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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 Centimeter Square," 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

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

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ABSTRACT

Androgens play a key role in maintaining women's health as well as men's. 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 health 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 crucial 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 women's 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), according to 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. Its 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 in the cortex of the adrenal gland, by conversion from 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, the vast majority are 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. It 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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Copyright© 2025 The Author. Published by Galenos Publishing House on behalf of National Society of Gynecology and Obstetrics. This is an open access article under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 (CC BY-NC-ND) International License. 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. 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 suddenly 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 Effects

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 they 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 stroma.^{19,20}

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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 well 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, we need to explore their up-regulation and down-regulation properties and understand how more than 1000 receptor mutations occur, so far mostly 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 in benefit of 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 can 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 lowdensity 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 essential. 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 secreted by the liver. 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 screening alone 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, either better or worse, 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, we can 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. Additionally, 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 μ g/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 Effects

Androgen and estrogen receptors (ER) are two important receptors regulating bone metabolism.⁴⁸ Androgens and estrogen-aromatized helices trigger significant effects on osteoblasts and osteoclasts by initiating 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 on whether testosterone treatment increases bone mineral density are inconclusive, and the findings are conflicting. Although there are studies showing better results in women given testosterone in combination with estrogen compared to estrogen alone⁵², 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 used. 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 It 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 body image. 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.^{56,59} 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 of United States of America 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 disease 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 6 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 their 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, as if trying to close the distance covered by estrogen in this regard. On the reproductive side, studies examining androgens and ovarian reserve, receptor polymorphisms, and reproduction from conception to sexual behavior will continue to be examined in depth. On the nonreproductive 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 crucial 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 issues, 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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The Importance of Exosomes in Gynecological Diseases

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Exosomes play significant roles in key functions of the female reproductive system, such as oogenesis, implantation success, embryo development, and proper fertilization. Exosomes are fundamental cell-derived structures involved in facilitating intercellular communication. 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 by encapsulating biological molecules from the cells of origin. With these features, they contribute both to the reorganization of cellular functions and to the collective functioning of cell populations. Additionally, the content of exosomes is used to monitor the diagnostic and therapeutic processes of various gynecological diseases. They contain genetic and proteomic data that can be utilized as biomarkers or therapeutic targets in gynecological cancers and pregnancy-related disorders.

In this context, the roles of exosomes in major female reproductive disorders-including endometriosis, premature ovarian failure, polycystic ovary syndrome, Asherman's syndrome, endometrial cancer, cervical cancer, ovarian cancer, and preeclampsia are reviewed.

Keywords: Exosomes, infertility. women's reproductive diseases

INTRODUCTION

ABSTRACT

Exosomes are extracellular vesicles with a double-layered lipid membrane secreted by cells.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: exosomes.9,10 apoptotic bodies. microvesicles, and Exosomes effective paracrine are regulators of intercellular communication. In recent years, they have been shown to participate in biological functions such as metabolism 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 have been found to be secreted from various parts 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. Oggenesis, follicular development, implantation, fertilization, and embryo development are closely tied to maternalembryo 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 and thereby transferring their cargo.²⁹⁻³¹ Once exosomes are internalized, they initiate physiological processes by

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Copyright© 2025 The Author. Published by Galenos Publishing House on behalf of National Society of Gynecology and Obstetrics. This is an open access article under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 (CC BY-NC-ND) International License. delivering bioactive molecules such as coding and noncoding RNAs, proteins, and lipids. These molecules the functions of the recipient cells.32-34 modulate Studies on 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 commonly affected signaling pathways by miRNAs include Wnt (Wingless), neurotrophin, epidermal growth factor receptor (EGFR), and TGF-B (transforming growth factor-beta) 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 their implications in polycystic ovary syndrome (PCOS), premature ovarian failure (POF), Asherman's 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. obesitv. insulin resistance. dvslipidemia. 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. Additionally, 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 mitogenactivated protein kinase signaling pathway, circadian rhythm regulation, endocytosis, and overall PCOS risk.46-⁴⁸Another study reported increased expression of S100-A9 in the exosomes of PCOS patients.⁴⁹ S100-A9, a calciumbinding 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 infertilitv disorder characterized bv 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 exosomebased 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, MSCderived 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, 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-a-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 IncRNA profiles. Decreased expression was noted in IncRNAs 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 such as 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 crucial 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 were shown to influence the adhesive and migratory capacities of trophoblast cells via exosomal signaling.77 miRNAs like 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 through 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 critical for 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 exosomederived data in endometrial cancer research. Additionally, 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 could 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 the pathogenesis of human papillomavirus infection.94,95 Early diagnosis is crucial 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 play a role in 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 MYCBP (c-Myc binding protein) and hTERT (human telomerase reverse transcriptase) 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 guality of life among ovarian cancer patients are partly attributed to the absence of effective early diagnostic tools. Therefore, the development of novel diagnostic and therapeutic strategies is essential for reducing disease incidence 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.¹⁰⁹⁻¹¹³Otherenzymes-suchasaldehydereductase, phosphoglycerate isomerase, fatty acid synthase, PRDX1, and major histocompatibility complex class I and II antigensalso play roles in 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 have 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 such as miR-21, miR-184, miR-193b, miR-200a, miR-200b, miR-200c, miR-203, miR-214, and miR-215 have shown potential as diagnostic cancer.112,114,121-123 biomarkers for ovarian Additional miRNAs like 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 is critical for understanding disease pathogenesis and improving 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 crucial 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 were shown to distribute into all body compartments bypassing blood-brain barrier, blood testis barrier and blood follicle barriers.¹⁴⁴⁻¹⁴⁶ This enables them to be used as diagnostic agents of different diseases as well as direct therapeutic agents and possibly as drug delivery cargos. 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 adipose tissue derived adult MSCs, human BMSC MSCs, 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 unlike stem cell therapie.¹⁵⁰

Advancements 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 meaningful insights for diagnosis, treatment, and disease monitoring. In conclusion, exosome-based studies are expected to make

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

Footnote

Authorship Contributions

Literature Search: N.Ç., H.S., Writing: N.Ç., H.S.

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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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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, leda 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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ABSTRACT

shown promise, but their application to human embryos has yet to be thoroughly explored (Rebollar-Lazaro and Matson⁶). 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 postfertilization, 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/m²)	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 Group 1, Group 2. Variable p-value (n=80) (n=80) Number of oocytes retrieved 14.1 ± 6.7 14.4 ± 6.2 0.76 (n) Number of 2PN (n) 8.9±4.2 8.7±3.6 0.79 0.26 Fertilization rate (%) 81.6±14.5 79±14.5 Number of cleaved embryos 8.7±4.2 8.5±3.6 0.79 (n) Cleavage rate (%) 97.5±5.7 97.9±6.04 0.58 Day 2 good quality embryos 5.84 ± 3.3 5.35 ± 2.9 0.32 (n) Rate of day 2 good quality 66.3±22.2 62.1±23.1 0.25 embryos (%) Day 3 good quality embryos 5.35 ± 3.5 0.23 4.72±3.15 (n) Rate of day 3 good quality 59.6±23.9 53.9 ± 25.8 0.15 embryos (%) 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* 0.04* Rate of top quality blasts (%) 38.5 ± 22.2 31.1 ± 23.3 3.16±2.9 2.43 ± 2.3 Cryopreserved blasts (n) *p<0.05 statistically significant; 95% confidence interval

Table 3. Clinical and cumulative outcomes of both groups **Clinical outcomes (all** SC group, GC group, p-value transfers) (n=74) (n=77) 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 53.2 47.5 0.68 cycles)

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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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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Purpose: This study aimed 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, a total of 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 found to be 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 reproductiveaged 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 Kistner⁷ 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 is 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 6 cycles and were considered CC-resistant cases were included in this study.

Women with bilateral tubal occlusion confirmed with hysterosalpingography or with hypo/hypothyroidism, hyperprolactinemia, or other endocrinopathies (e.g., 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 more than 8 points by the Ferriman-Gallwey scores) were recorded.¹³ Body mass index was calculated as the following formula: Weight (kg) divided by the height squared (m²). 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 the CC induction, low-dose (37.5-75 IU) gonadotropin treatment was started when a dominant follicle (\geq 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 swimup 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 regarded 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 as 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 \pm 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, whereas 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 analysis.

RESULTS

This study retrospectively examined 75 women who were infertile due to PCOS and needed IUI cycle treatment. They were divided into the, SP (group 1, n=37) and MSP groups (group 2, n=38) according to the ovulation induction protocol.

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

A statistically significant difference was not noted between the groups in terms of the start of CC treatment and the total dose of CC (p=0.5; 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). Twelve 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 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. 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 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

Table 1. Demographic features and baseline characteristics of two groups				
	Group 1 (n=37)	Group 2 (n=38)	р	
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 (x10 ⁶)	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)	р
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: Claminhana sitrata bCC: Human abariania ganadatranhi	no. Il III Introutoring incomination		

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	р
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

who underwent IUI, while 6 (17.6%) clinical pregnancies were detected, 4 cases (11.8%) had ongoing pregnancy, and 3 of the 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

Our study revealed that MSP can reduce the risk of multifollicular development with 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, comparable clinical and ongoing pregnancy rates.

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 emphasized that continuing to support the cycle with exogenous FSH during the gonadotropindependent 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.^{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 literature¹⁹ 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 our 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 ovulation induction success 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 showed a lower rate of multifollicular development compared to SP in our study. 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 hMG⁷; for example, the OHSS risk was 0.2% in SP that used a CC and hMG combination⁸ 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 than with ovulation induction with gonadotropin in CCresistant 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. It has been shown that pregnancy rates are similar in PCOS patients regardless of the ovulation induction protocol used. 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 this 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 inclusion of a population with 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 is 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 in PCOS patients resistant to CC. 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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A Double Blind Randomised Controlled Trial Comparing Two Panty Linerswith Different Surfaces with Respect to Microbial Colony Per Centimeter Square

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Purpose: The aim of the study is to compare microbial colonies per centimeter square of two different panty liner pad 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 sampling, and laboratory analyses. Participants were randomly assigned toone of the panty liner group according to internet based random number generator. Even numbers were assigned to pad group 102 and odd numbers were assigned pad group 103. The panty liners were identical in outlook and neither the patients, nor the clinicians and microbiologist were avare 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 randomisation. The vulva and panty liner contact time was similar in zinc coated and non-coated groups respectively ($m=280\pm65$, $m=275\pm72$, p<0.58). The zinc coated group coded as 102 had 60 (53.7%) patients without microbial growth which was significantly lower compared to the group 103 with non-coated regular panty liners (n=44, 37.6%, p=0.02). Number of colonies per centimeter square in zinc coated panty liners were significantly lower than non-coated group ($m=9324\pm24046$, $m=56663\pm99618$, p<0.001). Dermatological assessments of the vulva showed no notable between group and within group frequencies of vulvar erythema, and excoriation in either panty liner group.

Conclusion: The study confirms 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

ABSTRACT

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 skin allergens 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 current study aims to research any differences in microbial colony growth on zinc coated versus regular non-coated panty liners of the same company after 4-6 hours of vulvar skin and vaginal contact.

METHODS

A total of 250 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 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 mean number of colonies between the two panty liners per centimeter square. Secondary outcome measures were number of women with vulvar erythema or excoriation. Two hundred and sixty-eight women were eligible for the study and only women who rejected to use the ped (n=18) were excluded from the study leaving 250 cases for allocation.

The study was a double blindstudy with blocked randomisation. The block size was determined to be the same for the number of people andthere wasn't an 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 closed envelopes. The envelopes were driven from the box at the time of randomization, 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. After allocation 12 patients with code 102 did not bring their panty liners or did not comply with the use and excluded from the analysis while 8 patients with code 103 were excluded due to same reasons. As a result 113 patients with code 102 and 117 patients with code 103 were analysed.

The panty liners were identical in outlook and neither the patients, nor the clinicians and microbiologist were avare of the technology until the study was finished. 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 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. Additionally, 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 grampositive 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 *Escherichia 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 which was evaluated for colony count evaluations. 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 cm² 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



Figure 1. Consort flow diagram

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 grampositive blastospores. Colony counting was performed, and species identification and antifungal susceptibility testing were 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-zinc coated regular panty liner group.

Statistical Analysis

The statistical analysis of the study was done using IBM^å SPSS^{\circ} Statistics version 21. Continuos variables were presented as means \pm standard deviation of the mean and categorical data was presented as numbers 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's test. The comparison of continuous data between the groups was done using independent samples t test. Probability (p) less than 0.05 was considered to be statistically significant for all comparisons.

RESULTS

The demographical data given in Table 1. The frequency of vaginal discharge, foul odor in the perineum, vulvar itching, erythema on the vulva, excoriation on the vulva were similar in Zinc coated panty liner group (n=113) vs. non-coated group (n=117). The frequency of bacterial vaginosis in 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 not statistically different from the 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.

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 (%)	р
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			

The vulva and panty liner contact time was similar in zinc coated and non-coated groups respectively (m=280±65, m=275±72, p<0.58). The zinc coated group coded as 102 had 60 (53.7%) patients without microbial growth which was significantly lower compared to the group 103 with non-coated regular panty liners (n=44, 37.6%, p=0.02). Number of colonies per centimeter square in zinc coated panty liners were significantly lower than non-coated group (m=9324±24046, m=56663±99618, p<0.001). The microbial proliferation according to different panty liners are 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. Also, within group change in the frequency of vulvar erythema and excoriation was not statistically different. Vulvar skin findings before and after panty liner use is given in Table 4. There was no important harm or unintended effect in each group.

DISCUSSION

In our study, we found that zinc coated panty liners bare less microbial colonies compared to the non-coated regular panty liners. While most studies in the literature² focus on isolating and analyzing individual pathogen species through separate cultures, our research 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 on Chinese women, comparing two pads with non-woven and perforated surfaces, no significant difference was observed between the two groups. Similarly, in our 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 mentioned 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 double-blind study design address vaginal microclimate, vulvar findings and symptomatology and panty liner microbial colony forming unit count. This rigorous approach lends greater reliability to our findings when compared to similar studies.

Table 3. Microbial proliferation according to different panty liners			
Finding	Zinc-coated group (n=113)	Non-coated group (n=117)	р
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***
*Not statistically significant, independent samples t-test, p>0.05			

**chi-square test, statistically significant, p<0.05

***Independent samples t-test, statistically significant, p<0.01

Table 4. vulvar skin findings before and after panty liner use			
Finding	Zinc-coated group (n=113)	Non-coated group (n=117)	р
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, not significant, p>0.05 **McNemar's test, not significant, p>0.05			

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, Streptococcus Gour A,B, D and Streptococcus viridans before and after six months of panty liner use. In our study, although no significant difference was observed in bacterial vaginosis between the two pads tested, the total colony count of cultured bacteria from the panty liners was significantly lower for the zinccoated group. It can be concluded that zinc has reduction effect on the number of colonies formed by the undesirable bacteria in the vaginal flora than 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 cytokines⁹ and 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

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 in Candida albicans.¹⁴ Also Zinc was shown to cause

direct bacterial cell membrane disruption and also indirectly boy inducing reactive oxygen species.^{15,16} On the other hand extended exposure to ZnO was suspected to play a reversible role in aminoglycoside resistance and ampicillin and other b-lactam resistance in *E. coli* via modifying cell drug efflux systems, switching the bacteria to anaerobic respiration state and increasing ribosomal protein production.^{17,18} In our study we did not study antibiotic resistance of bacteria as panty liner use was short lasting. Further studies may unravel if there is any increased number of antibiotic resistant microbial growth with zinc coated panty liners.

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. Additionally, our research assessed a single-day usage of 4-6 hours, whereas Giraldo et al.⁴ study spanned 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 and non-cancer risk.In our study, we did not conduct a research on this product produced in Turkey.

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, we found that women showed high compliance with the terms of use, although they were not evaluated 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 studied and were not specific to pathogen types.

CONCLUSION

Research into the effects of daily panty liners used during nonmenstrual periods-such as for vaginal discharge, spotting, and mild urinary incontinence-is crucial for enhancing patient comfort and quality of life. Studies examining these panty liners impacts on vulvar irritation, vaginal pH, microbiological outcomes, and potential triggers for discharge or itching provide valuable insights. Designing industrial 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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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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Purpose: The objective of this study was to 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 155 control 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: Women with PCOS had a significantly higher body mass index (26.5 ± 6.2 kg/m² vs. 24.6 ± 4.7 kg/m², p=0.003) and later menarche (12.7 ± 1.3 years 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 mm vs. 20.7 ± 4.9 mm, p=0.001), shorter AGD (21.2 ± 13.3 mm vs. 26.1 ± 7.7 mm, p<0.001), and reduced labia majora length (62.7 ± 19.8 mm 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 mm 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 demonstrates 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



ABSTRACT

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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 is to compare a wide array of external genital measurements between women with PCOS and healthy controls. In addition, we seek 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 from as a as a multicenter study from January until between January and December 2024.

The study included 155 women with PCOS and, as control cases, 155 women with mild male factor infertility or unexplained infertility, all between 18 and 45 years of age and admitted to 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. 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 \geq 12 follicles measuring 2-9 mm

in diameter and/or an ovarian volume >10 cm^{3,3} All ovarian ultrasound evaluations were performed transvaginally using a high-frequency transducer to assess for polycystic ovarian morphology. Notably, 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 a situation where 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 $(AGD_{\rm AF})$] 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 $\leq 26.55.^{20}$ 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). Categorical data are

presented as numbers and percentages, and continuous variables as mean \pm 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 p-value <0.05 was considered statistically significant.

RESULTS

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.

The data revealed that 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). Additionally, 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 Ferriman-Gallwey scores, acne scores, and alopecia scores between the groups is shown in Table 1. In the PCOS group, the average Ferriman-Gallwey 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. This indicates 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 $(18.3\pm7.02 \text{ mm})$ in comparison to the control group $(20.7\pm4.9 \text{ mm})$ (p=0.001). The clitoris length was slightly longer in the PCOS group $(18.5\pm7.1 \text{ mm})$ compared to the control group $(17.1\pm6.7 \text{ mm})$, although this difference was not statistically significant (p=0.07). The clitoris glans width was significantly larger in the PCOS group $(6.5\pm2.8 \text{ mm})$ compared to the control group $(5.8\pm1.5 \text{ mm})$ (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

Table 1. Demographic variables of polycystic ovary syndrome cases and controls			
Variable	Polycystic ovary syndrome cases (n=155)	Control group (n=155)	р
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 complex that p <0.0	F		

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

Statistically significant, chi-square test, p<0.05

Table 2. Genital measurements of polycystic ovary syndrome and controls			
Variable	Polycystic ovary syndrome cases (n=155)	Control group (n=155)	р
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*
Perineal body lenght (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			

and minora between the groups is summarized as follows: 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, p=0.3, respectively). However, the PCOS group had significantly higher arousal and orgasm scores (3.8±1.6 and 4.1±1.2, respectively) compared to the control group $(3.3\pm1.2 \text{ and } 3.6\pm1.8, \text{ respectively})$ (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 aroup (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 an FSFI score below 26.55. Nevertheless, there was no statistically significant difference observed between the two groups (p=0.3).

DISCUSSION

This study provides new insights into the anatomical differences in external genital measurements between women with PCOS and healthy controls, along with their implications for sexual function and genital self-image. The results suggest that women with PCOS exhibit notable differences in genital morphology, particularly in clitoral size, labial dimensions, 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 deeper insights into 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 contrasts 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. Additionally, factors such as amount of intrauterine androgen exposure and the timing of hormonal imbalances during development could play pivotal roles in determining AGD outcomes.

Beyond the findings on AGD, our study aligns 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 clitoris prepuce length, and a larger clitoris glans width compared to the control group, both of which were statistically significant. 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

Table 3. FSFI and FGSIS measurments in polycystic ovary syndrome and controls			
Variable (mean ± SD)	Polycystic ovary syndrome cases (n=155)	Control group (n=155)	р
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

measurements may serve as valuable markers of androgen excess in PCOS.

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 can 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. In fact, in our 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.

This 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

In conclusion, this study reveals significant anatomical differences in genital morphology among 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. Additionally, 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 guality 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.

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Emergency Blood Transfusion in Gynecology Cases: A Multicenter Analysis

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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 our country.

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 scanned, 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<0.05).

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

ABSTRACT

The first blood transfusion was performed in the 17th century; however, since blood group distinction could not be made, 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 approximately 20% of the blood volume is lost. Transfusion is



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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 was fluid replacement to restore blood volume. 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-related circulatory overload, and electrolyte imbalances.5

METHODS

The study was conducted in eight hospitals from January 1, 2023, to January 1, 2024. Ethical approval for our 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. A total of 12,176 gynecological surgeries were performed, of which 399 required blood transfusions. The study included all patients over the age of 18 who received blood or blood products before, during, or after gynecological surgery. Among these transfusions, 80 (20.1%) were performed in general state hospitals, 180 (45.1%) in training and research hospital, and 139 (34.8%) in private hospitals. 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 "emergent" 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 IBM SPSS Statistics 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 were presented as mean \pm 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. 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 g/dL), 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 (p < 0.05). 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 (p>0.05). 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 (p≤0.05). 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

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 \pm 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 \pm standard deviation or n (%)	where appropriate					

a. Independent samples t-test, b. Fisher's exact test, c. Pearson chi-square test Hb: Hemoalobin

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

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 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 our study, no significant difference in mean age was observed between emergency and elective transfusion groups (p>0.05), 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 (p=0.9), 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 could aid in developing

targeted transfusion management strategies, especially for patients requiring emergency interventions. Anemia remains a critical determinant of transfusion necessity. Zhou et al.¹¹ found that anemia significantly increases transfusion needs prior to surgical interventions. In our 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 reduces 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, our study showed that 3.2% of patients required transfusions for gynecological indications, with a higher transfusion rate (6.5%) among those with gynecologic malignancies. This is likely attributable to the invasive nature and increased bleeding risk associated with oncologic surgeries. 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 emphasized greater FFP utilization 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 cases, 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. Additionally, 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. **Conflict of Interest:** No conflict of interest was declared by the authors.

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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) syndrome is a rare congenital condition characterized by multiple organ systems, including the vertebrae, anus, cardiac system, trachea, esophagus, renal system, and limbs. Mullerian duct anomalies are infrequently observed in VACTERL syndrome, with only a few cases reported in the literature. We present a rare case of a 20-year-old female with VACTERL syndrome, 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. MRI 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 syndrome and underscores the need for tailored surgical approaches to address coexisting congenital anomalies.

Keywords: VACTERL syndrome, 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 Mullerian 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 requires 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 Mullerian anomalies in patients with VACTERL syndrome, 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. Additionally, 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 supported.

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. Additionally, 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 seen 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. The 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 noncommunicating 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 IV 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 followup, 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. The objective was to demonstrate the multidisciplinary approach required to address the complexities of this case and to explore how the rare coexistence of these abnormalities in a patient with VACTERL syndrome presents 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-yearold 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 like hematometra and endometriosis. The second case involved a 14-year-old airl 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 crucial for ensuring 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 syndrome 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 contributes 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.,

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