgery. Its main strengths include a relatively large sample size and detailed evaluation of transfusion practices and complications. However, the retrospective design and the predominantly young patient population limit the generalizability of the findings. In addition, the lack of standardized measures for surgical complexity and intraoperative blood loss represents a potential confounding factor. Future prospective studies are needed to address these limitations.

CONCLUSION

This research investigated blood transfusion approaches in gynecological surgeries, focusing on the comparison between emergency and elective interventions. The results demonstrated that emergency cases are more frequently associated with thrombocytopenia and require larger volumes of transfusion. These findings point to the necessity of efficient blood product planning and swift intervention in urgent clinical scenarios. Moreover, they highlight the importance of establishing standardized transfusion guidelines, enhancing preoperative risk assessment, and ensuring seamless communication with blood banks. The data presented here contribute to the existing body of knowledge and may serve as a basis for improving transfusion practices in the field of gynecological surgery.

Ethics

Ethics Committee Approval: Ethical approval for our study was obtained from the Bolu Abant İzzet Baysal University Ethics Committee (approval number: 2024/49, date: 02.04.2024).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Ş.Ç., N.E.A., İ.Y., Concept: Ş.Ç., M.Ç.K., Design: Ş.Ç., M.Ç.K., B.A., Data Collection or Processing: N.E.A., N.Y., İ.Y., Analysis or Interpretation: N.Y., B.A., Literature Search: Ş.Ç., İ.Y., B.A., Writing: Ş.Ç., N.E.A., B.A.

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REFERENCES

1. Sturgis CC. The history of blood transfusion. Bull Med Libr Assoc. 1942;30(2):105-112.
2. Lotterman S, Sharma S. Blood transfusion. 2023 Jun 20. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025.
3. Carson JL, Triulzi DJ, Ness PM. Indications for and adverse effects of red-cell transfusion. N Engl J Med. 2017;377(13):1261-1272.
4. Szczepiorkowski ZM, Dunbar NM. Transfusion guidelines: when to transfuse. Hematology Am Soc Hematol Educ Program. 2013;2013:638-644.
5. Nissen-Meyer LSH, Seghatchian J. Donor health assessment - When is blood donation safe? Transfus Apher Sci. 2019;58(1):113-116.
6. Mansour D, Hofmann A, Gemzell-Danielsson K. A review of clinical guidelines on the management of iron deficiency and iron-deficiency anemia in women with heavy menstrual bleeding. *Adv Ther.* 2021;38(1):201-225.
7. American Society of Anesthesiologists Task Force on Perioperative Blood Transfusion and Adjuvant Therapies. Practice guidelines for perioperative blood transfusion and adjuvant therapies: an updated report by the American Society of Anesthesiologists Task Force on Perioperative Blood Transfusion and Adjuvant Therapies. *Anesthesiology.* 2006;105(1):198-208.
8. Stanhier M, et al. Blood transfusion practices in gynecological surgery. *J Gynecol Surg*. 2017;34(2):121-129.
9. Smith J, et al. The impact of age on blood transfusion needs in gynecological surgeries. *Int J Gynecol Surg*. 2019;28(4):200-206.
10. Demir S, et al. Smoking and its effects on blood transfusion in gynecological patients. *J Clin Transfus Med*. 2018;15(3):188-194.
11. Zhou X, Li Q, Zhang H. Preoperative anemia and its effect on surgical outcomes in gynecologic surgery: a systematic review and meta-analysis. *Eur J Obstet Gynecol Reprod Biol*. 2018;224:97-104.
12. Caldwell CE, Lee SL, Greenberg A. Effect of preoperative iron therapy on the need for blood transfusion in gynecologic surgery: A randomized controlled trial. *J Gynecol Surg*. 2019;35(1):45-50.
13. Stanhiser J, Chagin K, Jelovsek JE. A model to predict risk of blood transfusion after gynecologic surgery. *Am J Obstet Gynecol.* 2017;216(5):506.e1-506.e14.
14. Brenner B. Haemostatic changes in pregnancy. Thromb Res. 2004;114(5-6):409-414.
15. Sanghani HV, Rutherford C, Mangano DT, et al. Fresh frozen plasma in massive transfusion: clinical use and indications. *Transfusion*. 2014;54:134-139.
16. Hickok DE, Hulka JF, Peterson HB, et al. Massive blood loss and transfusion in obstetrics and gynecology. *Obstet Gynecol*. 2000;95:1035-1040.
17. Ferraris VA, Hochstetler M, Martin JT, Mahan A, Saha SP. Blood transfusion and adverse surgical outcomes: the good and the bad. Surgery. 2015;158(3):608-17.
18. Efe S, Demir C, Dilek İ. Distribution of blood and blood components, indications and early complications of transfusion. *Eur J Gen Med*. 2010;7(2):143-149.

Case Report

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Endometrioma Excision in a Patient with VACTERL Syndrome and a Rudimentary Uterine Horn: a Case Report

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ABSTRACT

Vertebral anomalies, anal atresia, cardiac malformations, tracheoesophageal fistula, renal anomalies, and limb defects (VACTERL) association is a rare congenital condition characterized by multiple organ systems, including the vertebrae, anus, cardiac system, trachea, esophagus, renal system, and limbs. Müllerian duct anomalies have been infrequently found in VACTERL association, with only a few cases reported. We present a case of a 20-year-old female with VACTERL association, presenting with a right adnexal mass diagnosed as an endometrioma. The mass measured 83x64x38 mm and exhibited typical features of endometrioma, including a hypoechoic 'ground glass' appearance. Magnetic resonance imaging also revealed a right non-communicating rudimentary uterine horn. A laparotomy was performed for the excision of the endometrioma and the removal of the rudimentary uterine horn. The patient had an uneventful postoperative recovery, with discharge on postoperative day three. Follow-up visits showed satisfactory healing and resolution of symptoms. This case highlights the challenges in managing gynecological conditions in individuals with VACTERL association and underscores the need for tailored surgical approaches to address coexisting congenital anomalies.

Keywords: VACTERL association, endometrioma, mullerian duct anomalies, rudimentary uterine horn, surgical management

INTRODUCTION

Vertebral anomalies, anal atresia, cardiac malformations, tracheoesophageal fistula, renal anomalies, and limb defects (VACTERL) association is a rare, non-random constellation of congenital malformations that affects multiple organ systems, including the vertebrae, anus, cardiac structures, trachea, esophagus, kidneys, and limbs.[1,2] While Müllerian duct anomalies are infrequent in patients with VACTERL, the rudimentary uterine horn is a rare form of such anomaly. Often associated with other congenital malformations, it can present significant surgical challenges, especially when complications like endometriosis or endometriomas.[3-5]

Managing endometriomas in patients with VACTERL association is challenging, especially when Müllerian defects like a rudimentary horn are present.[6] Altered reproductive anatomy can complicate surgical access and treatment. The rare coexistence of a rudimentary horn and endometriomas in VACTERL patients required careful surgical planning.[7]

This report highlights the surgical management of an endometrioma in a patient with VACTERL association and a rudimentary uterine horn. It emphasizes the challenges of diagnosis and treatment, the importance of a multidisciplinary approach and the need for awareness of Müllerian anomalies in patients with VACTERL association, as their presence can significantly impact the clinical course and management of associated gynecological conditions.

CASE REPORT

A 20-year-old female with a known history of vertebral malformations, tracheoesophageal fistula repair in infancy, and renal hypoplasia was referred to our clinic with a complaint of progressively worsening right lower abdominal pain, dysmenorrhea and irregular bleeding over the last few months. Upon review of her medical history, it was found that she had undergone multiple surgical interventions, including tracheoesophageal fistula repair during infancy, anal transposition, and rectal dilation procedures in later years. In addition, she had been treated for vesicoureteral reflux with cystoscopy and subureteral injections. Other noted anomalies included butterfly vertebrae, left renal hypoplasia, right aortic

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arch, and aberrant left subclavian artery. Based on these findings, the diagnosis of VACTERL association was strongly suggested.

On initial examination, the patient was alert, oriented, and afebrile, with stable vital signs. Abdominal examination revealed tenderness in the right lower quadrant without signs of peritoneal irritation. No palpable masses were felt, and the abdomen was soft and non-distended. The pelvic examination did not reveal any abnormalities, though the patient did report mild discomfort upon deep palpation of the right adnexal region. Further evaluation through transabdominal ultrasonography revealed a well-defined, cystic mass measuring approximately 8 cm in diameter located in the right adnexa. The mass demonstrated typical features of an endometrioma, including a homogenous, hypoechoic appearance, with a "ground glass" pattern, a hallmark of endometriotic tissue. No internal vascularity was detected on Doppler imaging, which is consistent with a benign cystic lesion. Moreover, the surrounding ovarian tissue appeared intact, without signs of torsion or rupture. These ultrasonographic findings were suggestive of an endometrioma, which was later confirmed by magnetic resonance imaging (MRI).

The MRI confirmed the presence of an endometrioma and additionally identified a rudimentary uterine horn on the right side. This rudimentary horn with an endometrial cavity and was not connected to the cervix. In contrast, the left uterine horn was normally developed and was found to be connected to the cervix, with a well-formed endometrial cavity, consistent with a unicornuate uterus. The anatomical findings supported a diagnosis of a unicornuate uterus with a non-communicating rudimentary horn, an anomaly rarely reported in VACTERL association.

Given the complexity of the patient's case, consultations with nephrology and anesthesiology were sought to evaluate the associated risks and plan for optimal perioperative care. The nephrology consultation focused on assessing the patient's renal function, as she had a history of renal hypoplasia, and to ensure that her kidney function was adequate for surgical management. The anesthesiology consultation was essential due to her history of tracheoesophageal fistula, which required a thorough assessment of her airway and anesthetic risks. The team was particularly concerned about potential challenges with intubation, given her anatomical anomalies.

Routine preoperative laboratory tests, including hemoglobin, renal function, and coagulation profiles, were within normal limits. The patient received intravenous cefazolin (1 g) prophylactically before surgery to minimize the risk of infection.

A laparotomy was selected due to the patient's altered anatomy and history of previous surgeries, which increased the complexity of laparoscopic access. Under general anesthesia, a Pfannenstiel incision was made. Upon entry into the peritoneal cavity, extensive pelvic adhesions were noted, particularly between the bowel and adnexa. Sharp and blunt adhesiolysis was performed to improve visualization. The right adnexal mass was identified and confirmed as an endometrioma. The cyst was carefully dissected from ovarian tissue, and complete excision was performed using meticulous hemostasis to preserve ovarian function. The right non-communicating rudimentary uterine horn was then mobilized and excised at its base. The excised horn was non-communicating with the endometrial cavity and had a fibrous attachment to the main uterus.

Hemostasis was ensured, and the peritoneal cavity was irrigated with warm saline. A drain was placed in the right adnexal region due to the extent of adhesiolysis. The abdominal wall was closed in layers, and the patient was extubated without complications (Figure 1).

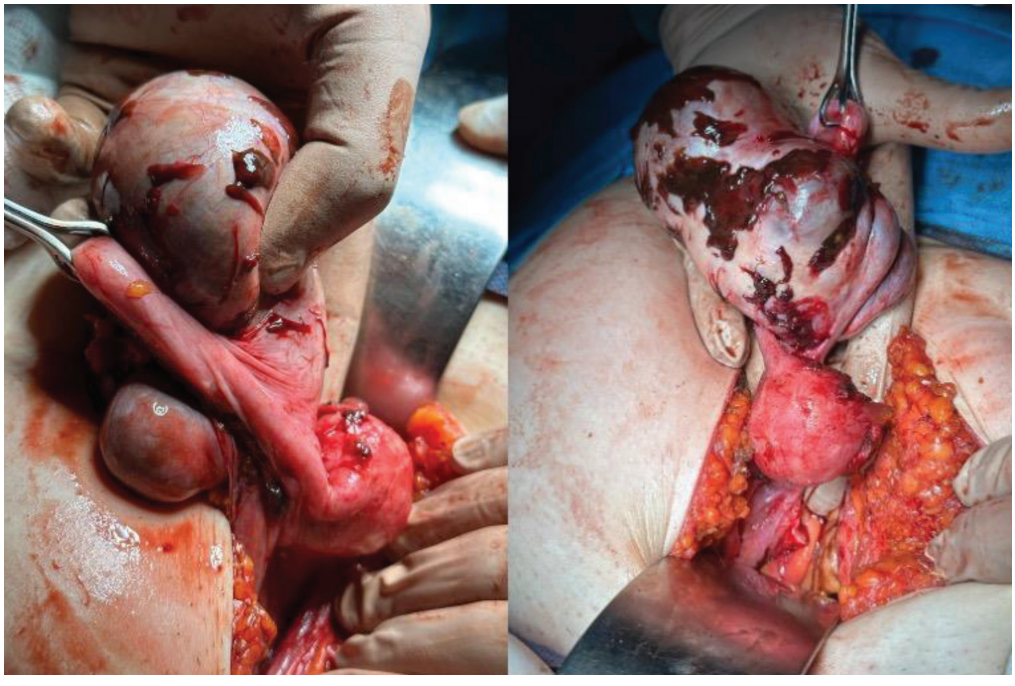

Figure 1. Endometrioma and right rudimentary horn

The patient was monitored in the postoperative unit for 24 hours, with stable vital signs and adequate urine output. Pain control was managed with paracetamol and tramadol as needed. Deep vein thrombosis prophylaxis was provided with low molecular weight heparin (enoxaparin 40 mg subcutaneously daily) until discharge. The surgical drain was removed on postoperative day two, with minimal serous output. The patient tolerated oral intake on postoperative day one, ambulated early, and had an uneventful recovery. She was discharged on postoperative day three with instructions for wound care, pain management, and scheduled follow-up. Histopathological examination confirmed an endometriotic cyst and fibromuscular tissue consistent with a rudimentary uterine horn.

The patient was treated with dienogest after surgery to prevent recurrence of endometrioma, reduce inflammation, and manage residual pain. At the one-month follow-up, the patient reported resolution of pain, and at the three-month follow-up, she reported regular menstrual cycles. No complications or recurrent symptoms were noted, indicating a favorable response to treatment. Informed consent was obtained for this case report at the post-discharge outpatient clinic controls.

DISCUSSION

The aim of this case report was to highlight the challenges in diagnosing and managing a patient with VACTERL association who presented with an endometrioma in the presence of a rudimentary uterine horn, a rare Müllerian anomaly. This case expands on the existing literature by presenting a unique combination of congenital anomalies that complicate both diagnosis and surgical management. A further aim was to demonstrate the multidisciplinary approach required to address the complexities of such cases and to explore how the rare coexistence of these abnormalities in a patient with VACTERL association presented specific clinical challenges.

Several studies have shown that patients with VACTERL association often have reproductive system abnormalities, including uterine malformations like unicornuate uterus or, less frequently, rudimentary horns.[7-14] For example, a 17-year-old girl with VACTERL presented with severe dysmenorrhea and was found to have a unicornuate uterus and a non-communicating rudimentary left horn.[9] After pelvic MRI and surgery, her symptoms resolved. Delayed diagnosis contributed to prolonged symptoms, highlighting the importance of timely intervention. Similarly, a 14-year-old with left renal agenesis and anorectal malformation presented with cyclical abdominal pain, which was later diagnosed as a right unicornuate uterus with a rudimentary left horn and hematosalpinx. Surgical intervention resolved her symptoms.[12] Lavoie et al.[11] reported a 10-year-old with recurrent abdominal pain, eventually diagnosed with uterine didelphys, hematometrocolpos, and hematosalpinx, which was also successfully treated with surgery. These cases emphasize the importance of early imaging and intervention in managing reproductive tract anomalies in VACTERL patients.

However, the combination of VACTERL association, a rudimentary horn, and an endometrioma is an extremely rare clinical scenario that has not been well documented.[7] Bhadwal et al.[7] presented two cases of uterine rudimentary horn and ovarian endometriosis in patients with VACTERL association. One involved a 12-year-old girl with a unicornuate uterus and an obstructed right uterine horn, leading to hematometra, hemosalpinx, and an endometrioma in the right ovary. This case emphasizes the importance of considering Müllerian anomalies in adolescents with gynecological symptoms and VACTERL, as they can lead to complications, including hematometra and endometriosis. The second case involved a 14-year-old girl with abdominal pain initially diagnosed as a right ovarian cyst, which was later reclassified as a rudimentary horn with hematometra. This case highlights the need for thorough radiological evaluation, as Müllerian anomalies can be easily overlooked on standard imaging. Both cases underscore the rarity of VACTERL-associated Müllerian duct anomalies and emphasize the need for a high index of suspicion. Management typically involves surgical excision of the obstructed uterine horn and removal of endometriotic lesions to prevent complications like infertility and chronic pain.[7]

A distinguishing feature of our case was the need for a multidisciplinary approach. Given the complexity of the patient's anatomy, consultations with nephrology and anesthesiology were essential for preoperative evaluation. Nephrology assessed the patient's renal function due to the presence of renal hypoplasia, while anesthesiology evaluated potential airway complications arising from her previous tracheoesophageal fistula repair. These consultations were critical to ensure the patient's safety during surgery, highlighting the importance of comprehensive preoperative planning in complex cases such as this.

The management of patients with VACTERL association requires a tailored, multidisciplinary approach due to the complexity of the associated anomalies. A combination of gynecological, surgical, and radiological expertise is often necessary to address the challenges presented by these cases. Surgical intervention, as demonstrated in this case, can offer significant symptomatic relief and improve the quality of life for affected individuals.

CONCLUSION

This case presents a unique and rare combination of VACTERL association, a rudimentary uterine horn, and an endometrioma, highlighting the complexities in diagnosis and management when multiple congenital anomalies coexist. The presence of a rudimentary uterine horn in a patient with VACTERL association is infrequently documented, and its coexistence with an endometrioma further complicates both the clinical picture and surgical approach. This report adds to the limited body of literature on such complex cases and underscores the importance of a multidisciplinary approach in the management of these patients.

Ethics

Informed Consent: It was obtained.

Authorship Contributions

Surgical and Medical Practices: A.A., C.K., Concept: B.K., Design: C.K., Data Collection or Processing: A.A., Analysis or Interpretation: B.K., C.K., Literature Search: B.K., Writing: A.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. Solomon BD. The etiology of VACTERL association: Current knowledge and hypotheses. Am J Med Genet C Semin Med Genet. 2018;178(4):440-446.
2. Forero LT, Henderson R, Galarreta C, Swee S, Bird LM. Expansion of the core features of VACTERL association to include genital anomalies. Am J Med Genet A. 2024;194(9):e63587.
3. Hambraeus M, Börjesson A, Ekmark AN, Tofft L, Arnbjörnsson E, Stenström P. Genital malformations in children with VACTERL-has time come to include "G" in the acronym? J Pediatr Surg. 2024;59(10):161575.
4. Teo XL, Narasimhan KL, Chua JH. Müllerian agenesis in the presence of anorectal malformations in female newborns: a diagnostic challenge. Singapore Med J. 2015;56(5):e82-e84.
5. Forero LT, Henderson R, Galarreta C, Swee S, Bird LM. Expansion of the core features of VACTERL association to include genital anomalies. Am J Med Genet A. 2024;194(9):e63587.
6. Bhamidipaty-Pelosi S, Kyei-Barffour I, Volpert M, et al. Mullerian anomalies and endometriosis: associations and phenotypic variations. Reprod Biol Endocrinol. 2024;22(1):157.
7. Bhadwal A, Sharma S, Ranga S. Uterine rudimentary horn and ovarian endometriosis with a special emphasis on VACTERL association: a report of two cases. International Journal of Reproduction, Contraception, Obstetrics and Gynecology. 2024;13(7):1868-1872.
8. Nunes N, Karandikar S, Cooper S, Jaganathan R, Irani S. VATER/VACTERL syndrome (vertebra/anus/cardiac/trachea/esophogus/radius/renal/limb anomalies) with a noncommunicating functioning uterine horn and a unicornuate uterus: a case report. Fertil Steril. 2009;91(5):1957.
9. Obeidat RA, Aleshawi AJ, Tashtush NA, Alsarawi H. Unicornuate uterus with a rudimentary non-communicating cavitary horn in association with VACTERL association: case report. BMC Womens Health. 2019;19(1):71.
10. Roman JD. Co-existence of a rudimentary non- communicating horn with a unicornuate uterus in association with 2 components of the vacterl association: a case report. Case Rep Obstet Gynecol Rep. 2021;3(2):1-4.
11. Lavoie C, Melanie Au, Syed H, Christine Do, Baker Z, Vasquez E. Surgical management of an obstructive Müllerian anomaly in a patient with VACTERL association: a case report. Clin Case Rep Int. 2024;8:1668.
12. Theodorou CM, Trappey AF, Chen SA, McCracken K, Saadai P. SSurgical management of an obstructive Müllerian anomaly in a patient with anorectal malformation. J Pediatr Surg Case Rep. 2021;71:101908.
13. Meena J, Bharti J, Roy KK, Kumar S, Singhal S, Shekhar B. Bicornuate uterus with complete cervico-vaginal agenesis and skeletal deformity: a case report. J Obstet Gynaecol India. 2019;69(Suppl 1):67-70.
14. Pariza PC, Stavarache I, Dumitru VA, et al. VACTERL association in a fetus with multiple congenital malformations - Case report. J Med Life. 2021;14(6):862-867.