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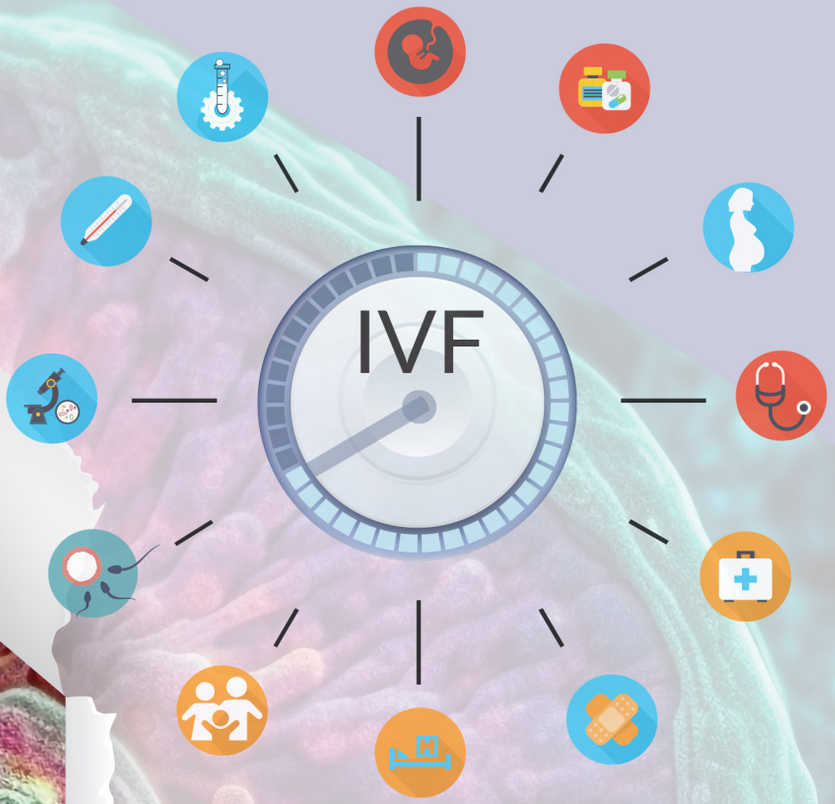

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5TH INTERNATIONAL MEDICAL CONFERENCE "INFERTILITY 35+" SUPPORTING COMPANIES

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CONTENTS

We are pleased to announce that the 5th International Scientific Conference, “Infertility 35+”, will take place on September 21-22, 2024, at the Sheraton Grand Tbilisi Metechi Palace in Tbilisi. The event is hosted by the Georgian-German Reproduction Medicine Center (GGRC) and supported by Georgian urological, obstetric-gynecological, endocrinological, reproductive, and oncofertility associations.

Within the framework of important meetings, reports will be presented by some of the world’s leading doctors and experts from the USA, Canada, Italy, Germany, Israel, Egypt, Austria, Kazakhstan, Armenia, Turkey, and, of course, Georgia. Residents in obstetrics-gynecology and reproductive medicine will also present their findings. The main topics will include reproducology, obstetrics and gynecology, urology, clinical oncology, endocrinology, and onco-surgery. Hundreds of doctors will hear fascinating reports and learn about the latest news and research in each of these fields.

It is important to note that certificates will be issued to pre-registered participants at the conference, and they will be awarded 8 credit points of continuing professional education (CPE). Scientific articles and abstracts presented at the conference will be published in “Medical Times,” a peer-reviewed journal dedicated to reproductive medicine. For the first time, the conference will include the launch of the journal’s e-version, which will be available on a website with a digital object identifier (DOI) to ensure prestigious dissemination of research.

We are delighted to offer Georgian doctors and researchers the opportunity to publish their articles in the e-journal at no cost and to attend the conference free of charge. Additionally, each participant will receive the 3rd edition of “Medical Times” and a new guidebook developed by me as a gift. This year, 600 doctors have already registered, including international attendees who are traveling to Georgia specifically for the event.The conference will span two days, with three separate halls dedicated to different aspects of the program. There will also be a workshop featuring specialists from Turkey, the USA, England, and Armenia discussing genetics and endometriosis. This workshop will address interesting cases and current international trends and approaches.We are particularly excited about the participation of various eminent associations.

Additionally, a working meeting will be held with the presidents of the International Fertility Federation (IFFS) and the associations from Georgia, Kazakhstan, Armenia, and Italy.

Finally, on behalf of GGRC, I would like to extend our gratitude to the Ministry of Internally Displaced Persons from the Occupied Territories, Health, Labour and Social Affairs of Georgia for their support.

Sincerely,

Nino Museridze
GGRC Clinical Director

PREMATURE OVARIAN INSUFFICIENCY DETERMINED BY X CHROMOSOME ANOMALIES

***JENARO KRISTESASHVILI**, MD, PhD, Prof.;[1] **ELENE ASANIDZE**, PhD, Prof.;[2] **JIBLADZE A**. PhD student[3]*
[1] Iv. Javakhishvili Tbilisi State University, Faculty of Medicine, Department of Obstetric, Gynecology and Reproductology. Center for Reproductive Medicine "Universe."
[2] "Geomedi" University, Faculty of Medicine, Center for Reproductive Medicine "Universe."
[3] Tbilisi State University, Center for Reproductive Medicine "Universe". Tbilisi, Georgia

SUMMARY

Background: Premature ovarian insufficiency (POI) is a condition defined by loss of ovarian activity before the age of 40 years, accompanied by menopausal symptoms. It is marked by amenorrhea or oligomenorrhea, absence of ovulation, elevated gonadotropins, and low estradiol levels. POI can be classified into spontaneous non-iatrogenic and iatrogenic forms. The prevalence of non-iatrogenic POI in the population ranges from 1% to 3.5%. The X chromosome's Numerical and structural abnormalities are key etiological factors.

Objective: To determine the clinical peculiarities and types of POI caused by X chromosome anomalies.

Material and Methods: The study included 26 patients aged 16 to 24 with non-iatrogenic spontaneous POI. Of these, 12 patients were diagnosed with numerical or structural abnormalities of the X chromosome based on clinical, laboratory, and instrumental studies. In some cases, x-chromosome abnormalities were detected using G-banding in peripheral blood lymphocyte cultures; fluorescence in situ hybridization (FISH) and molecular cytogenetic methods were employed.

Results: Among the 26 patients with non-iatrogenic spontaneous POI, four were diagnosed with Turner syndrome. These patients exhibited severe growth retardation, somatic anomalies typical of Turner syndrome, and sexual development according to Tanner's scheme (Ma0PaAx0Me0), including markedly elevated gonadotropins and decreased estradiol levels, By US – streak gonads and uterus, without follicles. Two patients were identified with a 45, X karyotype, while another had a 46, X, i(Xq) karyotype. Additionally, a mosaic karyotype with a 45, X cell line was detected in 5 patients (4 with 45, X/46, XX and 1 with 45, X/47, XXX). Structural abnormalities of the X chromosome, specifically deletions, were found in 2 patients. Patients with mosaicism and structural abnormalities of the X chromosome exhibited mild growth retardation and premature

ovarian failure, which was characterized by primary amenorrhea, hypergonadotropinemia, and a significantly reduced number of antral follicles. Only one patient with a 45, X /47, XXX mosaic karyotype experienced secondary amenorrhea and spontaneous puberty. One patient with tetrasomy X (karyotype 48, XXXX) and POI presented with tall stature, mental retardation, secondary amenorrhea, hypergonadotropinemia, and a few follicles in the ovaries. To initiate puberty, all patients were treated with monotherapy using natural estrogen analogs, followed by replacement therapy with estrogen-gestagens until the age of natural menopause.

Keywords: premature ovarian insufficiency, Turner syndrome, X chromosome abnormalities, numerical anomalies, structural anomalies, mosaicism, growth retardation

Conclusion:

- For all patients with non-iatrogenic premature ovarian insufficiency, despite the absence of subjective signs of estrogen deficiency and the characteristic signs of Turner's syndrome, karyotyping is recommended.
- Oocyte donation is the optimal method to achieve fertility in patients with premature ovarian insufficiency and numerical and structural anomalies of the X chromosome.
- In patients with X chromosome anomalies at an early stage of diagnosis of chromosomal anomalies and in cases of an acceptable number of ovarian follicles, cryopreservation of reproductive materials may be considered with the using genetic testing of the embryo.

INTRODUCTION

Premature ovarian insufficiency (POI) is a condition defined by loss of ovarian activity before the age of 40 years. POI is characterized by amenorrhea or oligomenorrhea, with elevated gonadotropins and low estradiol levels.[1] Distinguish spontaneous non-iatrogenic and iatrogenic forms of premature ovarian insufficiency. It's important to note that earlier studies indicated that the prevalence of non-iatrogenic POI is approximately 1%, while newer studies suggest it could be as high as 3.5%.[2,3,4]

Menstrual history of irregular periods <40 years of age is noteworthy for early diagnosis of POI. Menopausal symptoms may not always be present. Recommended investigations include hormonal tests (FSH, LH, AMH, anti-TPO antibodies, adrenal cortex hormones like cortisol, 17α-OHP, DHEA), 21-hydroxylase antibodies, karyotyping, Fragile X mental retardation (FMRI) gene premutation analysis, ultrasonography for antral follicle count, and bone densitometry (DXA)**.** To confirm the diagnosis of POI, it's necessary to measure FSH and AMH levels twice, with an interval of 4-6 weeks.[5,6]

The etiology of POI can be categorized as follows:

Spontaneous Non-Iatrogenic: Causes include genetic factors related to the X chromosome (such as XO Turner syndrome, X trisomies, X deletions, X translocations), Fragile X FMRI gene premutation (1-5%, rising to 13% with a positive family history), other gene mutations, autoimmune conditions (3-30%), and infections (parotitis, tuberculosis, malaria, cytomegaly).

Induced Iatrogenic: This form of POI is caused by medical interventions such as bilateral ovariectomy, cystectomy, chemotherapy (including anthracyclines), radiation therapy, and embolization of pelvic vessels.

Environmental toxins.

OBJECTIVE

To determine the clinical peculiarities and types of POI caused by X chromosome anomalies.

MATERIALS AND METHODS

A total of 26 patients aged 16-24 years with the spontaneous non-iatrogenic form of premature ovarian insufficiency (POI) were examined. Clinical, laboratory, and instrumental investigations were performed, including:

- **Hormonal investigations:** Prolactin, Estradiol, FSH, LH, TSH, FT4, Anti-Tg, Anti-TPO, AMH.
- **Genital organs ultrasound (US) examination.**
- **Cytogenetic investigation:** Determination of the karyotype in peripheral blood lymphocyte cultures using G-banding.

Fluorescence In Situ Hybridization (FISH) and molecular cytogenetic methods were also used in some cases.

Ethical considerations

All the adult participants and parents of adolescent individuals signed a written consent form**.**

RESULTS

Out of 26 investigated patients with non-iatrogenic POI, 24 patients had primary amenorrhea, and two patients – had secondary amenorrhea. None of the patients had subjective menopausal symptoms. Patients with primary amenorrhea had a delay in sexual development and developed secondary genital signs on the background of hormone replacement therapy before presenting to us. All patients had an elevated level of FSH – more than 25 IU/ml, with 2-fold testing and a decreased estradiol level. Ultrasonography showed a markedly hypoplastic uterus, and antral follicle counts reduced to 1-2 (0.5-1.5mm), or the follicles were not visualized. By karyotyping, 14 patients were diagnosed with a normal female karyotype and 12 patients with numerical or structural anomalies of the X chromosome.

Diagnosis of Turner syndrome with karyotypes 45, X and 46, X, i(Xq) is not difficult against the background of characteristic clinical manifestations (severe retardation of growth and sexual development, typical somatic anomalies, increased gonadotropins and decreased estradiol levels, by US streak gonads and uterus.[7, 8]

Admission of such patients in reproductive clinics before the manifestation of hypogonadism is relatively rare because they are admitted to the growth centers due to growth retardation.

Out of 12 patients with premature ovarian insufficiency due to X chromosome anomalies, only four patients had typical Turner syndrome with 45, X (2) and 46, X,i(Xq) (2) karyotypes, primary amenorrhea was presented in all four patients with Turner syndrome.

In cases of 45, X cell line containing mosaicism (45, X/46, XX,45, X/47, XXX) and X chromosome structural anomalies, the leading clinical manifestations were premature ovarian insufficiency on the background of mild growth retardation and single somatic anomalies. According to literature data, the degree of symptoms expression in cases of mosaicims is variable and in some cases spontaneous menarche and even pregnancy can be expressed.[9] Among the patients examined by us, spontaneous menarche and secondary amenorrhea were diagnosed in one patient with 45, X/47, XXX mosaicism, and growth retardation, which is also described in the literature.[9]

According to our data, two patients with POI and structural anomalies of the X chromosome had mild growth retardation. One of them (16 yr.) had delayed sexual development. Another 24-year-old patient was treated with estrogen-gestagens before referring to us. The growth of mammary glands and induced menstruations were expressed on the background of hormone replacement therapy.

In both cases, a two-time study determined a sharp increase in the FSH level and a decrease in the estradiol level. Ultrasonographic examination revealed a hypoplastic uterus and a markedly reduced number of antral follicles in the ovaries (1-2 follicles).

The situation is different in cases of polysomy X. The clinical manifestations of patients with triple X are characterized by a widely expressed polymorphism; therefore, the diagnosis is made in only 10% of cases, or in other cases, the diagnosis is delayed.[10]

Based on individual case reports, women with triple-X have been shown to have decreased AMH values, reduced ovarian reserve, and have an increased risk of early menopause and POI.[11] The risk of POI is estimated to be five times higher in women with 47, XXX than in women with 46, XX karyotype.[12]

An 18-year-old patient examined by us was diagnosed with tetraploidy X, which is a rare condition. Only 60 cases of tetraploidy X have been described in the worldwide literature. This disorder is characterized by a comprehensive clinical polymorphism, and considering that the literature more often represents individuals in early puberty, the types and frequency of sexual development disorders in these patients are not specified. [13,14,15,16]

In contrast to Turner's syndrome, our patient with 48, XXXX karyotype showed rapid and intense height growth (179 cm). The patient had secondary amenorrhea, sexual development according to Tanner Ma4P4Ax3, clinodactyly, microgenia and polysomy X-specific mental retardation (IQ- 59), and premature ovarian insufficiency. The FSH level by two-time testing was > 25 IU/ml, and the ultrasonography showed a markedly hypoplastic uterus and ovaries with single follicles.

After the diagnosis, the issue of treatment with sex hormones was considered for all patients with POI. In the cases of Turner's syndrome, in combination with the use of growth hormone, after reaching the appropriate growth for the initiation of puberty, small doses of monotherapy with natural estrogens were prescribed for 1-2 years, and then hormone replacement therapy with natural estrogen-gestagens until the age of natural menopause. In other cases, depending on the need, monotherapy with natural estrogens was used at the initial stage, and estrogen-gestagens combination for long-term treatment, as recommended in the literature, for prevention of long-term negative consequences of estrogen-deficient conditions (Osteoporosis, CVD, Alzheimer's disease, etc.).

It is known that in cases of non-iatrogenic POI, spontaneous pregnancy is possible in up to 15 % of cases.[17,18,19]

Egg donation is considered an optimal method of achieving fertility in cases of X chromosome anomalies.[19]

However, on the background of secondary amenorrhea and mild decrease in the number of antral follicles in some cases of 45, X cell line mosaicism, and structural anomalies of the X chromosome in cases of early diagnosis, the possibilities of cryopreservation of reproductive materi-

als can be considered with using genetic testing of embryos. Spontaneous puberty and pregnancy, interestingly, are more common in patients with 45, X/47, and XXX karyotypes than in patients with 45, X/46, and XX karyotypes.[20]

Conclusion

- For all patients with non-iatrogenic premature ovarian insufficiency, despite the absence of subjective signs of estrogen deficiency and the characteristic signs of Turner's syndrome, karyotyping is recommended.
- Oocyte donation is the optimal method to achieve fertility in patients with premature ovarian insufficiency and numerical and structural anomalies of the X chromosome.
- In patients with X chromosome anomalies at an early stage of diagnosis of chromosomal anomalies and in cases of an acceptable number of ovarian follicles, cryopreservation of reproductive materials may be considered with the using genetic testing of the embryo.

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STUDY OF RHEOLOGICAL PROPERTIES IN PHYSIOLOGICAL PREGNANCY

***MAKA MANTSKAVA**, Prof.;*[1,2,3,4] ***NANA MOMTSELIDZE**, Prof.;*[1] ***GIORGI KUCHAVA**, Prof.*[1]
[1] *Ivane Beritashvili Center of Experimental Biomedicine, Tbilisi, Georgia*
[2] *Head of Department of Rheology and Diagnostic Analytical Services*
[3] *President of the International Society of Rheologists*
[4] *National Head of the World Society of Clinical Hemorheology and Microcirculation*

SUMMARY

We studied pregnant women in the I and II trimesters and a control group of women in the 2nd phase of the menstrual cycle. All women measured the rheological properties of the blood, such as red blood cell aggregation, red blood cell deformation, blood plasma viscosity, and hematocrit. It turned out that rheological changes (deterioration) advancing in the first trimester tend to stabilize in the second trimester. This says, from our point of view, about the participation of a rheological system in the adaptation mechanism.

Keywords: red blood cell, aggregation, deformation, plasma viscosity, rheology. I trimester, II trimester

INTRODUCTION

During pregnancy, a woman's body undergoes profound changes. These changes result from the coordinated work of almost all body systems and the interaction of the mother's body with the child's body. During pregnancy, many internal organs undergo significant restructuring, which is adaptive in nature.

The respiratory system works harder during pregnancy and the respiratory rate increases. This is due to the increased need of the mother and fetus for oxygen and the limitation of the diaphragm's respiratory movements due to the increase in the size of the uterus.

During pregnancy, the cardiovascular system is forced to pump more blood to ensure an adequate supply of nutrients and oxygen to the fetus. During pregnancy, the thickness and strength of the heart muscles increase, the pulse rate and the amount of blood pumped by the heart per minute increase, and the volume of circulating blood increases. The tone of the blood vessels

during pregnancy decreases, the supply of nutrients and oxygen to the tissues increases, and the network of vessels in the uterus, vagina, and mammary glands decreases sharply.

During pregnancy, hematopoiesis increases, as does the number of red blood cells, hemoglobin, and plasma. Changes in the coagulation system also promote hemostasis.

The kidneys work more intensively during pregnancy. Changes occur in the digestive system and the body's skeletal system changes. Increased concentrations of relaxin and progesterone hormones in the blood contribute to the leaching of calcium from the skeletal system.

A decrease in the excitability of the cerebral cortex accompanies changes in the nervous system. At the beginning of pregnancy, an increase in the tone of the vagus nerve is observed.

Significant changes occur in the activity of the endocrine glands, contributing to the correct course of pregnancy and childbirth.

All body systems change. This is what is meant by adaptation processes during gestation. Changes are reflected in the rheological system of the blood, which ensures adaptation. The rheological parameters of blood and plasma affect the state of processes of systemic hemodynamics and, microcirculation and hemostasis.[1,2,3] Therefore, the adaptation process is essential for the systemic bloodstream. Of particular interest is the period of quick. The growth of the placenta, but even more interesting, is how the blood reiology changes at the initial stages of pregnancy. Comparisons of these two periods (initial growth (I trimester) and rapid placenta growth (II trimester)) are informative. Comparison and identification of differences will make it possible to obtain fundamental reoological data during pregnancy, which will help plan the renewal of improvement of rheological properties during gestation.[1,2,4,5,6]

The main properties of blood reoology are blood viscosity, hematocrit, red blood cell aggregability, and red blood cell membrane deformation. It is these parameters that provide blood fluidity, which is one of the most critical components of the bloodstream. The study aimed to study adaptive reactions of hemoroological properties in the physiological course of gestation in the 1st and the II trimesters of pregnancy.

MATERIALS AND METHODS

We investigated pregnant women (20-28 years old) from 4-13 weeks of gestation (the first point of study) to 13-26 gestation weeks (the second point of study). The control group comprised 14 practically healthy women in the second phase of the menstrual cycle.

The research design was approved by the Ethics Committee of the Society of Rheology (405133029) and the Ivane Beritashvili Center.

Inclusion parameters:

First pregnancy. Cutting funds for more than one year was not two years old.

Exception parameter:

Hematological diseases in history, cancer, anemia of pregnant women.

Study of rheological properties:

The index of red blood cell aggregability (EAI).

The index of red blood cell aggregability represents aggregated e red blood cells' area ratio against the whole area of the red blood cells. Red blood cell aggregation was evaluated using the recently developed "Georgian technique," which provided direct and quantitative data. Blood samples (4ml) from the cubital veins were centrifuged, and about 0.1 ml of blood was diluted 1:200 in their own plasma in the Thoma pipettes preliminary rinsed with 5% sodium citrate solution without the addition of any other anticoagulants to the blood under study. After standard mixing, the diluted blood was placed into a glass chamber 0.1 mm high. The quantitative index of red blood cell aggregation, which was assessed with a unique program at the Texture Analysis System (TAS-plus, "Leitz, Germany), represented itself the relationship between the aggregated and unaggregated red cells.[8-10]

Red blood cell deformability index (EDI).

Evaluation of red blood cell deformability was performed with the aid of the nucleopore membrane filter method, which is based on assessing the velocity of the red blood cells passage through the tiny pores (5 µm, which is a diameter of the smallest capillary) of the filter, at constant pressure (10 cm of water column) and temperature (37°C). The pure red blood cells were obtained by centrifuging the blood sample at 3000 rpm for 15 min. The resulting plasma was aspirated with a micropipette, and the remaining blood cells were added to the phosphate buffer with bovine serum albumin (0.2 mg per 5 ml). Then, the blood was centrifuged a second time at 1000 rpm for 5 min. The precipitated red blood cells and a thin layer of leukocytes and thrombocytes were separated from the phosphate buffer. This procedure was repeated three times. Purified red blood cell mass was diluted in the phosphate buffer with a hematocrit of 10%. Evaluation of the deformability index implied measuring a velocity of the red blood cell passage through the filter (mm/min) was recorded. The high-quality polycarbonate filters (with 5 µm diameter pores) were used in measuring procedures.[8-10]

Plasma viscosity.

Blood plasma viscosity was examined in a capillary viscometer at 37˚ C. The Diameter of the capillary was about 1.8 mm. The gravity force related to the difference of levels of the plasma under study—about 65—induced displacement of plasma samples (without application of additional pressure). For evaluation of the plasma viscosity in centipoises (cP), we determined the calibration factor (F). Blood plasma viscosity was calculated by multiplying the time for plasma displacement through the capillary by the instrument calibration factor.

RBC concentration (Hct).

For the calculation, we used the automatic counter (Human Count 2.1, Germany), which gave the result as digital values of RBC concentration.

Statistical analysis:

Statistical significance was tested using one-way ANOVA and a two-sample test. Relationships yielding P-values less than 0.05 were considered significant. All values were expressed as the mean±standard error.

RESULTS

Our studies have shown that all rheological parameters change in the I trimester, and their increase stabilizes in the II trimester. You can see Table 1.

Table 1. *Rheological properties in the control group and pregnant women. M±m.*

Rheological Properties	Control	Pregnant woman (I trimester)	Pregnant woman (II trimester)
	N=14	N=20	N=18
EAI, %	25±2,5	31±5,4	26±2,9
EDI, %	2,1±0,01	2,2±0,03	2,1±0,04
Hct, %	45±4	50±8	48±5
Plasma Viscosity, sP	1,13±0,05	1,15±0,05	1,14±0.05

DISCUSSION

Studies indicate that hemorheological shifts in the physiological course of pregnancy can be due to a decrease in the magnitude of hematocrit, blood viscosity, and aggregation and deformation of red blood cells.[1.2] In the I trimester of pregnancy, red blood cells have high aggregation with the subsequent decrease in deformability and the appearance of more rigid red blood cells. But in II trimester this situation changed. This circumstance explains reducing the viscosity of the blood under study. It is known that due to a decrease in deformability, the ability of red blood cells to pass through tiny capillaries worsens, and they can accumulate. In the places of their bends, bifurcations with the possible development of stasis.[5] At the same time, against the backdrop of a decrease in the deformation ability of red blood cells, tissue oxygenation may deteriorate, followed by the development of tissue hypoxia.[5] However, with the physiological course of pregnancy, despite the decrease in the deformability and plasticity of red blood cells, there is a fact of development that we have established adaptive reactions aimed at improving the microrheological properties of blood and microhemodynamics in the tissues of a pregnant woman. These reactions are manifested by a decrease in the aggregation ability of red blood cells, which decreases blood viscosity—an identified reduction of red blood cell aggregation.[11]

In the second trimester, a physiologically flowing pregnancy may be associated with a decrease in their deformability, an increase in the average size of red blood cells, and a reduction in concentration.

They have hemoglobin and, therefore, a change in osmotic properties. The described shifts inevitably change the structure of membrane phospholipids and proteins that form the binding places for adhesive molecules mediating intercellular interactions.[2] Plasma proteins compete for limited adsorption sites on red blood cells.

By their surface activity and mass concentration.[2,4]

Thus, the study's results indicate that with a physiologic leakage pregnancy under the influence of hormonal shifts,[3,12] changes in the body of a woman's qualitative and quantitative composition of peripheral blood occur. Arising in this case

Changes in the hemorheological parameters are the nature of adaptive reactions to improve systemic hemodynamics and microcirculation.

In all organs and tissues of the maternal body and, consequently, to maintain the ordinary course of the gestational process.

We continue the study in this direction. Naado to note that in this study, the first and second studied group was formed by the same and those of pregnant women (only two of them dropped out in objective prachi from the study); we will also explore these women with rheological pararances in the third trimester, which will, even more, explain as the rheological systems provide – body adaptation mechanism in gestation.

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AGE-RELATED ISSUES OF VITAMIN D DEFICIENCY AND COVID-RELATED HEALTH OUTCOMES IN GEORGIA

Dr. ***NIA METONIDZE****;*[1] *Dr.* ***DAVID TANANASHVILI***[2]
[1] *The University of Georgia, Tbilisi, Georgia*
[2] *BioStat ltd., Tbilisi, Georgia*

SUMMARY

Background: In patients hospitalized for COVID-19, vitamin D deficiency was highly prevalent. Elderly patients who survived COVID-19 took more time for a full recovery compared to other age groups.

Objective: This study aims to evaluate the age peculiarities of vitamin D deficiency and COVID-related health outcomes (hospitalization, transfer to ICU unit, requirement of oxygen therapy, and treatment by glucocorticoids).

Materials and Methods: Presented retrospective cross-section study was performed based on National Center of Disease control and Public Health (NCDC) data of Georgia. After obtaining the written informed consent form, 384 persons from the NCDC database were included in the study group. Study subjects were divided into two age groups: group 1 – patients aged 50 years – n=156; and Group 2 – patients aged < 50 years – n=228.

Results: The Mean serum 25(OH)D levels in the study groups did not differ significantly. However, these values were significantly lower in hospitalized patients of both groups. The odds of hospitalization and the requirement of oxygen therapy in group 1 were significantly higher compared to group 2 (OR = 3.79, p<0.001; OR = 5.10, p=0.002, respectively). The odds of the requirement of transfer to the ICU unit (OR = 2.22, p=0.387) and glucocorticoid treatment (OR = 2.73, p=0.077) between the groups were insignificant.

Conclusion: Our study revealed significantly worse COVID-19-related health outcomes in elderly patients than in younger age groups. However, the difference between groups in mean levels of serum 25-hydroxyvitamin D [25(OH)D] in hospitalized patients was not statistically significant.

Keywords: COVID-19; elderly; health outcomes; hospitalization; vitamin D.

INTRODUCTION

The COVID-19 pandemic was the outbreak following SARS in 2002 and MERS infections in 2012.[1,2] However, in contrast to previous ones, COVID-19 has higher transmission rates. It thus incurs more challenges in terms of prevention and treatment.[2] Mortality and other complications were the most susceptible adverse outcomes from COVID-19.[3] Their risk also increases in the presence of multiple comorbidities such as diabetes, cardiovascular disease, respiratory disease, malignancy and obesity.[3-6]

SARS-CoV-2 infection induces local and systemic inflammatory responses in humans.[7] Inflammation accompanied by an exaggerated immune response leads to pyroptosis and tissue damage in patients with COVID-19.[8] When SARS-CoV-2 infects the lungs, it causes alveolar epithelial cell death, endothelial disruption, increased lung permeability, and alveolar edema. It can lead to acute respiratory distress syndrome (ARDS) and multiorgan failure.[9]

In patients hospitalized for COVID-19, vitamin D deficiency was highly prevalent.[10] Therefore, it is rational to assume a beneficial role of vitamin D supplementation in preventing, reducing symptoms, or improving the prognosis of this disease. Several dozen studies have been conducted to determine the effect of vitamin D on COVID-19. Among them, a few have found promising results. An RCT of oral vitamin D3 (cholecalciferol; 60,000 IU daily), with a therapeutic target of serum 25(OH) D > 50 ng/mL, was found to significantly induce negative conversion of SARS-CoV-2-RNA and lead to a decrease in fibrinogen levels.[11] Other small-scale studies have also shown that vitamin D supplementation during or in the month preceding SARS-CoV-2 infections was associated with less severe outcomes, including lower mortality, even in elderly patients.[12] Asymptomatic or mildly symptomatic patients with COVID-19 given vitamin D showed improvement in related symptoms on day 14 but did not significantly reduce the time to negative transformation of SARS-CoV-2 RNA virus.[13] Another study found that a single high dose (200,000 IU) of vitamin D did not reduce the length of hospital delay or mortality in patients hospitalized for moderate to severe COVID-19.[14] Elderly patients survived after COVID-19 took more time for the full recovery compared to other age groups. The outcome of these conditions was rapid loss of muscle mass after hospital discharge due to immobilization, which can increase the risk of frailty, falls, fractures, and mortality.[15]

Therefore, our study aimed to investigate the age peculiarities of vitamin D deficiency and COVID-related health outcomes (hospitalization, transfer to ICU unit, requirement of oxygen therapy, and treatment by glucocorticoids).

METHODS

Study Design and Subjects

The presented retrospective cross-section study was performed based on the data of the National Center for Disease Control and Public Health (NCDC) of Georgia. 475 records of patients with determined serum 25-hydroxyvitamin D [25(OH)D] levels were randomly selected for the study. Researchers visited these patients, and after obtaining written informed consent, 384 persons from the NCDC database were included in the study group.

Study Parameters

The data on hospitalization, duration, transfer to the ICU unit, oxygen therapy requirement, glucocorticoid treatment, and symptoms were extracted from the NCDC database. The patients were

surveyed using particular structured questionnaires to provide information about the presence of vitamin D supplementation before the SARS-virus confirmation.

Study Groups

Study subjects were divided into two age groups: group 1 – patients aged 50 years – n=156; and Group 2 – patients aged < 50 years – n=228.

Statistical Analysis

The study results were statistically analyzed using SPSS 22.0 software (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean and standard deviation (SD), and differences were assessed by analysis of variance. Categorical variables were compared using Pearson's chi-square or Fisher's exact tests. Odds ratios (ORs) and 95% CIs within the presented study were estimated. P values of <0.05 were considered as statistically significant.

RESULTS

Study Characteristics

Table 1 gives the age, body mass index (BMI) data, and distribution by gender and body weight status of the patients in the study groups.

Table 1. *Age, BMI, and the distribution of patients by gender and body weight status in the study groups.*

#	Parameter	Group 1 (n=156)		Group 2 (n=228)	
		Mean	SD	**Mean**	SD
1	**Age, years**	**63.9**	7.9	**27.9**	10.4
2	**BMI, kg/m²**	**28.1**	9.0	**24.8**	4.0
3	**Body Weight Status**	**n=**	%	**n=**	%
	Normal	**32**	20.5%	**123**	53.9%
	Overweight	**91**	58.3%	**81**	35.5%
	Obesity	**33**	21.2%	**24**	10.5%
4	**Gender**	**n=**	%	**n=**	%
	Males	**33**	21.2%	**60**	26.3%
	Females	**123**	78.8%	**168**	73.7%

It is clear from the table that age did not differ between groups significantly. No significant difference was found between the groups according to the distribution by the age groups (chi2-test = 1.706, df=2, p=0.426). BMI mean values differed significantly between the groups (p<0.05). The same trend was found in the distribution by body mass between the groups – chi2-test = 43.36, df=2, p<0.001. Gender distribution was not significantly different.

Chart 1 shows the mean levels of serum 25(OH)D in the study groups. The difference between these values was not significant — t=0.695, p=0.487.

Chart 1. *Mean levels of serum 25-hydroxyvitamin D [25(OH)D]*

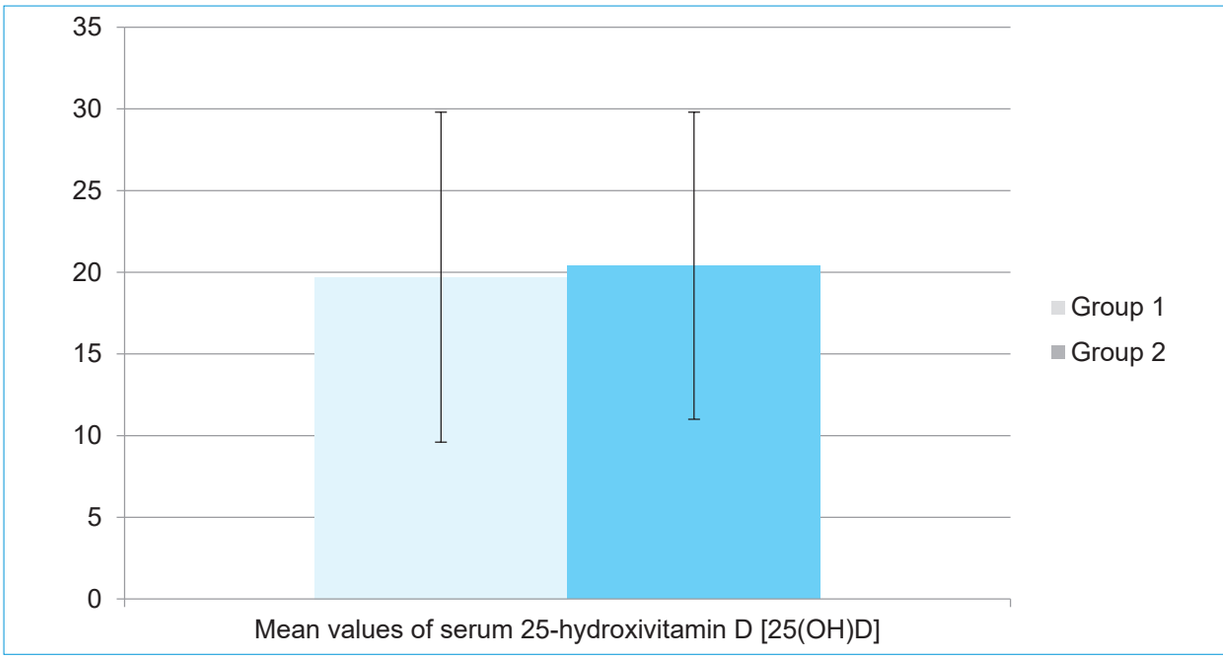


Table 2 gives the data on hospitalization rates, transfer to the ICU unit, oxygen therapy requirements, glucocorticoid treatment, and SARS-COV-2 infection symptoms extracted from the NCDC database.

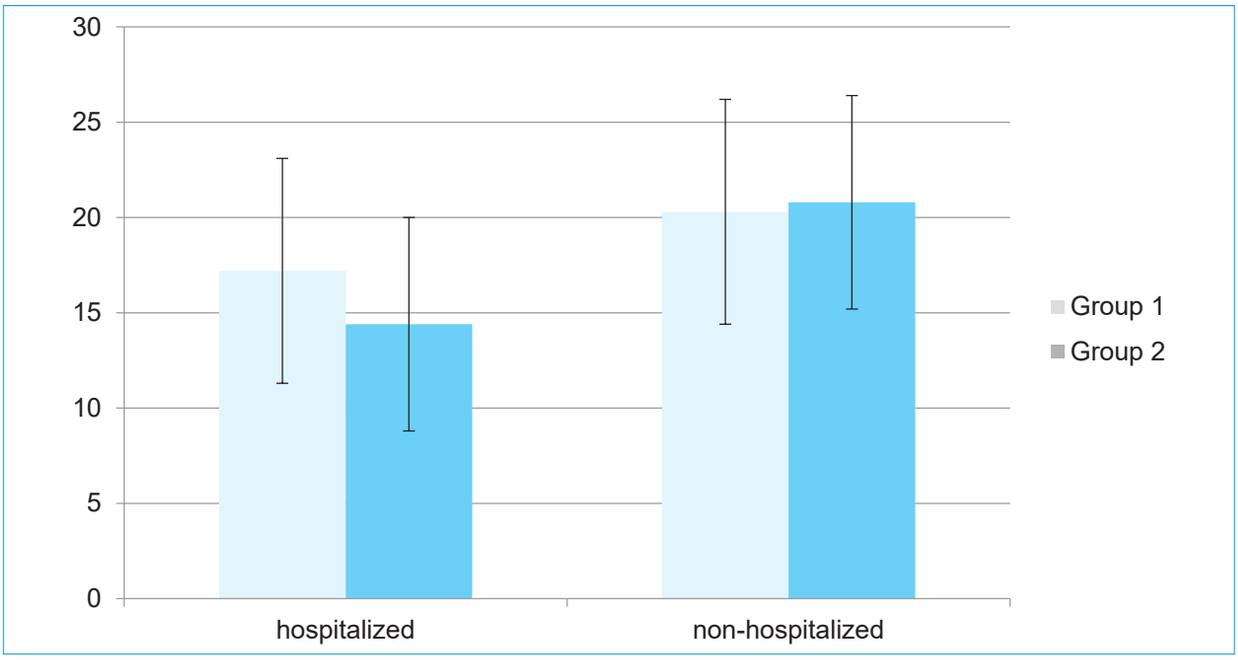
Table 2. *The distribution of patients by the hospitalization rates, transfer to the ICU unit, the requirement of oxygen therapy, the treatment by glucocorticoids, and SARS-infection symptoms in the study groups*

#	Health outcomes	Group 1 (n=156)		Group 2 (n=228)	
		n=	%	n=	%
1	Hospitalization	**31**	19.9%	**14**	6.1%
2	Transfer to ICU unit	**3**	1.9%	**2**	0.9%
3	Requirement of oxygen therapy	**16**	10.3%	**5**	2.2%
4	Treatment by glucocorticoids	**9**	5.8%	**5**	2.2%

The odds of hospitalization and the requirement of oxygen therapy in group 1 were significantly higher compared to group 2 (OR = 3.79, 95%CI 1.94 – 7.40, F-test = 3.91, p<0.001; OR = 5.10, 95%CI 1.83 – 14.22, F-test = 3.11, p=0.002, respectively).

The odds of the requirement of transfer to the ICU unit (OR = 2.22, 95%CI 0.37 – 3.42, F-test = 0.87, p=0.387) and the treatment by glucocorticoids (OR = 2.73, 95%CI 0.90 – 8.31, F-test = 1.77, p=0.077) between the groups were not significant.

Chart 2. *Mean levels of serum 25-hydroxyvitamin D [25(OH)D] in hospitalized and non-hospitalized patients in both groups.*



Mean levels of serum 25-hydroxyvitamin D [25(OH)D] in hospitalized patients were significantly lower in both groups compared to non-hospitalized patients ($p<0.05$). However, the difference in these levels between groups of hospitalized patients was insignificant ($p>0.05$).

DISCUSSION

There has been much discussion about the impact of vitamin D on SARS-COV-2 infection. Vitamin D may alter the disease manifestations depending on its influence on macrophage function and innate immunity. Vitamin D supplementation becomes relevant in the absence of highly effective prevention and treatment strategies for the pandemic. Considering the drugs' availability and very economical pricing, especially in developing countries (countries of Group A and B by Research4Life program16), vitamin D supplementation should be an important option for the populations at risk.

Previous systematic reviews have clearly shown an inverse association between 25(OH)D concentration and acute respiratory tract infections8,17, but these studies were not directly focused on SARS-CoV-2 infection. Similar to our findings, a study from the UK by Panagiotou et al. found that low serum 25(OH)D levels in 134 hospitalized patients with COVID-19 were associated with a more severe disease course.[18]

Conversely, a study using the UK Biobank looked at 348 598 participants, of whom only 449 had a confirmed diagnosis of COVID-19 as defined by a positive laboratory test for SARS-CoV-2 (only 0.13% of the study population), and they did not find any association between 25(OH)D and risk of COVID-19 infection.[19] In addition to the low number of patients with COVID-19, other weaknesses in this study included heterogeneity in severity and management of COVID-19 cases (likely a mixture of inpatient and community, instead of focusing on COVID-19 cases in only one

setting), serum 25(OH)D measurement between 2006 and 2010, and not contemporaneously with COVID-19 infection 10 to 14 years after recruitment to the UK Biobank, and no mention of validation of 25(OH)D measurement.

In terms of 25(OH)D and COVID-19 disease severity, a study from India of 154 patients admitted to hospital with COVID-19 reported that the mean 25(OH)D level was <30 ng/mL (insufficient range), and patients admitted to the intensive care unit and those that died from COVID-19 were more deficient in vitamin D than survivors.[20] Another study from Belgium (n = 186) reported similar findings of greater deficiency rates in patients with more severe disease.[21] Similarly, a study from Switzerland demonstrated that 25(OH)D concentrations were significantly lower in patients with COVID-19 than in those without the disease.[22]

Other studies have also demonstrated a correlation between vitamin D deficiency and COVID-19 infection, contrary to the study using patients from the UK Biobank. A study from Israel with 7807 subjects demonstrated that 25(OH)D concentrations were significantly lower among those who tested positive for COVID-19 than those who were COVID-19 negative.[23] A study from Wuhan, China, showed in a multivariable logistic regression that vitamin D deficiency (<30 nmol/L) was significantly associated with COVID-19 severity.[24]

It has long been clear that groups that traditionally exhibit vitamin D deficiency or insufficiency, such as older adults and nursing home residents and Black, Asian, and minority ethnic populations, are the same groups that COVID-19 has disproportionately impacted. Additionally, increased time spent indoors due to strict lockdowns and shielding triggered concerns that some people might not obtain the necessary physiological levels of vitamin D from sunlight.[25]

CONCLUSION

Our study revealed significantly worse COVID-19-related health outcomes in elderly patients compared to the younger age group. However, the difference between groups of mean levels of serum 25-hydroxyvitamin D [25(OH)D] in hospitalized patients was not statistically significant.

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Conflict of Interest:

The authors declare no conflicts of interest relevant to this study.

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Correspondence:

Nia Metonidze, MD

77a, Kostava str., University of Georgia, Tbilisi, 0177, Georgia

Email: n.metonidze@yahoo.com

DIAGNOSTIC VALUE OF 2D-SWE IN THE TREATMENT PROCESS OF DIFFUSE LIVER DISEASE

***TAMTA MOTSONELIDZE**, MD, PHD; **SOPHIO KAKHADZE**, MD, PHD;*
DUDANA GACHECHILADZE**, MD, PHD; **TEA CHANGELIA**, MD, PHD; **TEONA BUACHIDZE
P. Todua Medical Center, , Tbilisi, Georgia

SUMMARY

Background: Two-dimensional shear wave elastography (2D-SWE) is a modern diagnostic method for evaluating liver fibrosis. It is non-invasive, performed in real-time, and results are available immediately. 2D-SWE is integrated into the diagnostic apparatus of ultrasound imaging and allows us to determine the overall distribution of fibrosis in the liver during the examination. Therefore, it is possible to use this technique during the treatment process in chronic liver disease to monitor fibrosis assessment.

Objective: Revealing the diagnostic capabilities of shear wave elastography in the process of treating diffuse liver disease.

Materials and Methods: The examination included 52 patients with chronic liver disease. Before and 24 weeks after the treatment, we performed an ultrasound examination of the abdominal, 2D-SWE, conducted several laboratory analyses, and compared the obtained results.

Results: At 24 weeks after treatment, liver stiffness values detected by 2D-SWE decreased from 17.51 kPa to 15.45 kPa ($p < 0.001$). Also, spleen length ($p<0.05$), ALT ($p < 0.001$), and AST ($p<0.01$) in blood serum were decreased. There was a statistically significant increase in hemoglobin ($p<0.001$) and serum albumin ($p<0.001$) levels. Platelet count increased ($p<0.001$).

Conclusion: 2D-SWE helps monitor liver fibrosis during the treatment period.

Keywords: liver fibrosis, liver cirrhosis, ultrasound, elastography, shear-wave elastography.

INTRODUCTION

There are approximately 75 million heavy drinkers in the world, 2 billion people suffer from being overweight, and more than 400 million people suffer from diabetes. However, the global prevalence of viral hepatitis is still high, and the cases of liver damage caused by drugs and toxic

substances are still increasing. All these mentioned again and again create a prerequisite for the development of liver diseases.[1] Using antiviral drugs can cure more than 95% of patients, but there is also a risk of reinfection. This is why the hepatitis C virus (HCV) continues to be a global public health problem.[2]

The introduction of antiviral treatment in modern medicine has reduced the progression of liver fibrosis to cirrhosis and its decompensation during viral hepatitis. Patient hospitalizations and mortality decreased as hepatocellular injury and the necessity of liver transplantation decreased. This led to a significant improvement in the quality of life of the infected patient.[3]

The ultimate goal of antiviral treatment is sustained virological response (SVR). Initially, liver inflammatory processes improve, followed by liver structure changes. Then, the liver's metabolic function improves, reducing cognitive disorders and portal hypertension manifestations.[4] The mentioned processes are more effective the earlier the disease is diagnosed and treated.

Formation of cirrhosis from liver fibrosis sometimes takes decades. The process is slow if there is HCV infection or NASH. Still, this process progresses rapidly in cases of biliary obstruction, immunosuppression developed after liver transplantation, or co-infection with human immunodeficiency virus (HIV).[5]

Accurate staging of liver cirrhosis and fibrosis is crucial, as treatment recommendations sometimes differ depending on the type of chronic liver disease (CLD).[6]

To detect liver fibrosis and evaluate its degrees in modern radiology, the newly introduced two-dimensional shear wave elastography (2D-SWE) is used (2D-SWE), which assesses the liver fibrosis stage in kilopascals in real-time by quantitative stiffness estimation.[7-8]

2D-SWE, as a non-invasive, safe, and simple procedure, has dramatically reduced the number of biopsies, especially for the routine evaluation of viral hepatitis.[4]

2D-SWE – This tool has allowed clinicians to conduct timely and effective treatment, stop the progression of fibrosis, and promote its reverse development process.[9] Furthermore, 2D-SWE can be used to monitor fibrosis during treatment to evaluate the effect of therapeutic agents.

MATERIAL AND METHODS

The study included 52 patients (39 men and 13 women) with chronic liver disease, ages 18 to 77. It was conducted at P. Todua Medical Center (#13 Tevdore Mgvdeli St., Tbilisi 0112) from 2019 to 2023. Patients underwent abdominal ultrasonography, 2D-SWE to measure liver stiffness, and certain laboratory tests before and 24 weeks after treatment.

The study aimed to evaluate the diagnostic value of 2D-SWE in treating chronic liver disease and to determine clinical-biochemical data after treatment.

We carried out a retrospective study. We collected and entered the following patient data into the database: age, gender, disease etiology, liver stiffness measured in Kpa, liver size, structural changes of the liver, portal vein diameter, splenic size, splenic vein diameter, data on the presence of ascites, direct bilirubin µmol/L, ALT U/L, AST U/L, GGT U/L, INR, number of platelets 10^9/L, hemoglobin g/dL, albumin g/L.

Exclusion criteria were insufficient clinical data, acute hepatitis, hepatocellular carcinoma, encephalopathy, portal vein thrombosis, kidney, heart, lung, and blood diseases. As well as radiotherapy or chemotherapy received by extrahepatic tumors.

We reviewed and compared liver 2D SWE results and clinical-biochemical data before and after the respective treatment.

Statistical analysis was performed using SPSS 23.0 software.

Experienced specialists performed instrumental examinations in our study in accordance with the appropriate protocol.

Abdominal ultrasound examination

Abdominal ultrasound examinations were performed using the Canon Aplio i800 ultrasound system (Canon Medical Systems, Tokyo, Japan) with a 3.5 MHz convex probe with the patient in the supine position. The patient was fasting and followed the doctor's instructions to take deep breaths and hold his breath. Liver lobes, a nodular liver surface, echogenicity, parenchymal structure, portal vein diameter, splenic size, and splenic vein diameter were assessed, and the presence of ascites was determined.

Shear wave elastography

2D SWE studies were performed using a Canon Aplio i800 ultrasound system (Canon Medical Systems, Tokyo, Japan) with a 3.5 MHz convex probe. The patient was required to fast. Liver stiffness (LS) measurements using 2D-SWE were performed via a proper intercostal scan. The patient was in the supine position, with the right arm maximally extended. LS was assessed by short-breath holding and neutral breathing. Measured elasticity values were expressed in kilopascals (kPa). Stiffness was determined as the median of several successful SWE measurements...

RESULTS AND ANALYSIS

The etiology of chronic liver disease in our study was as follows: HCV–34; HBV-7; HCV/HBV–5; alcoholic-4; HCV/alcoholic-1; biliary-1;

In patients with chronic liver disease, liver stiffness increases along with the decrease in the elasticity of the liver tissue and the increase in fibrosis. In our study, the average liver stiffness was 17.51 Kpa. Changes were also noted in all clinical and laboratory data (liver size, portal vein, spleen length, splenic vein diameter, direct bilirubin, ALT, AST, GGT, INR, platelet count, hemoglobin).

Abdominal ultrasound examination revealed mild hepatomegaly and structural changes in 10 patients. There were no signs of portal hypertension, splenomegaly, or ascites. The remaining 42 patients had varying degrees of structural changes in the liver, incorrect edge contour, rounding of corners, nodules, vascular deformation, hypertrophy of the caudal lobe with a decrease in the size of the right lobe, signs of portal hypertension, and splenomegaly. Ascites were detected in 28 patients.

Patients with chronic liver disease were treated with appropriate, both symptomatic and pathogenic, as well as antiviral medicines. Abdominal ultrasound, 2D SWE, and follow-up analyses were conducted again 24 weeks after treatment. The obtained results were compared with the data before treatment, revealing that liver stiffness (LSM values) decreased. (Figure 1 a,b; Figure 2 a,b).

a ***b***

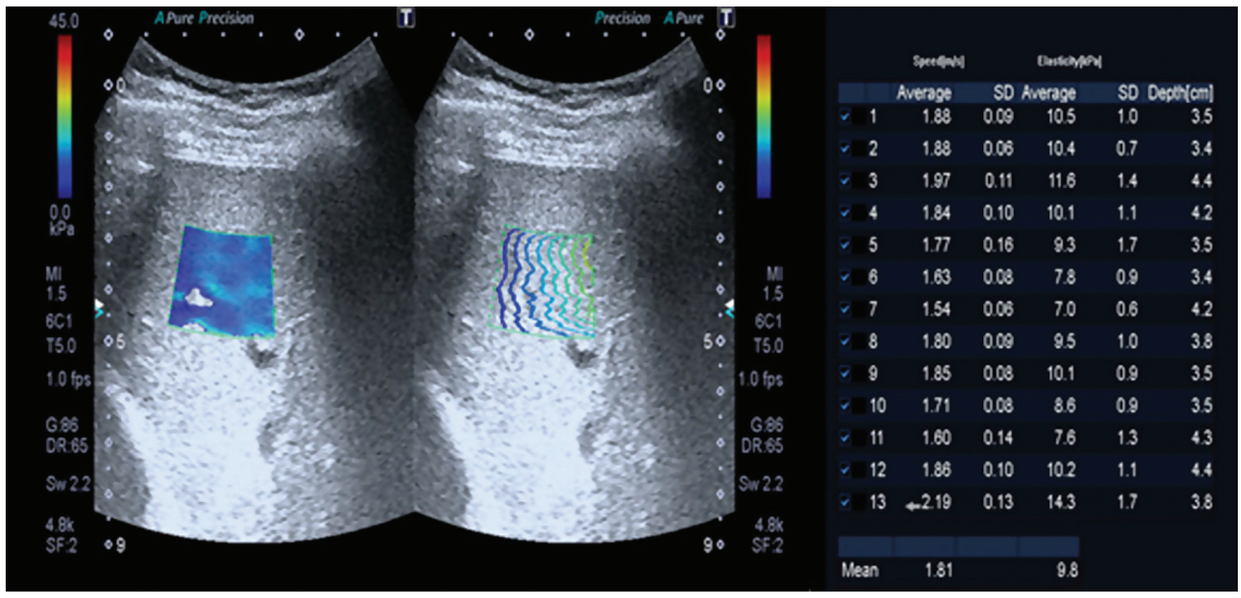

Figure 1. **Liver fibrosis of HCV etiology.**
Before starting treatment. a. Liver 2D SWE image during measurement of liver stiffness. b. In the calculation table, the liver stiffness is 9,8 Kpa.

a ***b***

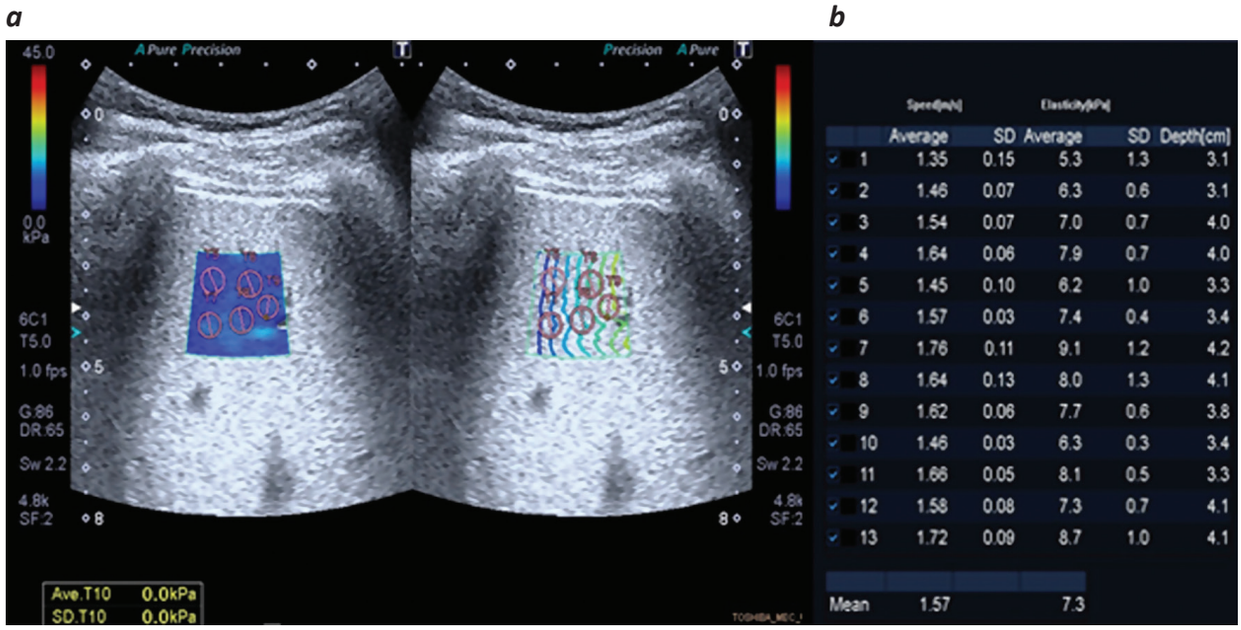

Figure 2. It shows the same patient 24 weeks after antiviral treatment. a. Liver 2D SWE image during measurement of liver stiffness. b. Calculation table: The liver stiffness is 7.3 Kpa.

During our study, at 24 weeks after treatment, mean LSM values decreased from 17.51 kPa to 15.45 kPa. LSM reduction was not associated with etiology, gender, or age. The condition of 44 patients improved after treatment, and the clinical-laboratory indicators improved; 5 patients did not have improvement, and the clinical and laboratory data were slightly changed; 3 patients had disease progression despite treatment. After the treatment, the diameter of the portal vein ($p = 0.42$) and the splenic vein ($p = 0.08$) were slightly changed. These changes were not statistically significant. The change in the spleen length was found to be statistically significant ($p < 0.05$). Significantly decreased blood serum ALT ($p < 0.001$) and AST ($p < 0.01$). There was also a

statistically significant increase in hemoglobin ($p < 0.001$) and serum albumin ($p < 0.001$) levels. Platelet count increased statistically substantial ($p < 0.001$). Statistically significant changes were not detected in the values of direct bilirubin ($p < 0.75$) and INR ($p < 0.43$) (Table N 1).

Table 1. *Bivariate analysis (T-test of independent variables)*

Feature	Mean	Mean difference	Standard deviation	P
Portal vein				
Before treatment	15.97	2.15	13.13	0.42
After treatment	13.81			
Spleen length				
Before treatment	156.71	1.26	4.24	<0.05
After treatment	155.44			
Splenic vein				
Before treatment	9.96	0.32	1.32	0.08
After treatment	9.63			
LSM				
Before treatment	17.51	2.05	2.22	<0.001
After treatment	15.45			
Direct bilirubin				
Before treatment	31.64	1.19	27.00	<0.75
After treatment	30.45			
ALT				
Before treatment	68.73	25.57	31.18	<0.001
After treatment	43.15			
AST				
Before treatment	105.31	50.14	113.25	<0.01
After treatment	55.16			
INR				
Before treatment	1.42	0.01	0.10	<0.43
After treatment	1.41			

Platelets				
Before treatment	181.79	23.59	20.90	<0.001
After treatment	205.38			
Hemoglobin				
Before treatment	11.96	0.41	0.65	<0.001
After treatment	12.38			
Albumin				
Before treatment	34.95	3.19	3.78	<0.001
After treatment	38.14			

DISCUSSION

During our study, liver fibrosis assessed by 2D SWE decreased after treatment, and mean liver stiffness decreased from 17.51 kPa to 15.45 kPa. ($p < 0.001$). These data are statistically reliable. Other studies confirm our study results.[10,11,12] Studies have shown that at the end of treatment, the liver stiffness assessed by 2D SWE decreases by several units compared to the initial stiffness. After 24 weeks, LSM rates continue to decrease, and at 36 weeks, LSM rates decrease further.11,13,14 According to the authors, the initial decrease in liver stiffness is caused by the improvement of inflammatory processes, and the subsequent decrease is related to the regression of fibrosis.11,12 During the study, it should be noted that regression of fibrosis after treatment was significantly higher in patients with LSM≥8.2 kPa than in the group of patients with LSM<8.2 kPa ($p<0.001$ and $p<0.001$).[12]

Our study shows that the reduction of liver fibrosis measured by 2D SWE after treatment was significantly associated with specific clinical-laboratory parameters.

At 24 weeks after treatment, serum ALT ($p < 0.001$) and AST ($p < 0.01$) decreased. Other authors obtained similar results.[15,13,14,12] A significant decrease in serum ALT after treatment should be associated with improving inflammatory processes.15,8 Our study found a statistically significant increase in serum albumin ($p < 0.001$) levels in a repeat analysis after 24 weeks of treatment. Other studies confirmed our results.[15,14] After 24 weeks of treatment, there was an increase in hemoglobin levels ($p < 0.001$) with statistical reliability. However, the opposite result was obtained by Suda et al., where the hemoglobin change was insignificant ($p = 0.47879$).[15] 24 weeks after treatment, our patient's platelet counts also increased statistically significantly ($p <0.001$). Regarding platelets, Kohla et al., in the study, state that, although platelet counts were not significantly different at 12 weeks of treatment, they increased significantly at 24 and 36 weeks. ($P <0.001$) (Kohla et al., 2020) According to the data of Yaraş et al., the number of platelets increased ($p < 0.05$) with statistical confidence 12 weeks after antiviral treatment.[14] These results contradict studies where platelet counts did not change significantly after antiviral treatment.[15] This can be explained by the fact that sometimes antiviral therapy worsens thrombocytopenia due to its side effects. Severe thrombocytopenia occurred in 6.1% to 41.1% of CHC patients receiving IFN-based therapy. However, after successful

IFN therapy and a certain period, the examination shows a significant increase in the number of platelets.[16] Statistically significant changes were not detected in the value of direct bilirubin ($p < 0.75$). Other authors found similar results[14,15] In our study, comparing the data before and after treatment at 24 weeks, no statistically changed data in INR values were revealed ($p<0.43$). A similar result was obtained in another study, where INR was measured in patients with chronic hepatitis B before starting antiviral therapy and at 24 and 48 weeks after treatment.[12] During the study, the portal and splenic vein diameter did not change statistically significantly after treatment. As for the length of the spleen, it decreased by an average of 12 mm. However, this change was statistically significant ($p<0.05$). Like our study, Olariu et al. revealed a positive relationship between the fibrosis degree and the spleen size one year after the end of therapy ($p < 0.001$). Moreover, the author reports that one year after treatment, patients with normal-sized spleens showed more improvement in the degree of fibrosis than patients with larger spleens.[10]

In our study, we could not correlate the regression of liver fibrosis after treatment with the patient's gender and age. However, Olariu et al., in their study, point out that better indicators of liver fibrosis regression in men are associated with the presence of such risk factors in women as childbirth, abortion, surgery, and blood transfusion. As for age, its correlation with changes in fibrosis after treatment was not observed in this study either.[10]

CONCLUSION

2D-SWE, a noninvasive and highly informative tool, can monitor liver fibrosis during treatment. Future studies on a more significant number of patients are desirable to expand the capabilities of 2D SWE.

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PROBLEMATIC ISSUES RELATED TO THE PHENOTYPIC CHARACTERISTICS, PERSISTENCE, AND PROGRESSION OF CHRONIC ENDOMETRITIS A CRITICAL REVIEW

***OLIKO MURGHULIA**, PhD Student;[1] **GEORGE TEVDORASHVILI**, MD, PhD, Prof.;[2]*
***NINO TEVZADZE**, Resident;[3] **GEORGE BURKADZE**, Prof.[4]*
[1] Tbilisi State Medical University
[2] Tbilisi State Medical University, Department of Obstetrics and Gynaecology
[3] Tbilisi State Medical University, Department of Anatomical and Clinical Pathology
[4] Tbilisi State Medical University, Head of the Department of Molecular Pathology, Tbilisi, Georgia

SUMMARY

Chronic endometritis is defined as mild persistent inflammation of the endometrium, characterized histologically by inflammatory cells in the endometrial stroma, including plasma cells, lymphocytes, eosinophils, and even lymphoid follicles. Diagnosing chronic endometritis is difficult for a variety of reasons. Most patients are asymptomatic, and ultrasound features are nonspecific. Microbiological examination is often not informative because most pathogens are non-cultivable. A hysteroscopy can diagnose chronic endometritis by detecting specific endometrial changes, such as focal or diffuse hyperemia, stromal edema, and micro polyps. Histopathological identification of plasma cells in endometrial biopsy specimens is considered the gold standard for the diagnosis of chronic endometritis. There is a hypothesis that chronic endometritis may be related to endometriosis, although studies in this direction are very scarce. There are different opinions about the persistence and progression of chronic endometritis, which require further research.

Keywords: chronic endometritis; plasma cells.

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Chronic endometritis is defined as mild persistent inflammation of the endometrium, characterized histologically by the presence of inflammatory cells in the endometrial stroma, including plasma cells, lymphocytes, eosinophils, and even lymphoid follicles.[1,2] Other histologic features include superficial edema, increased stromal density, and asynchronous differentiation of the endometrial epithelium and stroma.

The cause of the existing inflammatory process is not entirely known. Some cases of chronic endometritis show regression after antibiotic therapy, suggesting an infectious etiology.[3] The cases resistant to antibiotics might be due to another etiological agent. So far, the presence of plasma cells in the endometrial stroma is considered the most important diagnostic criterion.[4-6] A plasma cell is a differentiated form of a B lymphocyte that can produce immunoglobulins or antibodies and is, therefore, involved in humoral immunity. Chronic endometritis is often associated with infertility, endometriosis, implantation disorders, spontaneous abortion, and obstetric complications such as preeclampsia and premature birth. It is also connected to neonatal diseases in premature infants, including periventricular leukomalacia.[1,2]

Inflammatory processes damaging the endometrial cavity can affect the implantation of a fertilized egg, potentially resulting in infertility or spontaneous abortion.[3,4] Physiologically, the endometrial stroma contains various immunocompetent cells, including natural killer (NK) cells, macrophages, T cells, and neutrophils. The composition and number of these cells vary throughout the menstrual cycle.[7] While the leukocyte content does not exceed 10% of stromal cells during the proliferative and early secretory phases, their number increases dramatically starting in the mid-secretory phase. It remains elevated during the late secretory phase and early pregnancy. The cycle-dependent variation of these cell subpopulations is crucial for the implantation process. Studies reveal that the leukocyte population in the endometrium of women experiencing recurrent spontaneous abortions is notably different from that in women who achieve full-term pregnancies. Within the immune cell population present in the endometrial stroma, an increased number of NK cells and plasma cells is associated with recurrent spontaneous abortion, implantation failure, or in vitro fertilization failure. Generally, the causes, as mentioned earlier, often include anatomical defects of the uterus, parental karyotype abnormalities, and blood clotting disorders such as protein C deficiency, Factor V Leiden mutation, and antiphospholipid syndrome. Nonetheless, in 50% of cases involving miscarriage and failed in vitro fertilization, the underlying cause remains unexplained and may be related to chronic endometritis.

Infertility is a relatively common gynecological issue, with its frequency gradually increasing in recent years. Studies have revealed that the incidence of chronic endometritis among infertile patients ranges from 0.2% to 46%. Studies have also established that the frequency of chronic endometritis in cases of implantation defects following in vitro fertilization is 14%, and the overall implantation rate after endometritis treatment is 11.5%, which is notably low compared to cases where the presence of endometritis is not confirmed (32,7 %).[8]

It is important to emphasize that implantation occurs due to a complex interaction between the blastocyst and the endometrium.[9] Numerous signaling pathways are involved in this connection, and the endometrium must be suitable for successful implantation. Both embryonic and endometrial factors can easily disrupt this unstable balance. In this case, chronic endometritis is one factor that prevents successful implantation in the endometrium. According to some per-

spectives, inflammation is merely the trigger for a much more intricate and organized sequence of events. The altered secretion of cytokines and chemokines leads to changes in the leukocyte population. These modifications, in turn, affect the contractility of the uterus and the function of the endometrium in the process of decidualization and vascularization. Autophagy plays a crucial role and is essential for successful implantation.

As previously mentioned, the etiology of this pathology remains a subject of controversy. The literature presents conflicting data regarding endometrial cultures. In women with chronic endometritis, the endometrial microbiome is characterized by the presence of the following bacteria: Lactobacillus, Enterobacter, Pseudomonas, and Gardnerella, with their respective proportions being 33.21%, 7.17%, 7.32%, and 6.91%. Also noteworthy are E. faecalis, Streptococcus spp., Staphylococcus spp., Mycoplasma spp., and others.[10] Gardnerella is not detected in control samples of healthy women. Similarly, another study identified Bifidobacterium, Prevotella, and Gardnerella in infertile women with chronic endometritis, whereas these were not observed in infertile women without chronic endometritis.[11] Other research has reported significantly elevated levels of Gardnerella, Klebsiella, and Streptococcus in endometrial biopsies from infertile patients.[12]

The results of traditional methods for obtaining endometrial cultures depend on the specific laboratory performing the analysis. Additionally, the literature reveals that contamination with vaginal and endocervical contents cannot be entirely excluded, even when employing specialized devices intended to minimize contact with the vagina and cervix.

Finally, demonstrating an infectious agent in the endometrial cavity does not necessarily indicate its pathogenicity. Collecting an endometrial sample to detect infectious agents in the endometrial cavity is an invasive technique that can sometimes be difficult and painful. To avoid the necessity of obtaining endometrial specimens in suspected or confirmed cases of chronic endometritis, the compatibility of vaginal and endocervical cultures with infectious agents present in the endometrium was investigated. In the studies mentioned, it was revealed that the concordance of positive vaginal and endocervical cultures with endometrial cultures depends on the specific infectious agent. The overall concordance for endocervical canal samples is 48.3%, which varies significantly depending on the organism. For instance, there was no concordance between endocervical and endometrial cultures in cases where Staphylococcus spp. was present in the endometrium. In contrast, concordance was 100% and 58.3% for C. trachomatis and U. urealyticum cases. For vaginal samples, the overall concordance was 50.2%, ranging from 0% for Staphylococcus spp—cases to 16.7% for C. trachomatis cases and 48.8% for U. urealyticum cases. Therefore, neither vaginal nor endocervical cultures can be considered reliable predictors of endometrial microbial culture.

In patients with implantation defects and repeated spontaneous abortions, the presence of endometrial polyps is a common pathology alongside endometritis. Blood vessels play an essential role in the inflammatory process and, at the same time, are also the primary morphological component of polyps, with large-caliber blood vessels observed in the functional layer. According to some studies, signs of active endometritis have been detected in cases of polyps. In the part of the polyps, no vascular changes were detected in the presence of endometritis; only the presence of a vascular axis was noted. However, the incidence of polyps and endometritis cases was significantly higher than cases without vascular changes. This observation supports the hypothesis that

endometrial polyps may be part of a spectrum of changes contributing to chronic endometritis. Infertile women with endometrial polyps exhibit elevated levels of cytokines, particularly interferon-γ, suggesting an inflammatory etiology. According to these studies, the localized growth of the endometrium, which characterizes polyps, may be secondary to an inflammatory reaction. Furthermore, these studies have demonstrated that vascular changes significantly increase the risk of developing new vascular axes and polyps. According to this scenario, polyps are not the direct cause of infertility but are instead a consequence of vasculopathy, which is attributable to an underlying inflammatory or autoimmune etiology. This may account for the high rate of successful pregnancies observed following polypectomies.

There is a hypothesis that chronic endometritis may be related to endometriosis, although studies exploring this connection are minimal. Existing research indicates that the frequency of chronic endometritis is significantly higher in patients with endometriosis compared to those without endometriosis. Specifically, chronic endometritis was observed in 52.94% of cases with endometriosis. Chronic endometritis was noted in 40.0% of grade I endometriosis cases, 50.0% of grade II endometriosis cases, 70.0% of grade III endometriosis cases, and 46.7% of grade IV endometriosis cases. These indicators do not correlate with the stage, and deep endometriotic lesions may not be associated with chronic endometritis. According to these results, chronic endometritis may represent an independent complication of endometriosis or may be involved in its pathogenesis, given that endometritis is observed in the early stages of endometriosis.

Plasma cells in the eutopic endometrium play a role in eliminating bacteria and newly formed neoplastic cells. However, chronic endometritis may lead to tumor development. In general, endometriosis is not classified as a tumor but resembles the process of tumor metastasis. Several studies have indicated that tumors may exploit the plasticity of immune cells to their advantage. The overproduction of early inflammatory mediators (such as IL-12, TNF, and reactive oxygen species) activates the adaptive immune response to eliminate tumor cells. However, the same process can also facilitate neoplastic transformation. Chronic endometritis in the uterine cavity may contribute to transforming normal eutopic endometrium into endometriotic tissue, which can invade the pelvic cavity.

Studies have established that endometriosis and chronic endometritis have a similar immune background. For example, an unusual infiltration by B cells and endometrial stromal plasma cells (ESPCs) has been described during both processes in parallel with the local production of some proinflammatory cytokines, such as IL-6 and TNF-α. IL-6 is known to act as a differentiation factor for immature B cells. At the same time, TNF-α induces estrogen biosynthesis locally in endometrial glandular cells, which transforms endometrial cells into a proliferative phenotype and may contribute to the development of endometrial polyposis.[13] At the same time, overexpression of specific immunoglobulins (IgG1, IgG2, and possibly IgA) is frequently observed in the eutopic endometrium when endometriosis and chronic endometritis coexist. This overexpression is attributed to the increased production of immunoglobulins by endometrial stromal plasma cells.[14]

An increased inflammatory response could potentially be associated with the onset and progression of both diseases.

The role of the microbiome in tumor progression has been most thoroughly studied in colorectal carcinomas, where dysbiosis results in a reduction of regulatory commensal species and an

ensuing inflammatory process.[15] Endometrial cancer is similarly associated with proinflammatory conditions. Several studies have investigated the microbial environment of the uterus and its role in tumor development. Considering the inflammatory profile of endometrial tumors, it is suggested that a microbial component is also involved in malignant processes.[16]

Chronic endometritis is challenging to diagnose for a variety of reasons.[10, 13] Most patients are asymptomatic, and ultrasound features are nonspecific. Microbiological examination is often uninformative because most pathogens are not culturable. Additionally, during the collection of endometrial samples, it is impossible to prevent contamination of the material with cervical and vaginal flora. Chronic endometritis can be diagnosed through hysteroscopy by identifying specific endometrial changes, such as focal or diffuse hyperemia, stromal edema, and micropolyps.[10] However, the accuracy of this diagnostic method is contingent upon the operator's experience.[17]

Histopathologically identifying plasma cells in endometrial biopsy specimens is considered the gold standard for diagnosing chronic endometritis.[18] However, identifying plasma cells by conventional tissue staining alone is challenging. There is an apparent lack of standardized methods for histological evaluation of plasma cell infiltrates, although several options have been proposed in the literature.[1,2] According to some authors, the presence of one or several plasma cells in endometrial biopsies is sufficient to confirm the diagnosis of chronic endometritis. In contrast, others believe a specific number of plasma cells is required for this diagnosis. Additionally, plasmacytes are typically large cells with a high nuclear-to-cytoplasmic ratio, basophilic cytoplasm, and a "clock-face" pattern of heterochromatin in the nucleus.[19] These morphologic features of plasma cells are not always evident upon microscopic examination, as plasma cells often resemble endometrial stromal fibroblasts and mononuclear leukocytes.[17] Immunohistochemical study using the plasmacytic marker CD-138 (also known as syndecan-1, a transmembrane type heparan sulfate proteoglycan) is currently the most reliable and time-efficient diagnostic method. CD-138 immunostaining is more sensitive and specific than routine hematoxylin and eosin staining (sensitivity: 100% vs. 75%; specificity: 100% vs. 65%) and is characterized by less interobserver variability (93% vs. 47%).

Despite these advantages, this research method should be used cautiously, as CD-138 is also expressed on the plasma membrane of endometrial epithelial cells, which may lead to false-positive results.[19] Consequently, searching for new methods to detect plasma cells remains justified. Multiple myeloma antigen 1 (MUM-1) is a protein typically expressed in plasma cells and activated B and T cells. MUM-1 is essential at certain stages of B-cell development, including differentiating mature B cells into antigen-producing plasma cells. Considering the need for additional staining techniques, it is possible to test the utility of MUM-1 immunohistochemistry for identifying endometrial plasma cells.

Chronic endometritis can also disrupt the hormonal profile of the endometrium. Changes in the number and ratio of estrogen and progesterone receptors, along with other endometrial pathologies, can contribute to infertility, amenorrhea, and menstrual disorders. In one study, the expression of estrogen and progesterone receptors in endometrial glands and stromal cells was significantly higher in chronic endometritis cases than in the control group. Failure to decrease the expression of hormone receptors indicates a defect in endometrial maturation and the subsequent inability to support blastocyst implantation. Consequently, inflammation, especially diffuse inflammation, inhibits the expression of estrogen receptors in the endometrial glands and

stromal cells. Since the number of progesterone receptors depends on the expression of estrogen receptors, this disruption impairs the optimal conditions for the implantation of a fertilized egg.

The mean expression index of Ki-67 in endometrial glandular and stromal cells is significantly higher in chronic endometritis compared to the control group, similar to the pattern observed with hormone receptors. Therefore, the expression of Ki-67 correlates with the index of hormone receptor expression, i.e., with delayed maturation of the endometrium.[20]

As mentioned earlier, the persistence and progression of chronic endometritis are associated with infertility, endometriosis, and atypical hyperplasia of the endometrium. However, opinions on these issues differ, highlighting the need for further research.

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ADVANCES IN STEM CELL THERAPY FOR REPRODUCTIVE MEDICINE: APPLICATIONS, TECHNIQUES, AND POTENTIAL OUTCOMES

***NINO MUSERIDZE**, MD, PhD; **ANNA CHOKHONELIDZE**, MD*
Georgian-German Reproduction Center, Tbilisi, Georgia

SUMMARY

Stem cell therapy, a revolutionary approach in reproductive medicine, is making significant strides, offering new avenues for addressing infertility and related reproductive challenges. This article delves into the impact of stem cells, particularly mesenchymal stem cells (MSCs), and their pivotal role in regenerative medicine. We explore various methods for obtaining stromal cells from adipose tissue, including direct approaches such as adinizing and enzymatic techniques and indirect methods like emulsification and microfragmentation/micronization. Direct methods, such as adinizing, preserve the fat tissue for graft use, while enzymatic methods discard the fat due to potential contamination. Indirect methods aim to increase the relative proportion of stromal cells by removing parenchymal cells. Additionally, we discuss the application of MSCs in treating conditions such as premature ovarian failure and Asherman's syndrome, highlighting their role in tissue repair and fertility restoration. Our findings underscore the transformative potential of stem cell therapy in reproductive health, offering promising solutions for individuals facing infertility and other reproductive challenges. The continued research and refinement of these techniques, along with the exploration of new methods and technologies, hold promise for advancing reproductive medicine, providing a wealth of knowledge for professionals and individuals interested in this field.
Keywords: stem cells, mesenchymal stem cells, adipose tissue, regenerative medicine, infertility, PRP, reproductive health

Stem cell therapy is an emerging and transformative field in reproductive medicine. With its potential to regenerate and repair tissues, it offers a new paradigm in fertility treatments, providing hope for individuals and couples struggling with infertility.

The study explores the roles of parenchymal and stromal cells, adipose-derived stem cells (ADSCs), and Platelet-Rich Plasma (PRP) in regenerative medicine. Methods for obtaining stro-

mal cells from adipose tissue are classified as direct (adinizing, enzymatic techniques) and indirect (emulsification, microfragmentation). PRP separation and activation processes are also detailed.

Parenchymal Cells:

Definition: Parenchymal cells are the functional cells of an organ or tissue. They carry out the organ's specific functions.

These cells are the workhorses of the organ responsible for its primary physiological functions. For instance, in the liver, parenchymal cells, known as hepatocytes, perform tasks like detoxification and protein synthesis.

Stromal Cells:

Definition: Stromal cells are the supporting connective tissue cells that provide structure and support for the parenchymal cells. They constitute the framework or matrix of an organ or tissue.
Function: Stromal cells are crucial in maintaining the structural integrity of tissues and organs. They also participate in various physiological processes, such as immune responses, tissue repair, and communication with parenchymal cells.

Adipose cells and adipocytes are terms used to describe the cells that make up adipose tissue, a type of connective tissue that specializes in storing energy in the form of fat. Adipose cells refer to the cells that make up adipose tissue. These cells are specialized for the storage of fat.
Function: The primary function of adipose cells is to store energy as triglycerides (fat). They also play a role in insulation, cushioning organs, and serving as an energy reserve.

Adipocytes are the specific cells within adipose tissue responsible for storing and releasing fat.
Structure: Adipocytes have a unique structure characterized by a large, centrally located lipid droplet (vacuole) that occupies most of the cell volume. The nucleus and other organelles are pushed to the periphery of the cell. The primary function of adipocytes is to store excess energy in the form of triglycerides when the body has more power than it needs. These stored fats can be released when the body requires additional energy.

Adipose tissue is essential for normal physiological function and plays a critical role in energy balance, insulation, and protection of organs. However, excess adipose tissue, especially visceral fat (fat around internal organs), is associated with various health risks, including cardiovascular diseases and metabolic disorders.

White adipose tissue (WAT) is the body's most common type of adipose tissue and is crucial in energy storage. It is mainly composed of adipocytes (fat cells) that store triglycerides. Two major types of white adipose tissue are subcutaneous and visceral.

Subcutaneous Adipose Tissue:

Subcutaneous adipose tissue is found beneath the skin (subcutaneous layer).

It is an energy reserve that provides insulation to regulate body temperature and a cushion that protects organs and tissues from physical trauma.

Subcutaneous fat is the fat that you can pinch between your fingers. It is distributed throughout the body but is commonly found in areas like the thighs, buttocks, and abdomen.

Visceral Adipose Tissue:

Visceral adipose tissue is located around internal organs in the abdominal cavity, such as the liver, pancreas, and intestines. While it also serves as an energy store, visceral fat is metabolically active

and associated with increased health risks. It produces hormones and cytokines that can affect metabolic processes, and excess visceral fat is linked to conditions like insulin resistance, cardiovascular diseases, and metabolic syndrome.

Adipose tissue has gained attention in regenerative medicine due to its potential as a source of regenerative cells and therapeutic applications. The process involving adipose tissue for regeneration is often called "liporegeneration." Here are some critical aspects of adipose tissue in the context of regeneration:

Adipose tissue is rich in a type of stem cell called adipose-derived stem cells (ADSCs). These stem cells can differentiate into various cell types, including adipocytes, myocytes, chondrocytes, and osteocytes.

The abundance of ADSCs in adipose tissue makes it a valuable source for regenerative purposes.

Adipose-derived stem cells, rich in regenerative properties, are believed to contribute significantly to tissue repair and regeneration. Their potential applications in various regenerative medicine fields, including wound healing, tissue engineering, and treatment of degenerative diseases, provide a wealth of knowledge for professionals and individuals interested in this field.

Receiving stem cells from Adipose Tissue

However, there is no accepted classification in terms of methods. In this presentation, a new classification is proposed for the first time. Accordingly, stromal cells can be obtained from adipose tissue by two approaches: direct methods for the bonds between parenchymal and stromal cells and indirect methods, which target parenchymal cells rather than strong bonds and increase the stromal cell ratio relatively. These methods can also be subclassified as fat (+), fat (–), and fat (±) in terms of using the remaining fat in the final product as a graft. Direct methods include adinizing and enzymatic techniques; indirect methods include emulsification and micro-fragmentation/ micronization techniques. In the enzymatic method, the fat tissue in the final product is considered dirty because it contains enzymes and must be discarded. That is why it is a fat (–) method. The adinizing method using ultra-sharp blades is fat (+) because the adipose tissue can be used after the procedure. Because the fat tissue is exposed to blunt pressure in emulsification techniques, it cannot be used as a graft. Thus, these are fat (–) methods. There may still be intact adipocytes in micronization techniques using filter systems; therefore, it should be classified as fat (±). Adinizing provides the highest efficiency and the full use of the end product. This detailed classification will guide clinicians in choosing the right product, making them feel more informed and knowledgeable.

DIRECT METHODS

Direct methods separate the stromal cells without killing the directly affected parenchymal cells by the bonds and bridges between parenchymal and stromal cells.

Fat (+) methods: Adinizing is a method where adipocytes and stromal cells are separated with ultra-sharp blades. The fat tissue can be used after the procedure, and the ECM is preserved. Copcu and Oztan named the final product obtained using TOST (total stromal cell).

Fat (–) methods: These involve obtaining stromal cells via enzymatic methods. The final product is called SVF (stromal vascular fraction); the fat obtained during this process is not used. It is

considered waste because of the potential risk of enzymes being in it. The loss is about 90% and almost ten times as many cells can be obtained by mechanical methods.

INDIRECT METHODS

These methods aim to remove the parenchymal cells entirely or partially and ensure that relatively more stromal cells remain in the final product.

Emulsification: Adipocytes, which are extremely sensitive to trauma, are emulsified by passing fat tissue between two syringes with the help of a nanofat connector stromal cells, which are more resistant to trauma, remain in the environment.

Microfragmentation/micronization: The aim is to fragment and eliminate the parenchymal cells, decrease their efficiency, and increase the relative stromal cell rate. For this purpose, filter, bead, centrifuge, membrane systems, and blunt pressure systems are used. There is little or no amount of adipocyte spring in the final product. Although these can be fat (±) depending on the extent of the pressure applied in the procedure, there will be some adipocyte death; so, most adipocytes will never survive, as in adinizing and Supplemental Digital Contents.

Ultra-sharp blades not only allow adipocytes to reach the desired diameter but also reveal their regenerative properties.

Enzymatic methods, such as collagenase or trypsin, aim to release stromal cells by dissolving the bonds between parenchymal and stromal cells.

Enzymatic methods are much more expensive, time-consuming, and complicated, requiring more equipment and staff than mechanical methods.

Enzymatic methods are considered dirty; they must be discarded after obtaining the stromal cell.

In mechanical methods, the aim is to cut the ligaments directly with ultra-sharp blades, defined as “adinizing” by Copcu et al. Mechanical methods are classified as “emulsification” and “micronization/microfragmentation.”

Copcu and Oztan have published a rather detailed review of mechanical methods. This study showed the evolution of mechanical processes and presented them in four steps.

These are:

1. Nanofat, defined by Tonnard,
2. “beads” system defined by Tremolada,
3. a “connector and filter system” defined by Cohen
4, and, finally, ultra-sharp blade systems defined by Copcu and Öztan, whose extensive literature review showed that the highest efficiency in terms of cell number and viability is in the exact blade systems.

They called cutting fat tissues with ultra-sharp blade systems “adinizing.” This method is not microfragmentation because it directly targets the stromal and parenchymal intercellular bonds, and while obtaining stromal cells, the parenchymal cells are not damaged.

Sources of Regenerative Cells: Regenerative cells refer to cells that have the potential to repair or replace damaged tissues in the body. These can include various types of stem cells, which can differentiate into different cell types.

Blood PPP and PRP: PPP stands for Platelet-Poor Plasma, and PRP stands for Platelet-Rich Plasma. Both are components of blood.

PRP is derived from a patient's own blood and contains a higher concentration of platelets than normal blood. Platelets contain growth factors that can potentially stimulate tissue repair and regeneration. PRP is sometimes used in regenerative medicine, particularly orthopedics and sports medicine, to promote healing in injured tissues.

Adipose Tissue: Adipose tissue is fat tissue. It is a rich source of mesenchymal stem cells (MSCs). MSCs have the potential to differentiate into various cell types, and they are being explored in regenerative medicine for their ability to promote tissue repair.TOST (total stromal-cell).

PRP + Mesenchymal stem cells + PPP is called Supercharged MEST

Stem Cells Hierarchy: Totipotent cells are the only cells that can form all the cell types in a body. They have the extraordinary ability to give rise to all cell types in an organism, including both embryonic and extraembryonic tissues. The term "totipotent" is derived from the Latin words "toti" meaning all and "potens" meaning potential.

During the early stages of embryonic development, totipotent cells emerge from the fusion of sperm and egg to form a zygote. The zygote undergoes successive cell divisions, and each resulting cell remains totipotent, meaning it has the potential to develop into a complete organism.

Pluripotent cells are a pivotal stage in cellular differentiation, possessing the ability to develop into many, but not all, cell types in the body. Unlike totipotent cells, which can give rise to both embryonic and extraembryonic tissues, pluripotent cells are primarily capable of forming the three germ layers: ectoderm, mesoderm, and endoderm.

Embryonic stem cells (ESCs) are a classic example of pluripotent cells. They are derived from the inner cell mass of the blastocyst during early embryonic development. Pluripotent cells have the remarkable potential to differentiate into various cell types, including neurons, muscle cells, and blood cells, among others.

Multipotent mesenchymal cells are a fascinating category of cells with a notable capacity for differentiation. These cells, often called mesenchymal stem cells (MSCs), exhibit the ability to differentiate into multiple, but more limited, cell types within a specific lineage.

Derived from various tissues such as bone marrow, adipose tissue, and umbilical cord, multipotent mesenchymal cells can give rise to cells like osteoblasts (bone cells), chondrocytes (cartilage cells), and adipocytes (fat cells). This differentiation potential makes them particularly valuable for regenerative medicine applications.

26 – Mesenchymal stem cells (MSCs) hold promise in regenerative medicine, particularly in addressing premature ovarian failure (POF). POF, characterized by the loss of ovarian function before the age of 40, poses significant challenges for affected individuals.

In the context of POF, MSCs can play a crucial role in regenerating ovarian tissues and restoring normal ovarian function. Studies suggest that MSCs, when administered either systemically or directly into the ovaries, may contribute to follicular development, enhance vascularization, and modulate the inflammatory microenvironment associated with POF.

The immunomodulatory and regenerative properties of MSCs make them potential candidates for promoting the repair of damaged ovarian tissue. Additionally, MSCs may stimulate the activation of dormant follicles, leading to the release of mature eggs. This offers hope for fertility restoration in women facing premature ovarian failure.

While research in this field is ongoing, the therapeutic potential of MSCs in addressing premature ovarian failure represents a promising avenue for the development of innovative and effective treatments. As we continue to delve into the intricacies of regenerative medicine, MSCs stand out as a beacon of hope for individuals grappling with the challenges of premature ovarian failure.

27- Mesenchymal stem cells (MSCs) present a compelling avenue for addressing the challenges posed by Asherman's syndrome. This condition, characterized by the formation of intrauterine adhesions or scar tissue, can lead to menstrual abnormalities, infertility, and recurrent pregnancy loss.

The regenerative properties of MSCs make them a potential therapeutic option for restoring the damaged uterine lining in Asherman's syndrome. By promoting tissue repair and modulating the inflammatory response, MSCs may contribute to the regeneration of healthy endometrial tissue, reducing adhesions and restoring the normal function of the uterus.

Research and clinical studies exploring the use of MSCs in Asherman's syndrome have shown promising results. Whether administered locally or systemically, MSCs have demonstrated their ability to differentiate into various cell types, including endometrial cells, fostering tissue regeneration and promoting a more conducive environment for pregnancy.

As we delve deeper into the potential applications of regenerative medicine, MSCs stand out as a promising tool in the quest to address the challenges of Asherman's syndrome. They offer hope for improved reproductive outcomes and quality of life for affected individuals.

31 - Mesenchymal stem cells (MSCs) exert their therapeutic effects through various mechanisms, making them a versatile tool in regenerative medicine. Here are critical aspects of their mechanisms of action:

1. Differentiation: MSCs can differentiate into various cell types, including bone, cartilage, adipose tissue, and more. This ability allows them to replace damaged cells and contribute to tissue repair.

2. Immunomodulation: MSCs possess immunomodulatory properties. They influence the immune system by suppressing inflammation and regulating immune responses, making them valuable in treating conditions where excessive inflammation plays a role.

3. Tissue Homing: MSCs have the capacity to migrate to sites of injury or inflammation in the body. This homing ability enhances their effectiveness in targeting and repairing specific tissues.

4. Paracrine Effects: MSCs secrete various bioactive molecules, such as growth factors, cytokines, and chemokines. These paracrine signals can stimulate tissue regeneration, reduce inflammation, and promote a healing environment.

5. Angiogenesis: MSCs can induce the formation of new blood vessels (angiogenesis), facilitating improved blood supply to damaged tissues. This is crucial for tissue repair and regeneration.

6. Anti-Apoptotic Effects: MSCs have anti-apoptotic (anti-cell death) properties, which can protect existing cells from undergoing programmed cell death in a damaged or inflamed environment.

7. Exosome Release: MSCs release extracellular vesicles, including exosomes containing proteins, nucleic acids, and other bioactive molecules. These exosomes contribute to MSCs' therapeutic effects, influencing neighboring cells and promoting regeneration.

40 - COMPONENTS OF PLATELET-RICH PLASMA (PRP)

Platelet-rich plasma (PRP) is a novel biomaterial that has garnered attention across diverse fields, including sports medicine and dermatology. This substance primarily comprises three key components, each playing a significant role in its therapeutic efficacy.

1. Platelets: Platelets are tiny, disc-shaped cell fragments that play a pivotal role in blood clotting and wound healing. In PRP, platelets are concentrated to levels higher than those found in normal blood. These platelets are rich in growth factors, which stimulate cell proliferation, tissue repair, and regeneration.

2. Plasma: Plasma is the liquid component of blood that carries blood cells and platelets throughout the body. In the context of PRP, plasma serves as a vehicle for delivering concentrated platelets to targeted tissues. It contains proteins, nutrients, hormones, and electrolytes that support cellular functions and healing.

3. White Blood Cells: While some PRP preparations focus solely on platelets, others include white blood cells. White blood cells are integral to the immune system's response and contribute to the body's defense against infections. In PRP, they may enhance the immune-modulating properties of the treatment.

These three components work synergistically in PRP to promote tissue repair, reduce inflammation, and accelerate healing. Whether used in orthopedics for joint injuries, dermatology for skin rejuvenation, or other medical fields, PRP is a testament to the potential of harnessing the body's own healing mechanisms for therapeutic purposes.

As we delve deeper into the applications of PRP, we witness a paradigm shift in how we approach healing and regeneration. This innovative approach has the potential to reshape the landscape of medical treatments, offering patients new avenues for recovery and well-being.

41 - PRP Separation: The journey begins with a small sample of the patient's own blood, usually extracted from the arm. This blood undergoes a meticulous separation process, often employing a centrifuge. The centrifuge spins the blood at high speeds, causing its components to separate based on their densities. Through this process, the platelet-rich portion is isolated, creating PRP.

Platelet Activation: However, the true magic lies in activating these concentrated platelets. Once separated, the PRP contains inactive platelets ready to unleash their healing power. Activation is often achieved by introducing substances like calcium chloride and thrombin or physical means like laser light exposure. This activation prompts the release of growth factors and other bioactive molecules from the platelets.

Growth Factors Unleashed: Activated platelets release a cascade of growth factors, including platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), insulin-like growth factor (IGF), and many others. These growth factors play a pivotal role in tissue repair, angiogenesis (formation of new blood vessels), and modulation of the inflammatory response.

In essence, PRP separation and platelet activation transform a small blood sample into a potent elixir rich in growth factors, ready to be strategically applied in various medical fields. From orthopedics for tissue regeneration to dermatology for skin rejuvenation, the applications of activated PRP are diverse, offering a glimpse into the future of personalized and regenerative medicine.

As we explore the realm of PRP, we witness not just a separation of components but a convergence of science and healing, unlocking the body's innate capacity to regenerate and repair.

Transvaginal PRP Intraovarian Injection: In this approach, a minimally invasive procedure is employed, where a thin needle is introduced through the vaginal wall to access the ovaries. The targeted delivery of PRP directly into the ovaries aims to stimulate follicular development, enhance vascularization, and create a more conducive environment for healthy egg maturation.

Laparoscopic PRP Intraovarian Injection: For those cases requiring a more detailed intervention, laparoscopic injection of PRP offers a surgical option. A laparoscope, a thin tube with a camera, is inserted through small incisions in the abdomen to precisely guide the injection of PRP into the ovaries. This method allows for a more comprehensive assessment of the reproductive organs while delivering the regenerative benefits of PRP.

Mechanism of Action: The regenerative properties of PRP, with its rich source of growth factors, may contribute to repairing damaged ovarian tissue, promoting follicular development, and improving overall ovarian function. This approach holds potential for women facing challenges such as diminished ovarian reserve or premature ovarian failure.

While this field is still evolving and further research is underway, the prospect of PRP intraovarian injection signifies a paradigm shift in fertility treatments. The personalized and regenerative nature of this approach aligns with the growing trend of tailored interventions in reproductive medicine, offering hope to those navigating the intricate journey of fertility challenges.

As we delve into this frontier, let us anticipate the continued progress and the transformative impact that PRP intraovarian injection might bring to the lives of individuals and couples aspiring to build their families.

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INNOVATIONS IN OOCYTE PRESERVATION TECHNIQUES

***NIA NIZHARADZE**, BSc*
Agricultural University, Tbilisi, Georgia
Georgian-German Reproduction Center, Tbilisi, Georgia

SUMMARY

In contemporary society, many women are prioritizing their careers and personal growth, often leading to the postponement of parenthood. This delay, however, presents challenges due to the biological limitations imposed by age on fertility. Oocyte cryopreservation has emerged as a vital technique for women to safeguard their reproductive potential. This paper reviews recent innovations in oocyte preservation, focusing on advancements in cryopreservation techniques, including vitrification and closed system vitrification, novel cryoprotectants, and the integration of emerging technologies such as automation and nanotechnology. Additionally, it explores the development of ovarian tissue cryopreservation and its applications in preserving fertility for women undergoing medical treatments or facing premature ovarian failure. The review also highlights cutting-edge techniques like artificial ovaries and in vitro activation (IVA), which promise to revolutionize fertility preservation. By examining these advancements, the paper aims to provide insights into how modern innovations address delayed parenthood challenges and enhance fertility preservation options.

Keywords: Oocyte Cryopreservation, vitrification, cryoprotectants, ovarian tissue cryopreservation, in vitro activation (IVA), artificial ovaries, microtechnology.

INTRODUCTION

In the contemporary landscape, many women are redefining traditional life paths by prioritizing education, career advancement, and personal growth. This shift towards self-fulfillment and career ambition often leads to the postponement of major life decisions, such as starting a family. While this approach allows women to achieve significant personal and professional milestones, it

also presents challenges related to fertility, as the biological clock continues to tick regardless of individual aspirations. As women delay parenthood, the risk of age-related infertility increases, underscoring the critical need for effective fertility preservation methods.

Oocyte cryopreservation has emerged as a pivotal solution, empowering women to preserve their reproductive potential. This technique involves the ultra-low temperature preservation and storage of oocytes for future reproductive attempts, offering a way to circumvent the natural decline in fertility associated with aging. Over the past few decades, significant advancements have been made in the field of oocyte preservation, resulting in improved techniques and greater success rates.

Key innovations in oocyte cryopreservation, such as the development of vitrification, a rapid cooling method that has substantially enhanced survival rates compared to traditional slow-freezing techniques, have significantly advanced the field. The refinement of cryoprotectants, including introducing novel and natural alternatives, has also contributed to better preservation outcomes. Moreover, integrating advanced technologies such as automation and nanotechnology has revolutionized the preservation process, increasing precision and efficiency. These advancements provide a promising outlook for the future of fertility preservation.

This paper explores these recent innovations and their impact on fertility preservation. It delves into the historical development of oocyte cryopreservation, examines current techniques and their efficacy, and considers future directions in the field. By highlighting these advancements, the paper aims to provide a comprehensive overview of how modern science addresses the challenges of delayed parenthood and enhancing reproductive options for women.

Vitrification

The history of oocyte cryopreservation, or the freezing of eggs for future use, has evolved significantly since its inception. The Development of oocyte cryopreservation was a hot topic in the 1980s and 1990s.

The first attempts at human oocyte freezing started in 1886 when researchers began experimenting with freezing human oocytes. Still, initial attempts using slow-freezing methods resulted in low survival and fertilization rates. The slow-freezing method involves gradually lowering the temperature of the oocytes at a controlled rate, typically around 0.3 to 2 degrees Celsius per minute.[2] “Pregnancy after human oocyte cryopreservation.)

Let’s go through some key innovations in oocyte preservation that are actively still used and developed in the modern world:

By 1997, cryo-biologists introduced vitrification in IVF, known as the extra-rapid cooling process. This flash-cooling method prevents the formation of ice crystals, which can damage the oocyte’s structure. This technique has dramatically improved the survival rates of thawed oocytes compared to traditional slow-freezing methods, with better fertilization outcomes and higher pregnancy rates.

A few years later, in 2004, the first live birth from vitrified oocytes was reported, showing the potential of this method.[4] After that, vitrification began to be widely adopted in fertility clinics by bringing higher survival rates and better developmental potential of oocytes. In the 2010s,

oocyte vitrification was recognized practice by important societies, like ASRM (American Society for Reproductive Medicine).[17]

The following invention was closed-system **vitrification**, a cryopreservation method in which oocytes are vitrified in a completely enclosed system, preventing direct exposure to liquid nitrogen. This method enhances the safety and sterility of oocyte preservation, avoiding potential contamination risks. Closed-system vitrification represents a significant innovation in the field of cryopreservation, addressing some of the critical concerns associated with traditional open vitrification methods. This advanced technique ensures greater safety and sterility.

Cryoprotectants

Novel Cryoprotectants have been a big push to success, as they are produced with Reduced Toxicity, minimizing cellular damage caused by osmotic and oxidative stress.[15] "Freezing of living cells: mechanisms and implications.). New formulations of cryoprotectants aim to reduce stress factors on oocytes during the freezing and thawing processes. These innovations help maintain the integrity and viability of the oocytes. Natural cryoprotectants have been developing, which contain biocompatible solutions; some of the most promising natural cryoprotectants include Trehalose, a disaccharide sugar found in many plants, fungi, and invertebrates.[5] **Proline is** an amino acid naturally present in many organisms, including plants and bacteria.[21] **Antifreeze proteins (AFPs)** found in various cold-adapted organisms such as fish, insects, and plants can be used alone or in combination with other cryoprotectants to enhance cell viability. They don't penetrate the membrane, avoiding its structure modification, unlike permeable cryoprotectants, which include dimethyl sulfoxide (DMSO), glycerol, and ethylene glycol. While effective, these compounds can be toxic to cells at high concentrations and require careful handling and optimization.[7]

Combining Natural Cryoprotectants with Traditional Methods is a promising innovation; natural cryoprotectants are often used in combination with traditional cryoprotectants to balance efficacy and toxicity. This synergy can optimize cryopreservation outcomes, offering enhanced protection with reduced adverse effects. For instance, combination strategies include Trehalose and Glycerol. Trehalose can be combined with glycerol to provide effective cryoprotection while reducing glycerol's required concentration, thus lowering toxicity.

Proline and Ethylene Glycol: Proline can be added to ethylene glycol-based solutions to enhance osmotic stability and protect against oxidative stress.

AFPs and DMSO: Incorporating AFPs into DMSO-based protocols can minimize ice formation, lower DMSO concentrations, and reduce toxicity.

Future research on natural cryoprotectants aims to optimize concentrations, deepen understanding of their molecular mechanisms in organisms and cells, and make them clinically approved for use.

Ovarian Tissue Cryopreservation

Ovarian tissue cryopreservation (OTC) is an advanced technique used to preserve fertility in women who may lose ovarian function due to medical treatments, such as chemotherapy or radiation,

or due to premature ovarian insufficiency.[6] The procedure involves the removal, freezing, and storage of ovarian tissue, which can later be thawed and reimplanted to restore fertility and hormonal function. OTC is particularly valuable for Cancer Patients: Women undergoing treatments that may impair ovarian function.

Autoimmune Diseases: Conditions requiring treatments that could harm the ovaries.

Genetic Conditions: Women with genetic disorders that predispose them to premature ovarian failure. Prepubescent Girls: The only fertility preservation option for young girls who cannot undergo other methods like egg freezing.

OTC has become a critical technique in fertility preservation. Recent advancements have focused on improving its efficiency, safety, and success rates.

Slow Freezing and Vitrification

Slow Freezing is a traditional method involving the gradual cooling of ovarian tissue. Post-thaw survival rates are generally good, but follicular viability varies. Studies report survival rates of 60-70% for follicles and tissues.

Vitrification is an ultra-rapid cooling technique that prevents ice crystal formation. Compared to slow freezing, vitrification shows higher follicular survival rates, often exceeding 80% in some studies. This technique is gaining popularity due to its improved efficiency and reduced ice crystal damage.

The processes still require precise control of the freezing rate, as any deviation can lead to suboptimal results. Additionally, the use of permeable cryoprotectants must be carefully managed to avoid toxicity.

Artificial Ovaries

Artificial Ovaries create a bioengineered scaffold to host ovarian tissue or isolated follicles. These scaffolds are implanted back into the patient to restore ovarian function.

Artificial ovaries represent a cutting-edge approach to fertility preservation and ovarian tissue cryopreservation (OTC). According to Oktay (2010), this innovative technique involves creating a bioengineered scaffold or structure that can support the growth and development of ovarian follicles. The goal is to provide a safe environment for immature follicles to mature and eventually produce viable oocytes without reimplanting ovarian tissue directly into the patient's body, thereby reducing the risk of reintroducing malignant cells in cancer patients.

Advantages of Artificial Ovaries:

Safety in Cancer Patients: Artificial ovaries minimize the risk of reintroducing malignant cells, which can be a concern when transplanting cryopreserved ovarian tissue. By isolating and maturing follicles in a controlled environment, the technique reduces the potential for cancer recurrence.

Preservation of Ovarian Function: Artificial ovaries aim to restore fertility and the endocrine functions of the ovaries, which are essential for overall health and delaying menopause.

Customization: The technique allows for the customization of the microenvironment, potentially optimizing conditions for follicle development based on individual patient needs.

Challenges and Current Limitations

Technical Complexity: Creating artificial ovaries is technically demanding, requiring sophisticated techniques to isolate follicles, construct scaffolds, and ensure proper follicular development and biosafety.

Limited Clinical Application: Although promising, artificial ovaries are still mainly experimental. Clinical trials are ongoing, but widespread clinical application has not yet been achieved.

Vascularization: Ensuring adequate blood supply to the follicles remains a significant challenge. Without proper vascularization, follicles may not receive the necessary nutrients and oxygen needed for development.

Regulatory and Ethical Considerations: As with any new medical technology, the development and use of artificial ovaries raise ethical and regulatory issues that must be carefully considered. This includes informed consent, long-term effects, and potential off-target effects.[9]

The development of artificial ovaries holds the potential to provide a safer alternative to traditional ovarian tissue transplantation, with the added benefit of reducing the risk of cancer recurrence. Continued research and clinical trials are essential to bringing this technology into clinical practice and offering new hope to infertility patients.

Future Directions

Improved Scaffold Design: Research is focusing on developing more sophisticated scaffolds that better mimic the natural ovarian environment. This includes incorporating materials that promote vascularization and support long-term follicular development.[20]

Clinical Trials: Ongoing clinical trials aim to assess the safety and efficacy of artificial ovaries in humans. These trials are crucial for moving the technology from the lab to the clinic.

Integration with Other Technologies: Combining artificial ovaries with other fertility preservation techniques could enhance outcomes and provide more options for patients.

Ethical Frameworks: As technology advances, developing ethical frameworks and guidelines for the use of artificial ovaries will be essential. This includes addressing issues of consent, long-term outcomes, and accessibility.

In Vitro Activation (IVA)

In Vitro Activation (IVA) is an advanced technique in the field of fertility preservation, particularly in conjunction with Ovarian Tissue Cryopreservation (OTC).[10] IVA involves activating dormant primordial follicles within ovarian tissue in a laboratory setting, promoting their growth and development into mature oocytes that can be fertilized. This technique offers hope for women with conditions such as primary ovarian insufficiency (POI) or those who have undergone treatments that severely affect ovarian function, like chemotherapy.

Method involves activating dormant follicles in vitro before transplantation. This technique includes treating ovarian tissue with specific growth factors and drugs. When the patient is ready

for IVA, the ovarian tissue is thawed and carefully fragmented. This fragmentation disrupts the Hippo signaling pathway, a critical regulator of follicular dormancy, thereby encouraging the activation of dormant follicles. The fragmented ovarian tissue is cultured in vitro in a medium supplemented with specific growth factors and signaling molecules. These factors further stimulate the growth of the activated follicles. Mature oocytes are retrieved from the cultured tissue and can be fertilized via in vitro fertilization (IVF).[1] The resulting embryos can be transferred to the patient's uterus or cryopreserved for future use.

Since IVA involves the activation and maturation of follicles in vitro, it reduces the need for multiple ovarian stimulation cycles and invasive oocyte retrieval procedures. While IVA is a promising technique, it is still relatively new, with limited clinical experience and long-term data. More research and clinical trials are needed to establish its efficacy and safety. Future developments in IVA may involve personalized approaches, where the activation and culture protocols are tailored to individual patient characteristics, improving the likelihood of success. IVA has the potential to improve fertility treatments with ovarian tissue transplantation.

Automation and Microtechnology

Integrating automation and microtechnology represents the future of oocyte preservation, offering unprecedented levels of precision, safety, and efficiency. Automated systems streamline the cryopreservation process, reducing human error and variability, while microtechnology introduces innovative solutions for protecting and enhancing oocytes during freezing and thawing. As these technologies continue to evolve, they will likely lead to even greater success rates in fertility preservation, providing more effective and reliable options for women looking to safeguard their reproductive potential.

Automated Vitrification/Thawing Systems

Are robotic platforms designed to standardize and streamline the oocyte cryopreservation process? These systems control the vitrification steps with high precision and reproducibility, minimizing human error and variability. Automated thawing systems precisely control the warming rates and conditions to ensure the optimal recovery of oocytes after cryostorage.[19]

Robotic Handling and Cryostorage

They automate the handling, loading, and storage of oocytes during cryopreservation. These systems manage oocytes with extreme care, reducing the risk of mechanical damage and contamination.

AI-Driven Quality Control

AI systems are increasingly used to monitor and control various aspects of the oocyte preservation process, including assessing oocyte quality before and after cryopreservation. AI-driven algorithms can analyze data from imaging systems and sensors to predict oocyte viability, optimize cryopreservation protocols, and detect anomalies during the process.

Microparticle-Based Cryoprotectants

They offer superior protection to oocytes during the freezing and thawing processes. These cryoprotectants can be designed to enter the cell more efficiently and provide better protection against ice formation. Microparticle-based cryoprotectants reduce the toxicity often associated with traditional cryoprotectants.[3]

Mechanisms of Action.[14]

Encapsulation of CPAs: Microparticles can be engineered to encapsulate CPAs like DMSO, releasing and controlling them during cooling. This reduces the need for high concentrations of free CPAs in the surrounding solution, minimizing toxicity.

Ice Recrystallization Inhibition: Certain microparticles can prevent ice recrystallization, which can cause significant damage during thawing. These particles effectively inhibit ice growth, preserving the integrity of the oocytes.

Surface Coating: Microparticles can be coated with substances that interact with the oocyte membrane, providing a barrier against ice formation and reducing thermal shock.

Microtechnology-Enhanced In Vitro Maturation

Microtechnology involves using microscale devices and systems to create more controlled and precise environments for biological processes. In the context of IVM, microtechnology can enhance oocyte maturation by providing better control over the microenvironment in which the oocytes develop.[19]

Key Applications:

Microfluidic Systems: These systems allow for the precise control of fluid flow and the delivery of hormones, nutrients, and other factors that are critical for oocyte maturation.[11] Microfluidic devices can mimic the dynamic conditions of the ovarian follicle.

3D Culture Systems: Microtechnology enables the development of three-dimensional (3D) culture systems that can replicate the structure of ovarian follicles. These systems provide realistic mechanical and biochemical cues, possibly improving oocyte maturation.[17]

CONCLUSION

The field of oocyte preservation has witnessed remarkable progress over recent decades, driven by technological advancements and a deeper understanding of reproductive biology. Techniques such as vitrification and closed-system vitrification have significantly improved the survival and viability of cryopreserved oocytes, offering women more reliable options for preserving their fertility. The development of novel cryoprotectants, including natural alternatives, has further enhanced the effectiveness of these preservation methods while minimizing cellular damage.

Innovations such as automation and microtechnology pave the way for more precise and efficient cryopreservation processes, potentially increasing success rates and reducing human error. Meanwhile, advancements in ovarian tissue cryopreservation, including the use of artificial ova-

ries and in vitro activation (IVA), offer promising solutions for women facing fertility challenges due to medical treatments or premature ovarian insufficiency. These technological and methodological advancements represent a significant leap forward in fertility preservation, providing women with greater opportunities to plan their parenthood on their terms. As research continues and new techniques are refined, the future of oocyte preservation holds the potential for even more significant improvements in efficacy and accessibility, ultimately helping more women achieve their reproductive goals despite the constraints of biological age.

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PHENOTYPIC CHARACTERISTICS OF PERITONEAL TUMOR IMPLANTS IN OVARIAN EPITHELIAL TUMORS: A CRITICAL REVIEW

***NIKOLOZ SARAULE**, MD;[1,2] **REMA GVAMICAVA**, MD, PhD, Prof.;[1] **NINO KANTARIA**, MD, PhD;[1]*
***SHOTA KEPULADZE**, MD;[1] **GIORGI BURKADZE**, PhD, Prof.[1]*
[1] Tbilisi State Medical University
[2] Georgian-German Reproduction Center, Tbilisi, Georgia

SUMMARY

Ovarian borderline malignancies are heterogeneous in 80-90% of cases and are characterized by a favorable prognosis, while in 10-20% of cases, peritoneal implants form and relapse occurs. The presence of peritoneal implants has uncertain predictive value. According to some authors, they undergo regression, and in some instances, long-term survival is observed despite the presence of disseminated implants. Implants are also classified into invasive and non-invasive types. Such a classification may have predictive value, so it is an active study area. According to recent studies, cytokines secreted by macrophages induce angiogenesis by ovarian tumors and evade immune surveillance. The frequency of macrophage distribution in the mesothelium may indicate disease spread and be associated with broader tumor dissemination. The role of the peritoneum in tumor dissemination processes is an active area of research. The development and metastasis of ovarian epithelial carcinoma are associated with fibrosis, one of the driving forces in the epithelial-mesenchymal transition process. Therefore, deciphering the regulators of epithelial-mesenchymal transition in ovarian epithelial tumors is necessary to develop new therapies to prevent metastatic spread and improve patient survival rates.

Thus, the correct identification of peritoneal implants is an essential factor. Although there are histological criteria to distinguish invasive from non-invasive implants, differentiation can be difficult. Additionally, little is known about the molecular-genetic basis of implants. This issue requires further research to determine diagnosis, treatment methods, and prognosis accurately.

Keywords: peritoneum; implants; microenvironment; prognostic markers; ovarian epithelial tumors.

Ovarian cancer is one of the most common pathologies among gynecological malignancies. Each year, there are approximately 210,000 new cases of ovarian epithelial carcinoma, with

128,000 resulting in death.[1] In Georgia, according to 2021 data from the NCDC, 274 new cases of malignant ovarian tumors were recorded[2].

Despite the treatment provided, the 5-year survival rate for ovarian cancer is approximately 46-49%.[1] The incidence is significantly lower before menopause and increases post-menopause, leading to an average age of diagnosis of 63 years. The risk of developing ovarian cancer is 1 in 70, but in women carrying germline mutations in the BRCA1 and BRCA2 tumor suppressor genes, the risk increases significantly.[3]

There is a dualistic model for the development of ovarian epithelial tumors, which is widely accepted. This model divides ovarian tumors into Type I and Type II groups.[4] Type I tumors include low-malignancy serous, endometrioid, clear cell, mucinous, and seromucinous carcinomas. Type II tumors include high-grade serous carcinoma, carcinosarcoma, and undifferentiated carcinoma. This group is characterized by a more advanced stage, a higher age group at diagnosis, and greater genetic instability compared to Type I. Both types of tumors differ in their origin cells, precursor lesions, and variations in molecular-genetic mutations. Intermediate precursors of Type I tumors are borderline malignant tumors, often arising from cystadenomas, whereas Type II tumors develop from serous tubal intraepithelial carcinomas of the fallopian tube. Consequently, serous borderline tumors (SBT) precede the development of low-grade serous carcinoma (LGSC). Most cases are detected in the 20-50 age group (average age 46).[4]

Although most serous borderline malignant tumors have a benign course, some cases progress to serous carcinoma, significantly increasing mortality. This process is not well understood. Some studies indicate that in 2/3 of serous borderline ovarian tumor cases, there is somatic activation of KRAS or BRAF mutations, playing an essential role in their progression.[5] This could be used as a biomarker to assess the risk of progression from borderline malignant ovarian tumors to low-grade serous carcinoma.

Ovarian borderline malignant tumors are heterogeneous in 80-90% of cases and are characterized by a favorable prognosis, whereas in 10-20% of cases, peritoneal implants form and relapse occurs. According to one study, in patients with serous borderline malignant ovarian tumors with invasive implants in the peritoneum, more than 30% showed progression to serous carcinoma.[6]

The distinguishing criterion between ovarian borderline malignant tumors and serous carcinoma is mainly the presence of stromal invasion, regardless of extrinsic ovarian existence. The presence of peritoneal implants has uncertain predictive value. According to some authors, they undergo regression, and in some cases, long-term survival is observed despite the presence of disseminated implants. Implants are also classified into invasive and non-invasive types. Such a classification may have predictive value, so it is an active study area.

There is a hypothesis that implants with invasive properties are characteristic of both ovarian serous tumors and borderline malignant tumors, and their presence may indicate disease progression. Research is limited, providing information on the phenotypic characteristics of invasive and non-invasive implants.

As noted, primary tumors of the peritoneum are rare. Secondary tumors of the peritoneum are more common and complicate the course of most intra-abdominal tumors.[7] Their prognosis depends on the nature of the primary tumor. Without intervention, the prognosis for peritoneal

carcinomatosis of any etiology is poor, with a survival rate of only a few months. Peritoneal carcinogenesis can be explained by mechanisms such as lymphatic or hematogenous spread, serous migration, spontaneous or traumatic (surgical) dissemination, and perforation.[8]

The peritoneum's characteristic structure, distinguishing it from other fat-rich visceral tissues, is its well-vascularized immune cell structures, predominantly represented by lymphocytes and macrophages and often colonized by tumor cells. Interestingly, colonization of the peritoneum by ovarian cancer cells in immunocompromised experimental mice (lacking T, B, and NK cells) occurs as successfully as in non-immunocompromised models, indicating the involvement of non-lymphoid tissues in this process.

Cytokines Secreted by Macrophages Cause Angiogenesis and Immune Surveillance Evasion by Ovarian Tumors

According to recent studies, the frequency of macrophage distribution in the omentum may indicate disease dissemination and is associated with more extensive tumor spread. However, the characteristics of ovarian tumor spread in the omentum cannot be fully explained by macrophage quantity alone, as they constitute the dominant cell population in peritoneal fluids (60%).

The role of the peritoneum in tumor process dissemination is a subject of active study. It is hypothesized that signaling pathways associated with peritoneal metastasis formation include several key molecules: 1) E-cadherin and epithelial-mesenchymal transition, which are involved in settlement of tumor cells; 2) the actin microfilament system, involved in the transport of tumor cells within the peritoneum; 3) intercellular adhesion molecule 1 (ICAM-1), vascular adhesion molecule 1 (VCAM-1), tumor cell receptors such as CD44, and cytokines like tumor necrosis factor-alpha (TNFα), interleukin-beta, and interleukin-gamma, which facilitate tumor cell dissemination; 4) metalloproteinases and integrins, which mediate tumor cell invasion; 5) epidermal growth factor receptor (EGFR), epidermal growth factor (EGF), transforming growth factor-alpha (TGFα), insulin-like growth factor-1 (IGF-1), vascular endothelial growth factor and its receptor (VEGF and VEGFR), which are involved in tumor cell proliferation and angiogenesis.[9]

Once tumor cells dissociate from the primary tumor site as single cells or cellular clusters, they metastasize via passive mechanisms, i.e., transported to the peritoneal surface and omentum through the physiological movement of peritoneal fluid. A significant molecule that aids tumor cells in separating from the primary site is E-cadherin. The expression of E-cadherin is significantly lower in peritoneal metastatic cells of ovarian tumors compared to cells in the primary tumor site. This fact may indicate that low E-cadherin expression confers a more invasive potential to the tumor, and the absence of its expression is associated with lower survival rates.

After dissociating from the primary tumor site, ovarian cancer cells are present in peritoneal fluid as multicellular spheroids or single cells. In tumor cell spheroids, cells maintain an epithelial phenotype and express Sip1, a regulator of E-cadherin and matrix metalloproteinases (MMP-2).[10] At this stage, integrins such as α5β1 and its ligands, fibronectin, are located on the surface of tumor cells and play a crucial role in binding to other ligands, such as α6β1 and α2β1. These molecules modify the tumor cells' microenvironment in the peritoneal ascitic fluid. The various characteristics of the microenvironment determine the interaction of tumor cell spheroids' surface receptors with the peritoneum or omentum surface.[11]

Proteolytic activity is also essential in the spread of tumor cells. Matrix metalloproteinases such as MMP14 and MMP2 may facilitate the disaggregation of tumor cell spheroids and their adhesion to peritoneal mesothelial cells.

Integrins are critical mediators in the signal transduction between ovarian carcinoma cells and the mesothelium, contributing to the spread, invasion, and peritoneal metastasis of ovarian cancer cells. Integrin αvβ6 binds to the RGD peptide, which is presented in the LAP peptide, associated with TGF-β1 as part of the latent transforming growth factor-beta binding protein 1 (LTBP1), causing conformational changes in the TGF-β1-LAP-LTBP1 complex. This complex, known as the latency-associated complex, is released by integrin αvβ6, which then binds to its receptor, activating the signaling pathway. Studies show that Wnt5A induces αv integrin expression in ovarian cancer cells, indicating a positive correlation between Wnt5A, αv, and β6 expression in metastatic serous ovarian carcinoma samples. Research also demonstrates that Wnt5A is an essential mediator in the initial stage of epithelial-mesenchymal transition in ovarian carcinoma metastasis.[11]

The development and metastasis of ovarian epithelial carcinoma are associated with fibrosis, one of the driving forces in the epithelial-mesenchymal transition process. Therefore, understanding the regulators of epithelial-mesenchymal transition in ovarian epithelial tumors is essential for developing new therapies to eliminate metastatic spread and improve patient survival rates.

The Wnt signaling pathway is critically important, and its dysregulation is closely associated with tumor progression.[12] β-catenin-independent Wnt signaling, known as the non-canonical pathway, includes the Wnt/Ca2+ and Wnt/planar cell polarity (PCP) pathways, which mediate cell polarity, movement, and cytoskeletal reorganization. Wnt5A is a key non-canonical Wnt molecule that can act as a tumor promoter or suppressor in various carcinomas. Wnt5A demonstrates tumor-enhancing effects and may be associated with epithelial-mesenchymal transition in the progression of ovarian carcinoma.[11]

The Role of TGFβ in Fibrosis and Epithelial-Mesenchymal Transition (EMT)

TGFβ plays a crucial role in fibrosis and subsequent EMT through various effects, including the Smad signaling pathway. Members of the TGFβ superfamily generate signaling pathways via type 1 and type 2 serine/threonine kinase receptors, which form a heteromeric complex.

Ovarian Tumors with Borderline Malignancy

Ovarian tumors with borderline malignancy are characterized by the absence of stromal invasion, and their primary prognostic factor is the type of peritoneal implants. These implants are considered invasive when cell proliferation involves underlying tissues (peritoneal surface, omentum, and intestinal wall) or non-invasive. Whether these implants represent metastasis from the primary site or de novo neoplastic transformation of the peritoneal surface is still unknown.[13]

Mitochondrial DNA sequencing was conducted to assess clonality in eight patients with both ovarian borderline malignancies and peritoneal implants.[13] In 37.5% of cases, similar mitochondrial DNA mutations were found in both the ovarian borderline malignancies and the implants, suggesting that the implants may originate from the primary tumor site.

Genetic and Molecular Analysis of Peritoneal Implants

Other sources suggest that peritoneal implants differ clinically and diagnostically from serous borderline ovarian tumors. Studies have been conducted to determine whether peritoneal implants and serous borderline ovarian tumors have a monoclonal origin. According to these studies, KRAS and BRAF mutations are present in two-thirds of low-grade serous tumors. However, little is known about the molecular-genetic basis of the implants.

Immunohistochemical studies were conducted to examine the presence and distribution of mesothelial cells, stromal fibrocytes, and myofibroblasts in invasive and non-invasive implants using the following antibodies: Calretinin, CD34, and α-SMA.[14] All cases of invasive implants revealed a loss of mesothelial cells and stromal fibrocytes, whereas most non-invasive implants retained mesothelial cells and stromal fibrocytes. Myofibroblast proliferation was present in all cases of invasive implants and approximately half of non-invasive cases. The loss of mesothelial cells and stromal fibrocytes in conjunction with myofibroblast proliferation was a specific indicator for differentiating invasive from non-invasive implants, providing an essential morphological diagnostic aid. According to the study above, these antibodies' combined sensitivity and specificity were 100% and 81%, respectively. However, this method may not be suitable for small biopsies of non-invasive desmoplastic implants.

Molecular Characteristics and Therapeutic Resistance

Research shows that high-grade metastases and invasive implants exhibit irregular expression of oncogenes and tumor suppressor genes, with different pathway-specific disruptions. Irregular tumor suppressor genes are enriched with DNA repair genes such as BRCA1/2 and MSH6, which are involved in developing high-grade serous carcinoma and low-grade malignant carcinoma of the ovary. Increased gene expression may result from gain-of-function mutations due to hypomethylation of regulatory regions. Reduced expression may be attributed to loss-of-function mutations or epigenetic silencing. Cell survival and proliferation may increase depending on the mechanism affecting oncogenes and tumor suppressor genes.

To evaluate the malignant potential of invasive implants, a study was conducted on genes including ABCB1, CDC2, CDKN1A, FAT1, MMP9, MSH2, NQO1, and TOP2A.[15] These genes are associated with chemotherapy resistance in ovarian cancer.[16] Additionally, ABCB1 is involved in cell migration and growth in vitro and correlates with poor prognosis in serous ovarian cancer. CDC2 and CDKN1A genes regulate the cell cycle. The FAT1 gene is a member of the cadherin superfamily and controls cell proliferation.[17] MMP9 participates in the progression of malignant tumors. It is believed to facilitate tumor progression, including invasion, metastasis, and angiogenesis, by mediating the degradation of type IV collagen in the basement membrane and extracellular matrix. NQO1 is a family member of NAD(P)H dehydrogenase (quinone). NQO1 regulates the ubiquitin-independent degradation of p53. NQO1 stabilizes p53, protecting it from degradation. Tumors with reduced NQO1 expression/activity exhibit decreased p53 stability, possibly leading to chemotherapy resistance. NQO1 is associated with poor prognosis in patients with serous ovarian carcinoma. Finally, TOP2A encodes DNA topoisomerase, an enzyme involved in DNA transcription and replication.

Implications for Diagnosis and Treatment

Proper identification of peritoneal implants is a critical factor. Despite the presence of histological criteria distinguishing invasive and non-invasive implants, their differentiation can be challenging. Additionally, little is known about the molecular-genetic basis of the implants. This issue requires further research to determine diagnosis, treatment methods, and prognosis accurately.

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COMPREHENSIVE AI-BASED SYSTEM FOR KPI ASSESSMENT IN IVF LABORATORIES: DEVELOPMENT AND IMPLEMENTATION

***SERGEI SERGEEV**, PhD;[1] **IULLIA DIAKOVA**;[2] **LASHA NADIRASHVILI**[3]*
[1] Georgian-German Reproduction Center, Tbilisi, Georgia; IVF and Genetics Center, Moscow, Russia
[2] ART-IVF Clinic, Moscow, Russia
[3] Georgian-German Reproduction Center, Tbilisi, Georgia

SUMMARY

Background: In vitro fertilization (IVF) success heavily relies on laboratory Key Performance Indicator (KPI) evaluation systems, but traditional analytical methods often fail to provide deep, actionable insights into KPI establishment and measurement.

Objective: This study aimed to develop a comprehensive AI-based KPI evaluation system for IVF laboratories, focusing on a novel Deep Neural Network (DNN) model for predicting clinical pregnancy rates (CPR).

Materials and Methods: We analyzed 3,888 IVF treatment protocols, utilizing consensus KPIs from guidelines, various analytical methods, such as descriptive statistics, time series analysis, machine learning algorithms, and a custom-developed DNN model.

Results: The DNN model demonstrated a high level of accuracy, with an AUC of 0.79 and a PRC of 0.69 in predicting CPR. Importantly, there was no significant difference ($p < 0.05$) between predicted and actual CPR, reaffirming the model's reliability. The model's performance was on par with PGT-A-tested embryo transfers and other commercial AI solutions in IVF, including time-laps systems. External validation in independent clinics yielded an AUC of 0.73, consistent with cross-validation reports from multiple clinics. The application of the DNN model in 4 clinics for quality assurance identified variations in individual staff performance, enabling targeted mentoring and quality improvement, further reinforcing the system's reliability and reproducibility.

Conclusion: The developed AI-based KPI assessment system is a significant leap forward for IVF analytics. It provides a comprehensive, accurate, and reproducible tool for internal quality assurance, external clinic audits, and individual staff competency assessment. By shifting the focus from traditional embryo selection to a deeper understanding of parameters influencing success-

ful IVF outcomes, it opens the door to more personalized and effective infertility treatments, offering hope for the future of IVF.

Keywords: in vitro fertilization; quality assurance; artificial intelligence; deep neural networks; key performance indicators; clinical pregnancy prediction; predictive modeling.

INTRODUCTION

In vitro fertilization (IVF) is a complex and critical process in assisted reproductive technology. The success of it heavily relies on the expertise and performance of embryologists. Implementing a robust Key Performance Indicator (KPI) evaluation system is essential to ensure optimal outcomes and continual improvement. This study aims to develop a comprehensive KPI evaluation system for an IVF clinic's embryology laboratory using artificial intelligence (AI) algorithms, detailing the data analysis process, its importance, and the advantages of such an approach.

Historically, the field of IVF has relied on a limited set of analytical tools for assessing laboratory performance and patient outcomes. Conventional data analysis typically involves basic descriptive statistics (means, medians, standard deviations), simple success rate calculations, and the occasional use of basic inferential statistics. While these methods provide a fundamental understanding of performance, they often fail to offer deep, actionable insights into the complex dynamics of IVF processes. A robust, continuous quality control system is paramount in the high-stakes field of assisted reproduction. Our AI-based solutions can find a place in this system as independent, reproducible, and highly effective algorithms for analytics and data-driven decision-making approaches.

In recent decades, neural networks and machine learning (ML) models have become vital tools in various fields, including computer vision, natural language processing, recommender systems, medical diagnostics, and the field of IVF.[1] These models serve as the foundation for creating algorithms capable of extracting complex dependencies from data, making predictions, and making decisions based on these dependencies. With that AI-based approach, we can transform our descriptive retrospective analytics into predictive prospective research. In some cases, neural networks can identify a broader spectrum of associations than other statistical methods, thanks to their ability to recognize highly nonlinear associations among input parameters. Therefore, the critical point of our study was the establishment of a new approach to KPI analytics utilizing deep learning network (DNN) architecture to predict the treatment cycle result – clinical pregnancy achievement (CPR).

The importance of the laboratory stage in IVF is detailed in consensus resolutions for quality control and the assessment of KPIs, constituting an integral part of internal quality control (QC) according to international standards.[2] Nevertheless, determining such indicators to identify potential problem areas in laboratory work is not always straightforward, and clear recommendations on which KPIs need improvement for real increases in successful IVF protocol numbers are lacking.[3,4] KPIs provide a comprehensive view of the embryologist's performance at various stages of the IVF process, allowing for a nuanced understanding of success rates and potential areas for improvement. In this work, we utilized consensus KPIs as a tool for outcome prediction and laboratory performance measurement.

METHODS

To create the KPI assessment system, we selected the Vienna Consensus[2] and the Maribor Consensus[5] as reference quality indicators, with adjustments for determining the total number of good-quality blastocysts and individualized KPI calibration according to patient population data from the publication by Zacà et al.[6] and the ASPIRE guidelines.[7]

We used retrospective data of 3888 IVF treatment protocols with known outcomes in "The Georgian-German Reproduction Center," Tbilisi, Georgia, from January 2022 to January 2024 to develop a data set for ML training and 394 protocols for model testing. For validation, a PGT-A dataset of 1600 cycles was used. For external model validation, we used data from 2 independent ART centers in Russia. All protocols with missing data values were discarded from the study.

Patient informed consent for that study was unnecessary because only retrospective and fully de-identified data from embryo development has been used. It is entirely non-invasive for patients or their embryos (no medical intervention was performed on the subject, and no biological samples from the patient were collected to develop that model). The ESHRE recommendations and Gardner blastocyst grading system were used for embryo evaluation, in which "good blastocysts" were identified as Bl3BB and higher grades.

Python 3.11, Scikit-learn 1.4.2, and Sklearn 1.4 were used to implement machine learning models and statistical modeling in DataSpell 2024.1.3 IDE. The neural network model has been developed and executed in the GPU PyCharm 17.0.10 environment with the Tensorflow 2.15.0 and Keras library 2.14.0. DNN calibration was performed using CalibratedClassifierCV from sci-kit-learn, which applies logistic regression to align probabilities. A comparative analysis of prediction errors was conducted with area under the receiver operating characteristic curve (AUC), accuracy, F-1 score, specificity (actual negative rate), sensitivity (recall), precision (positive prediction value), precision-recall curve (PRC) and Matthews Correlation Coefficient (MCC).

Statistical analysis of individual KPIs was conducted using StatTech software version 3.0.6. Descriptive statistics were chosen based on data distribution: for customarily distributed quantitative indicators, mean (M) and standard deviation (SD) with 95% confidence intervals (CI) were used, while median (Me) and interquartile range (IQR: Q1-Q3) were employed for non-normally distributed data, as determined by the Shapiro-Wilk test. The direction and strength of correlation between two quantitative variables were evaluated using Spearman's rank correlation coefficient. A p-value < 0.05 was used as the significance threshold for statistical analysis. Comparison of groups based on quantitative indicators was performed using one-way analysis of variance (ANOVA) for normally distributed data or the Kruskal-Wallis test for non-normally distributed data, followed by post hoc comparisons when significant differences were detected.

RESULTS AND DISCUSSION

Data preparation

The first step in developing our KPI evaluation system was to collect and prepare the relevant data. From the protocols of treatment cycles, we selected those executed by the current team of embryologists present in the laboratory. We established clear criteria for excluding protocols, including incomplete data, information provision errors, and procedures performed by multiple embryologists. We chose protocols that satisfied the Vienna consensus criteria to prepare the

dataset for individual staff KPI analysis. Our statistical analysis revealed that all parameters, except the patient's age, exhibited distributions different from usual (R-test), and according to the Dickey-Fuller criterion, their time series were non-stationary ($p > 0.05$). We analyzed the workload distribution in the laboratory among staff based on input (number of oocytes used in procedures) and output (number of obtained blastocysts) parameters. This analysis revealed no significant differences ($p > 0.05$) in pairwise comparisons between all embryologists. This approach to data preparation is crucial for the validity and reliability of our subsequent KPI evaluation and analysis, allowing us to draw meaningful Conclusion about the performance of our IVF laboratory and individual embryologists.

Descriptive statistical analysis

We conducted descriptive statistical analysis to gain insights into the overall performance and variability of the KPIs. The mean patient age was 34 (SD = 5.7, min 18, max 49) years; the number of follicles 16.3 (SD = 9.64); OCC number 13.04 (SD = 7.32); number of oocytes used for fertilization 11.19 (SD = 6.68); 2pN number 8.15 (SD = 5.37), number of cleavaged embryos 8.12 (SD = 5.36); total number of blastocyst D5-D6 3.33 (SD = 3.43); number of good quality blastocyst 3.14 (SD = 3.67); fertilization rate 0.74 (SD = 0.31); cleavage rate 0.99 (SD = 0.4); reasonable blastocyst rate 0.40 (SD = 0.35); oocyte retrieval rate 0.78 (SD = 0.5).

The KPIs we analyzed provide a comprehensive view of embryologists' performance at various stages of the IVF process, allowing for a nuanced understanding of success rates and potential areas for improvement. Our quarterly analysis for 2022-2023 focused on input parameters for the laboratory, with particular attention to the main criterion of properly selected stimulation - the MII rate. This analysis revealed stability in the obtained mature oocytes, with a median of 0.87 and an interquartile range (Q1-Q3) of 0.855-0.893. These values consistently surpassed the Maribor consensus competency threshold of≥74% and a target value of ≥90%.[5] Notably, we observed invariance in these rates across the studied years. Furthermore, we conducted a comparative analysis of the main clinic KPIs between IVF and ICSI procedures. Our statistical tests revealed no significant differences in several key areas: the fertilization rate (U statistic = 4687.0, $p = 0.2945$), blastocyst formation rate (U statistic = 6096.5, $p = 0.0528$), good-quality blastocyst formation rate (U statistic = 4920.5, $p = 0.9031$), and availability of MII oocytes (U statistic = 5404.0, $p = 0.8479$). These findings suggest consistency in performance across different fertilization techniques employed in our clinic, providing valuable insights into the uniformity of our laboratory processes and outcomes.

Individual staff performance analysis

Our analysis of individual embryologists' performance revealed no statistically significant differences ($p > 0.05$) in achieving KPIs (IVF polyspermy rate, ICSI degradation rate, ICSI and IVF fertilization rates, and reasonable blastocyst rate) across selected treatment cycles. Further examination of the distribution of individual embryologist KPIs yielded promising results. The median values for fertilization and blastocyst development rates exceeded the Vienna consensus benchmark for all conducted cycles.[2]

Moreover, these parameters' quarterly values (Q1-Q3) were consistently above the established competency level. This analysis provides a valuable tool for comparing performance across

different embryologists within our team. It allows us to identify top performers whose techniques and practices might be shared as best practices. Equally important, it helps us recognize individuals who may benefit from additional support or training, ensuring continuous improvement and maintaining high standards throughout our embryology laboratory.

Time-series analysis

While descriptive statistics provide a quick overview of the central tendencies and spread of KPIs, allowing for easy identification of typical performance levels and outliers, they fall short of capturing the dynamic nature of these metrics over time. We employed moving averages with confidence intervals for each KPI to address this limitation and gain a time-series understanding of changes in KPI shifts. This approach helps identify trends and patterns in KPI performance over time, with the confidence intervals offering insight into the stability and reliability of these trends. We analyzed the laboratory's KPIs stability, focusing on their prognostic influence on pregnancy rates.

Our observations revealed stable oocyte retrieval rates until the third quarter of 2023, followed by a decline in August 2023. Concurrently, we noted a significant decrease ($p < 0.05$) in the blastocyst formation rate, indicating a reduced probability of forming high-quality blastocysts from each retrieved oocyte. The period from early 2022 to the second quarter of 2022 showed a clear trend of increasing oocyte fertilization rates and blastocyst formation rates, correlating with an increased embryo implantation rate observed during this time. A similar trend of increased blastocyst formation rate was observed in 2023. However, while the decrease in blastocyst formation rate in the third quarter of 2023 may appear notable when comparing quarterly averages between years, this decrease is not statistically significant ($p > 0.05$) and amounts to less than 15%.

This finding suggests that no substantial changes in laboratory conditions reduce the overall number of high-quality blastocysts. Our time-series analysis thus provides a more nuanced understanding of KPI fluctuations, allowing us to distinguish between meaningful trends and normal variations in laboratory performance over time.

Regression analysis

We applied ML methods to our dataset to gain a deeper understanding of our data dependencies. After conducting regression analysis using the method of least squares (OLS) for the good blastocyst rate, the following statistical data were obtained: the R-squared (Coefficient of Determination) was 0.393. The F-statistic value was 322.6, and the significance level (F-statistic) was shallow (< 0.0001), leading us to conclude that the model was statistically significant. The coefficient for the number of blastocysts was 0.0670, suggesting the expected change in the frequency of forming blastocysts when the number of blastocysts increases by one. The Durbin-Watson coefficient was close to 2, indicating the absence of significant autocorrelation in the model's residuals and the correctness of the analytical system.

According to the regression analysis, negative dependencies of the blastocyst formation rate on the number of oocytes retrieved and the total number of zygotes were identified, which aligns with analytics from other IVF centers. Notably, the correlations of blastocyst formation rate with the number of retrieved oocytes, the number of inseminated oocytes, and 2pN were not sta-

tistically significant (p = 0.841, p = 0.842, p = 0.880, respectively). A substantial (p < 0.05) linear dependency was found with the total number of blastocysts. This is an essential indicator of laboratory parameter stability, as an increase in the total number of blastocysts does not deteriorate their quality but increases the probability (proportion) of forming high-quality blastocysts.

KPIs change forecasting

Calculating the laboratory's KPIs during the data preparation procedure allows for the utilization of ML methods to forecast KPI changes with a justified mathematical model. This approach helps determine reference threshold values for quality indicators and identify growth zones for performance improvement. The Seasonal AutoRegressive Integrated Moving Average (SARIMA) model is a time series method widely used for forecasting data with seasonal fluctuations. To achieve this, we integrated the SARIMA model into the laboratory's KPI calculation process to identify the structure of data changes, including seasonal fluctuations, trends, and noise.

This ML model involves determining the orders of autoregression (p), differencing (d), and moving average (q) for the time series components, as well as seasonal orders (P, D, Q). The SARIMA model has been trained on historical data to forecast future performance indicator values based on past observations. These forecasts can calculate target KPIs for the individual laboratory and analyze its effectiveness. Subsequent comparison of forecasted KPIs with actual data forms the basis for a systematic analysis of laboratory efficiency. This approach to internal quality control provides the laboratory with a tool for more predictable process management, early detection of potential issues, and optimization of operations based on data and analytics.

Neural network-based approach

The leading performance indicator of the IVF unit is the clinical pregnancy rate (CPR). The primary challenge in calculating CPR is determining its competence value for each patient population or individual case. According to Maribor's consensus, "Competence and benchmark values of clinical pregnancy rate should be set for a specific local context".[5] Unfortunately, this is not a simple task for an individual IVF center.

Most ML models applied in the field of IVF are based on regression and logistic regression algorithms to identify relationships between the target variable and input parameters.[8] The target variable typically represents the outcome metric of clinic success, such as the frequency of achieving clinical pregnancy, based on fitting data from patient medical history.[9] However, most models used for evaluating CPR are based on patient data and their previous IVF treatment cycles, often failing to track patterns in the changes of quality laboratory parameters relevant to the final transfer outcome.[10]

We developed and implemented our own Deep Neural Network (DNN) within a KPI framework for CPR prediction to address this. This model was trained on our data, with a mean CPR per embryo transfer of 61.93%. The metrics for the model after fitting were: test accuracy = 0.72, AUC = 0.79, PRC = 0.69, precision = 0.72, recall = 0.52, F1 score = 0.61, and MCC = 0.41. After completing the full training process, the predicted CPR was 56.18%, which showed no significant difference (p = 0.114) from the actual CPR in our clinic. Utilizing this DNN model, we can compare actual and predictive CPR across time intervals to understand the likelihood of achieving pregnancy. With

our neural network model, we established a lower threshold limit for the probability of clinical pregnancy occurrence for each year of operation. A significant difference ($p < 0.05$) was noted for patients in 2021-2022 years compared to 2023 year, with a decrease in the likelihood of clinical pregnancy ranging from 10% at the beginning of the year to 20% after the third quarter of 2023. The theoretically calculated probabilities using the DNN model align with the actual CPR reports in these specified time intervals. In other words, our KPI calculation and DNN model prediction analysis demonstrate that the decrease in CPR from the third quarter of 2023 is a process not directly related to the quality of stimulation (patient preparation) or laboratory work but depends on the patient's initial clinical data.

Using the independent ML algorithm XGBoost, we confirmed the correlation between selected parameters and pregnancy occurrence in our DNN model, highlighting their significance in predicting the outcome of embryo transfer. The parameters identified through linear regression analysis can be considered critical features for developing our DNN for predicting CPR. Utilizing them as the foundation for training the neural network allows for more accurate and reliable forecasting of the probability of clinical pregnancy. This approach ensures a more precise tracing of the mutual influence of laboratory parameters and their impact on the outcome of embryo transfer as part of the internal quality control system. Ultimately, our DNN model will provide clinic staff and patients with more precise and individualized predictions, contributing to more effective infertility treatment outcomes based on laboratory quality and performance indicators.

After training the neural network model, we utilize the probabilities obtained from it and apply logistic regression for calibration. This creates a model that takes outputs from the DNN and calibrates them to probability predictions, improving model performance. As a result, we achieved precision = 0.83 and recall = 0.66.

We chose linear regression to analyze the probabilities predicted by the DNN. The analysis yielded a mean squared error (MSE) value of 0.0027, indicating that our model has a deficient error. This suggests that the model's predictions are close to the actual values of pregnancy probability. The coefficient of determination (R-squared) was found to be 0.937, indicating that approximately 93.7% of the variance in pregnancy probability is explained by the independent variables in the model.

Explanation of DNN results with clustering analysis

To deepen our understanding of the predictive capabilities of the developed DNN for IVF pregnancy probability, we conducted a k-means clustering analysis on the model's predicted output values across a dataset to identify inherent patterns in the expected pregnancy probabilities, offering insights into the diverse patient profiles represented within the dataset. The clustering process partitioned the cases into three distinct clusters, each characterized by unique combinations of predictive features. For the effectiveness analysis of our DNN in predicting pregnancy probability in IVF, we compared predicted clusters with clusters based on accurate data on pregnancy occurrence in our clinic.

This comparison allowed us to identify consistency between predicted and real results and differences that may indicate essential aspects of the data. Both clusters contained similar features characterizing stimulation quality indicators and patient clinical data, such as age, number of follicles, and number of oocytes. We also observed similar trends in the distribution of feature

weights characterizing pregnancy probability. This comprehensive analysis demonstrates the robustness of our DNN model in predicting IVF pregnancy probability, providing valuable insights for model explanation and clinical decision-making and enhancing the effectiveness of quality assurance (QA) programs.

DNN validation

To understand the predictive capability of our DNN model, we selected a reference group from our dataset — single embryo transfer of euploid embryos after preimplantation genetic testing for aneuploidy (PGT-A). This selection achieved an initial balance of classes in our patient subpopulation, with an average implantation rate of 54.6%. An analysis of CPR in these protocols revealed a 0.27 error in predicting clinical pregnancy rate, with an AUC = 0.67 (CI = 0.62-0.75) and an accuracy of 0.77. The same metrics (AUC = 0.67) were obtained for embryonic and clinical outcomes of blastocysts with stratified AI scores from iDAScore Embryoscope™ [11] and with other CNN models and commercial AI solutions in IVF: AUC = 0.65 for fresh and frozen transfers and 0.63 for euploid transfers.[12] Consequently, the prediction capability of our DNN model can be used as an entirely noninvasive additional method for embryo selection, similar to other time-lapse systems.[13]

The external validation of our model in 2 independent clinics had comparative results of AUC = 0.73 with a hybrid AI model 3D-ResNet based on several TL technologies (MIRI, GERI, and EMBR-EMBR+) in a cross-validation report from 14 clinics (mean AUC = 0.73) and with video models along (AUC = 0.68) from the same data.[14]

Bayesian method application for prospective approach

However, all these predictions were performed on retrospective data. The Bayesian method offers a robust framework for integrating historical data with real-time predictions from neural networks, enabling a transition from retrospective to prospective analysis. We utilized it to enhance the prediction accuracy of IVF cycle success rates. We defined the prior distribution based on accurate data from IVF cycle successes in 2023-2024. The historical data comprised clinical pregnancy rates and the number of transfers per cycle, transformed into probabilities for each quarter. The quarterly probabilities were as follows: 0.55, 0.55, 0.49, 0.59, and 0.47. A predicted success probability of 0.64 was also obtained from the DNN model for a new cycle.

We initialized our Bayesian model with prior parameters based on historical success and failure counts, specifically with prior successes (α) set to 188 and prior failures (β) set to 106. These priors encapsulate our initial belief about the success rate derived from extensive historical data. Subsequently, we updated our posterior distribution iteratively using the quarterly probabilities. We calculated the equivalent success and failure counts for each probability by scaling the probabilities to a standardized sample size. Following integrating all quarterly probabilities, we incorporated the neural network's predicted success probability for the upcoming cycle. This was done by adjusting the posterior parameters again and adding the expected probability as an additional observation. The final posterior parameters were then used to derive the predicted success rate and associated credible interval for the next IVF cycle. The resulting posterior distribution yielded an expected success rate of approximately 0.58 (58% CPR chance) for the next cycle, with CI = 0.55 - 0.61.

This updated distribution reflects a more accurate estimate of the success rate of the treatment cycle for the future and can help evaluate our observations and CPR expectations. With the dynamic updating of new data, the Bayesian method allows for the seamless integration of historical data and real-time predictions, offering a precise measure of the uncertainty associated with forecasts. This makes the model more robust and adaptive. The combination of the DNN and Bayesian frameworks provides a robust, adaptable, and precise approach to predictive modeling, making it an invaluable tool in the ongoing effort to optimize IVF treatment quality control and data analytics.

Quality management with the help of DNN

The developed neural network model can serve as a unique and comprehensive tool for internal quality control of laboratory parameters and clinic performance by setting lower limits of CPR probability. In many cases, it is challenging to differentiate between patients seeking assistance from various doctors within the clinic, making the specification of competency boundaries in achieving quality targets quite blurry and uncertain. However, our DNN employs a grouped analysis of protocols, allowing their aggregation based on temporal criteria and individual staff members.

We compared the actual clinical pregnancy rates achieved individually by each reproductive specialist during 2023 in external audit programs across four different IVF centers. Through this approach, we identified three doctors (No. 1, No. 2, and No. 3) with actual CPRs of 34.0%, 42.5%, and 40.5%, respectively, which were higher than the theoretically calculated thresholds of 33.60%, 33.33%, and 38.01% for their patient groups ($p > 0.05$). Additionally, we identified three doctors (No. 4, No. 5, and No. 6) whose transfer outcomes (9.60%, 24.82%, 16.01%) require serious monitoring and verification, as their actual CPRs were significantly lower ($p < 0.01$) than those predicted by the model (33.53%, 33.33%, 37.25%) for these staff members. Based on that, it can be concluded that the competency level of doctors No. 4, 5, and 6 still does not allow them to work independently. Every procedure they conduct requires careful monitoring and mentoring from more experienced colleagues, such as specialists No. 1, 2, and 3 within the same center.

Thus, the developed DNN is an integral, accurate, and reproducible element of QA that can be used for both internal QA and external clinic audits, as well as for determining the individual competency of the staff. With its help, it is possible to define the boundaries of personal competency and identify staff members whose work requires increased scrutiny and those who can perform such monitoring. The importance of this analysis lies in the fact that the role of a mentor can be assigned to existing clinic staff without the need for additional external experts or auditors. This significantly simplifies organizational matters and reduces direct financial expenditures on staff training.

CONCLUSION

Our research represents a significant leap forward in applying data analytics to IVF practice. By integrating diverse analytical methods, we have developed a comprehensive framework that goes far beyond traditional descriptive statistics. This approach includes advanced KPI calculations personalized for individual staff members, time series analysis with moving averages, clustering and principal component analysis (PCA) for embryologist performance using machine learning algorithms, linear regression analysis for understanding KPI relationships, and neural network

prediction for the result of IVF treatment procedures. This multifaceted approach allows a deeper understanding of laboratory performance, embryologist efficiency, and process stability. It can be applied as a comprehensive quality management system in the embryology laboratory. It serves as a first step in integrating AI in IVF, shifting from the traditional concept of selecting the best embryo to understanding the parameters involved in successful outcomes.

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MENOPAUSAL TRANSITION PERIOD: HORMONAL CHANGES, CLINICAL SYMPTOMS, DIAGNOSIS, AND MODERN MANAGEMENT APPROACHES

***GEORGE TEVDORASHVILI**, MD, PhD;[1] **NIKOLOZ KINTRAIA**;[1] **MARIAM ANDGHULADZE**;[2] **NATO METSKHVARISHVILI**[1]*

[1] Tbilisi State Medical University

[2] "Medicare Georgia", Tbilisi, Georgia

SUMMARY

Background: The menopausal transition (MT) marks the end of a woman's reproductive years, characterized by hormonal changes, irregular menstrual cycles, and various symptoms impacting health and quality of life.

Objective: To understand the hormonal fluctuations during MT and the clinical implications for managing symptoms.

Method and Materials: The article synthesizes findings from various studies and consensus workshops, particularly the Stages of Reproductive Aging Workshop (STRAW), detailing hormonal changes and clinical presentations during early and late MT.

Results: MT involves erratic estradiol levels, decreased progesterone, and increased follicle-stimulating hormone (FSH), leading to irregular cycles, with notable events like luteal out-of-phase (LOOP) cycles. Symptoms often include abnormal uterine bleeding, hot flashes, sleep disturbances, and mood disorders. Hormonal therapies, including estrogen and selective serotonin reuptake inhibitors (SSRIs), are effective but should be administered cautiously due to associated risks.

Discussion: The hormonal chaos of MT complicates infertility and symptom management. Non-hormonal therapies and lifestyle modifications are beneficial but often less effective than hormone therapy.

Conclusion: A personalized approach to managing MT is crucial, integrating hormonal and non-hormonal strategies, lifestyle changes, and mental health support. Continuous research aims to optimize these interventions for better outcomes in women during MT.

Keywords: menopausal transition, perimenopause, hormonal changes, menstrual irregularity, anovulatory cycles, symptom management, hormone therapy, reproductive aging.

INTRODUCTION:

The menopausal transition (MT), which is also called the advanced reproductive age, represents the final years of a woman's reproductive life[1] and is associated with profound reproductive and hormonal changes,[28, 60] a decrease in the consistency of ovulation and changes in menstrual patterns.[58]

MT begins with the first onset of menstrual irregularity[28, 60] and with variations in menstrual cycle length and a monotropic rise in follicle-stimulating hormone (FSH), and ends with the final menstrual period, classically confirmed only when followed by 12 months of amenorrhea, thereby definished the final menstrual period (FMP).[1, 6, 28]

Perimenopause, which literally means "about or around the menopause," begins at the same time as the MT and ends one year after the final menstrual period.[1] The median age at the FMP is 51.4 years,[1, 6, 10] but chronological age cannot be substituted for reproductive age, as women reach menopause at different ages.[45]

MAIN BODY:

Perimenopause, instead of being merely a period of declining estrogen, is marked by three significant hormonal changes that can start in regularly menstruating women as early as their mid-thirties.: 1. Erratically higher estradiol levels, 2. Decreased progesterone levels (normally ovulatory, short luteal phase or anovulatory cycles), and 3. Disturbed ovarian-pituitary-hypothalamic feedback relationships.[52]

The stages of reproductive aging have been well described in two workshops, with the acronym **STRAW - Stages of Reproductive Aging Workshop (STRAW)**.[28-30, 60]

Based on the proceedings of a consensus conference, the STRAW report further classifies reproductive and post-reproductive life into seven stages, with the MT accounting for two of those stages: early and late.[1, 49]

In the early MT (stage –2), previously, regular menstrual cycles become more variable, and cycle length changes by seven days or more.[1] By the time Stage -2, the early transition, is attained, the ovarian follicle cohort has shrunk to a critical level, and, usually, a woman will note her first missed menstrual period.[28]

FSH is more consistently elevated by this time, and ovarian reserve measures, such as Inhibin B, AMH, or an ultrasound-measured antral follicle count, are now critically low. Because the follicle cohort is still relatively preserved at these early stages of the transition, the rise in FSH causes folliculogenesis to appear more rapidly, and the follicular phase of the menstrual cycle becomes shorter. Follicles grow more quickly but seem to ovulate at a smaller size. An increase in follicle growth during the luteal phase has also been noted, indicating that the dominant follicle for the subsequent cycle has developed significantly before menstruation.[28, 31] Recent data show that approximately a third of all perimenopausal cycles have a significant surge in estradiol occurring de novo during the luteal phase. These types of cycles, in which ovulation follows rapidly upon one another, with minimal follicular phase length, have been named **luteal out-of-phase or LOOP events**[31] and may explain a large proportion of symptoms and signs for symptomatic perimenopausal women.[52]

These types of cycles contribute further to the menstrual irregularity of perimenopause and are associated with hormone secretory patterns that deviate from midreproductive-aged women's hormone patterns. Specifically, lower luteal progesterone and higher FSH have been ob-

served, and erratic estrogen secretory patterns have been associated with the transition. Thus, some hormone changes may be related to altering menstrual patterns and increasing cycle irregularity, which can be profound and contribute to symptomatology.[28, 31]

If we look at LH levels, it is clear that perimenopausal women have a short follicular phase, missing about 5-7 days.

Many of the marked increases in ovulatory cycle E2 and cycle irregularities during the menopausal transition may be due to **LOOP events** and appear to be triggered by prolonged high follicular phase FSH levels.[60]

While older women have higher levels of Estradiol, Progesterone levels are low in this age group. And despite the high activity of estradiol, we don't have many follicles because of enough progesterone. At this time, the luteal phase is practically unchanged in duration, but the progesterone level is reduced, and the estradiol level is increased. Another fact that attracts attention to this age group is that despite the high numbers of estradiol, there is an increase in the level of FSH. This is the result of the reduction of AMH and Inhibin levels.

Another pattern of this period is the **LAG cycle,** which involves a short follicular phase with altered folliculogenesis, high estradiol, and low or reduced progesterone levels.

The number of anovulatory cycles increases when the cycle becomes irregular in women with previously regular cycles. Progesterone levels show that only a few cycles are ovulatory cycles, and the other cycles are not ovulatory in perimenopausal women. During these cycles, estradiol levels were steadily elevated, and when ovulation occurred, estradiol levels were back to their normal range.

In some cycles, the level of estradiol is increased, and the level of FSH decreases, which means that the feedback sensitivity is maintained to some extent during the aging process; however, this sensitivity is not stable and constant. During anovulatory cycles of perimenopause, progesterone levels are low, and estradiol and FSH levels are elevated.

The presence of LOOP and LAG cycles complicates infertility interventions

According to the new model, the balance between estrogen and progesterone, which implies the presence of high estrogen and low progesterone levels in perimenopausal women, may be caused by the development of multiple follicles without ovulation.

In perimenopausal women, atypical dominant follicles may be very large and grow to >26 mm in size. This is associated with higher estradiol levels and significantly lower progesterone levels.

Therefore, the hypothesis implies that the suppression of progesterone production occurs due to the high estradiol level produced by the atypical dominant follicle of the luteal phase. This is an entirely different model from what we have known so far.

Late MT (stage 1) is characterized by two or more missed menstrual periods, at least one intermenstrual interval of 60 days or more, and an FSH level greater than 40 IU/L.

Circulating estrogen is more likely to be low during anovulatory cycles, and the long periods of amenorrhea are accompanied by a sharp increase in the prevalence of typical menopausal symptoms. However, when a woman does have a menstrual cycle, it may be **ovulatory, anovulatory with relatively high estrogen levels, or anovulatory with low estrogen levels.** This stage is the speed bump of the menopausal transition.[28]

For the average woman, the menstrual milestone of the early transition (Stage -2) is age 47, the late transition (Stage -1) occurs at age 49, and the FMP at age 51. However, there is substantial variability in the onset of these milestones.[28]

Menopause is determined in retrospect after a year of amenorrhea. For women with at least one intermenstrual interval of 60 days or more, the median menopausal time is 2.6 to 3.3 years. Cigarette smoking may alter the ovarian aging process and advances the age of menopause by as much as 2 years.[1]

Endocrine changes for diagnosis of Menopausal Transition Period

Secretion of reproductive hormones during the MT fluctuates widely.[1]

The variations in circulating **FSH** levels with increasing age are most probably due to changes in ovarian physiology affecting the secretory pattern of the gonadotrope, and the ovary becomes increasingly resistant to stimulation by gonadotropins, probably due to the decreased number of follicles, which leads to a decline in the production of both estrogens and inhibins.[50] Early follicular phase FSH, taken between cycle days 2-5, is the most sensitive and convenient time in the cycle to perform its measurement.[49]

In perimenopausal women, **Estradiol** production fluctuates with FSH levels and can reach higher concentrations than those observed in young women under age 35.[1]

Progesterone levels during the early MT are lower than in women of mid-reproductive age and vary inversely with body mass index.[1] These lower levels arise through three mechanisms: 1) decreased progesterone production within normal-length ovulatory cycles; 2) shortened luteal phase lengths within ovulatory cycles; and 3) more frequent anovulatory cycles.[52]

Levels of **Androstenedione, Testosterone, and DHEA decline after menopause and menarche** but do not decline sharply after menopause because the theca cells continue to produce androgens.[1]

The prime mover in the feedback disruptions that result in the hormonal changes of perimenopause is now confirmed to be Inhibin B.[52,61] **Inhibin B** is a TGF-beta superfamily peptide that is produced by the granulosa cells of the growing follicle cohort - by antral and dominant follicles and directly suppresses the pituitary secretion of FSH.[49] As the follicle cohort shrinks, less Inhibin B is produced, leading to the well-characterized monotropic rise in FSH, a cardinal feature of the menopausal transition.[28]

Studies confirm the role of declining Inhibin B levels in allowing FSH to rise in the follicular phase. Elevated FSH, in turn, stimulates the second estradiol peak called LOOP during the luteal phase.[38]

Antimullerian hormone or Mullerian Inhibiting Substance - AMH/MIS are also tgf-beta superfamily peptides[49] secreted by the granulosa cells of small secondary and preantral follicles.[11] Levels parallel the number of remaining ovarian follicles measured by antral follicle counts (on transvaginal ultrasound).[52] They are not just produced by follicles in their terminal stages of growth but also by primary, secondary, and early antral follicles and, therefore, reflect more completely the follicle cohort.[49]

Antimullerian hormone (AMH) and Inhibin B have been used as peripheral serum markers of ovarian reserve. AMH is very effective in predicting the probability of a poor outcome with fertility therapy when it is low.[28] Theoretically, measuring AMH/MIS is the most effective way to measure a woman's progress toward menopause.

Some physicians use serum FSH levels early in the cycle (say cycle day 3) as a test for perimenopause. For an individual woman, however, FSH is neither sensitive nor specific.[52] There are hopes that the AMH will prove helpful in deciding about proximity to menopause, but further validation is needed.

The hypothesis that elevated perimenopausal estradiol levels were behind perimenopausal experiences was based on clinical observations of estrogen-associated experiences (increasingly heavy flow, increased premenstrual symptoms, mastalgia, fluid retention, weight gain) in cycles documented with the Daily Perimenopause Diary and QBT.[52] Cycle lengths tend to shorten in older, regularly menstruating women, and the follicular phase becomes shorter. Shortened follicular phase lengths are associated with higher early-cycle serum estradiol levels and with higher urinary FSH levels in both follicular and late luteal phases.[52]

Relationships Between Cycle Characteristics and Clinical Correlations of Health in Midlife Women

Most women who are symptomatic during the MT present with frequent or excessive bleeding or with hot flashes and other symptoms of estrogen deficiency. [1]

Abnormal uterine bleeding (AUB) is expected during the MT, particularly once menses become irregular and unpredictable. Because the time interval surrounding menopause is characterized by relatively high acyclic estrogen levels[52] and relatively low progesterone production, women in the MT are at some increased risk for developing endometrial hyperplasia or carcinoma.[1,13]

SWAN findings suggest that unusually **heavy menstrual bleeding (HMB)** typically does not have a hormonal basis, and such patterns, especially when they are persistent, should be investigated for an underlying anatomical, gynecologic cause.[12, 49]

Recent epidemiological evidence indicates that **vasomotor symptoms or hot flushes** before or at the onset of the MT are common[16, 49], affecting 30-70% of premenopausal women (varies by race/ethnicity, BMI, smoking, anxiety, and depressed mood).[1, 16] They are likely to be mild in nature at these earlier stages of a woman's reproductive life.[32] Vasomotor symptoms cause a substantial amount of distress and reduction in health-related Quality of Life (HRQOL).[28, 33]

Lipid profiles and inflammatory markers in women with varying cycle lengths in SWAN, when controlled for body size, showed no difference except for triglycerides, which increased with increasing cycle length. Lower mean cycle estrone conjugates and pregnanediol glucuronide were associated with higher triglycerides, insulin, and inflammatory markers. In longitudinal SWAN studies, total perimenopause/early postmenopause and, falling E2 and rising FSH were independent of age, while HDL peaked in late perimenopause.[49]

Longitudinal studies have shown that hot flashes are associated with low exercise levels, smoking, high FSH and low estradiol levels,[1, 17] increasing body mass, ethnicity, socio-economic status, and a history of premenstrual dysphoric disorder (PMDD) or depression.[1,18]

Depressed mood disorders and increased anxiety also are increased during the MT. [1, 28] Community-based surveys indicate that perimenopausal women experience considerably higher levels of psychological distress and have an elevated risk of significant depression compared to both premenopausal and postmenopausal women.[1,19] **Major depression**, diagnosed using a Structured Clinical Interview for Diagnosis (**SCID**), was found to be more likely to occur in women during the late menopausal transition.[39, 40] Similarly, anxiety symptoms also appear to be more likely to be reported as women traverse menopause and may be linked to the onset of major depression.[28, 41]

Sleep disturbances are also widespread during the MT. Women begin to experience changes in their sleep patterns in their 40s, and these tend to worsen with entry into the menopausal transition.[28] Poor sleep is also related to aging and not only the menopausal transition period.[28, 34, 35] According to the **SWAN** data, difficulty sleeping was clearly associated with the perimenstrual phases of the cycle, with early perimenopausal women overall experiencing more poor sleep than women who had not yet experienced a break in their cycles.[28, 36] Overall, a 29% increase in the odds of reporting trouble sleeping was observed as women progressed from regular cycling into the early transition. Sleep quality was worse at the beginning and end of the menstrual cycle.[49] Women with metabolic syndrome experienced substantially less sleep efficiency by polysomnography.[28, 37]

Other common symptoms during the MT include **decreased libido, forgetfulness, vaginal dryness, dyspareunia, irritation, dysuria, and urinary incontinence.**[1,28]

The constellation of symptoms of vaginal dryness, irritation, and dysuria has been named **genitourinary syndrome of menopause (GSM)**.[38] The latter may be a more accurate reflection of the collective morbidity to the female genital tract caused by a lack of estrogen.[28]

Clinical Implications - Modern Management Approaches of Symptoms during the MT

Clinical strategies for addressing these issues typically include hormone therapy, which can be safely administered to most perimenopausal women for a short duration. Additionally, nonhormonal and behavioral therapeutic approaches can also be utilized.[28]

The most common strategies for managing perimenopausal hormonal chaos include: Continuous or cyclic oral contraceptives, use of standard HRT-replacement hormone therapy regimens, Estrogen only supplementation, TSEC - use of tissue-selective estrogen complex - bazedoxifene + conjugated estrogen, cyclic progestin-only therapy, GnRH agonist +HRT, Depo Medroxyprogesterone Acetate + estrogen (oral or non-oral), Progestin-containing IUD + estrogen (oral or non-oral), contraceptive patch, contraceptive ring and contraceptive injections or implants.

Long-term use of HT in older menopausal women has been associated with increased risks for venous thromboembolism, coronary events, stroke, and breast cancer.[1, 22] Although short-term treatment of symptomatic women during the MT likely poses significantly fewer risks, HT generally should be used in the lowest effective dose and for the shortest time required. Low-dose estrogen regimens (conjugated equine estrogens, 0.3 mg daily, or its equivalent) can achieve as much as a 75% reduction in vasomotor symptoms over 12 weeks, approaching the efficacy of standard-dose HT regimens. They may have fewer risks and side effects.[1, 23, 28] The decision to use HT should be made only after first carefully reviewing its risks and benefits for the individual.[1]

The relative safety of HT during the MT has not been thoroughly investigated. The results of one observational study have suggested that women who start HT near menopause had a decreased risk of coronary heart disease when taking estrogen alone (relative risk [RR] 1⁄4 0.66; 95% CI, 0.54– 0.80) or in combination with progestin (RR 1⁄4 0.72; 95% CI, 0.56–0.92) (24). A secondary analysis conducted by the investigators involved in the **Women's Health Initiative (WHI)** revealed that the risk for coronary heart disease was not significantly increased in women under age 60 years of age or within ten years of menopause.[25] Further studies to evaluate the safety and efficacy of HT during the MT and the early postmenopausal years are ongoing.[1]

When hormones are contraindicated or otherwise unacceptable to a patient, some other options are now on-label for treatment.[28]

Concerns about the risks of HT have increased interest in nonhormonal alternatives for treating symptoms in the MT. In some women, vasomotor symptoms during the MT can be reduced by wearing layered clothing, avoiding caffeine and alcohol, and keeping the ambient temperature a few degrees cooler. Herbal treatments such as black cohosh have been shown to have marginal or no benefit in placebo-controlled trials.[37, 45]

Neuroactive agents, including selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), alpha-adrenergic agents, and others, all have some efficacy in treating vasomotor symptoms.[1]

For vasomotor symptoms, paroxetine mesylate, a 7.5 mg long-acting salt of paroxetine, was recently approved by the FDA for this indication. For vaginal dryness, Ospemifene, 60 mg, a new selective estrogen receptor modulator (SERM), has also been FDA-approved. For adverse mood symptoms, the selective serotonin reuptake inhibitor (SSRI) class of drugs is a reasonable alternative for depression. Similarly, while there are no menopause-specific remedies for poor sleep, treatments ranging from behavioral modification for insomnia to melatonin receptor agonists may be tried. For women who have hot flashes that are bothersome only at night, Gabapentin in a small nightly dose of 100–300 mg may be highly effective.[28]

Both SSRIs and SNRIs may be effective, as norepinephrine and serotonin seem to play a role in the hypothalamic regulation of temperature homeostasis and are involved in the occurrence of hot flashes. Randomized placebo-controlled trials have shown that SSRIs (citalopram, sertraline, paroxetine) and SSNIs (venlafaxine) can help to reduce the severity and frequency of hot flushes.[1, 26, 27] Clonidine (an alpha-adrenergic agonist) and gabapentin also have some efficacy.[1, 26, 27] However, the effectiveness of neuroactive therapies does not equal that of HT.

Given that the onset of menopause cannot be predicted precisely, that women may ovulate up until their final menses, and that prescription drugs and alternative therapies may have potential adverse effects on pregnancy, clinicians should remain sensitive to the contraceptive needs of women during the MT.[1]

Nonpharmacologic or botanical remedies for menopausal symptoms have been largely ineffective in well-conducted clinical trials. These ineffective treatments include yoga,[42] omega-3 fatty acid supplementation,[43] and black cohosh.[28, 42-44]

Optimal hormonal management should slow the brain's aging process—estrogens maintain brain metabolism, synapses, mood, and cognition. Progestins increase irritability and decrease metabolism. The best product should suppress the endogenous function of the ovary, be the safest estrogen, be a safe and brain-neutral progestin, reduce the risks of developing withdrawal bleeding, and be in continuous mode.

Evidence-Based Additions:

Recent studies have highlighted several important considerations for managing MT:

Cardiovascular Health - HT has been associated with a reduced risk of coronary heart disease when started near menopause, particularly with estrogen alone.

Bone Health - HT can significantly reduce the risk of osteoporosis and fractures by maintaining bone density.

Mental Health - Cognitive-behavioral therapy (CBT) and mindfulness-based stress reduction (MBSR) have shown efficacy in managing mood disorders during MT.[1, 28, 35]

CONCLUSION:

The menopausal transition (MT) is a complex period characterized by significant hormonal changes and varied clinical symptoms. Effective management requires a personalized approach that may include hormonal and non-hormonal therapies, lifestyle modifications, and psychological support. The best product for managing the MT period should suppress the endogenous function of the ovary, be the safest estrogen, be a safe and brain-neutral progestin, reduce the risks of developing withdrawal bleeding, and be in continuous mode. Ongoing research refines these strategies to improve outcomes for women undergoing MT.

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IMPACT OF ORAL DHEA SUPPLEMENTATION ON ANDROGEN LEVELS IN WOMEN USING COMBINED ORAL CONTRACEPTIVES: A RANDOMIZED STUDY

***GEORGE TEVDORASHVILI**, Md, PhD, Prof.;*[1] ***NIKOLOZ KINTRAIA**, MD, PHD;*[1]
***MARIAM ANDGHULADZE**, Md;*[2] ***NATO METSKHVARISHVILI**, MD, PHD*[1]
[1] *Tbilisi State Medical University*
[2] *«Medicare Georgia», Tbilisi, Georgia*

SUMMARY

Background: Combined oral contraceptives (COCs) reduce androgen levels, particularly Testosterone (T), by inhibiting ovarian and adrenal androgen synthesis and increasing Sex Hormone-Binding Globulin (SHBG). This can lead to testosterone deficiency, which is associated with negative effects on well-being, mood, energy, cognitive function, sexual functioning, muscle mass, and bone density.

Objective: To evaluate the effects of adding oral Dehydroepiandrosterone (DHEA) to Combined Oral Contraceptives (COCs) on the hormonal profile in Caucasian women.

Materials and Methods: A randomized, double-blind, placebo-controlled trial was conducted with 35 healthy Caucasian women aged 21-35 years (BMI: 18.5-25 kg/m²) who used a COC containing 30µg ethinyl estradiol (EE) and 150µg Levonorgestrel (LNG). Participants discontinued COC use for three menstrual cycles before being randomized to receive either COCs with 50 mg/day DHEA (n=18) or placebo (n=17) for six cycles. Hormonal levels were measured at baseline and after 1, 3, and 6 months of treatment.

Results: COC use significantly reduced Total Testosterone (by 56.2%), Free Testosterone (by 65.7%), Dehydroepiandrosterone, Dehydroepiandrosterone Sulfate, and Androstenedione levels while increasing SHBG concentration ($P<0.001$). Adding DHEA to COCs restored both Free and Total Testosterone levels to baseline ($P<0.001$).

Discussion: The addition of DHEA to COCs was effective in maintaining physiological androgen levels, potentially counteracting the adverse effects of COCs on female sexual function and overall well-being.

Conclusion: Adding 50 mg/day DHEA to EE/LNG-containing COCs maintains physiological levels of Free and Total Testosterone in Caucasian women, potentially mitigating the adverse effects of

COCs on female sexual function. Further comprehensive clinical trials are warranted to evaluate these clinical effects.

Keywords: combined oral contraceptives, testosterone, dehydroepiandrosterone, androgen deficiency, sex hormone-binding globulin, female sexual function.

INTRODUCTION

Combined oral contraceptives (COCs) are known to reduce the levels of androgens, especially Testosterone (T), by inhibiting ovarian and adrenal androgen synthesis and by increasing levels of Sex Hormone-Binding Globulin (SHBG).[1]

As a result of the action of COCs, which involves the suppression of gonadotropins, they may have a direct inhibitory effect on the synthesis of ovarian and adrenal androgens and blood testosterone levels. Testosterone, the most potent circulating androgen in women, can decrease by up to 50% due to the action of COCs. [1,2]

Three possible underlying mechanisms may be held responsible for this effect: (i) Suppression of ovarian androgen synthesis, (ii) increased SHBG concentrations, and (iii) suppression of adrenal androgen synthesis.[1]

Testosterone deficiency is thought to be associated with a broad range of undesired effects, including diminished well-being and quality of life, mood changes (depression, irritation, moodiness), loss of energy, cognitive disturbances, interference with optimal sexual functioning [8], declining muscle mass and strength and lowering of bone density.[1]

A Significant reduction of androgen levels caused by Combined Oral Contraceptives (COCs) is a reliable mechanism by which COCs may adversely affect sexual function in a subset of women. [1,8]

Maintaining physiological levels of androgens, especially Free Testosterone (FT), may ameliorate these adverse effects of COCs. This can potentially be achieved by adding dehydroepiandrosterone (DHEA) to Combined Oral Contraceptives (COCs).[4-7]

Dehydroepiandrosterone (DHEA) and Dehydroepiandrosterone Sulfate (DHEAs) are endogenous steroid hormone precursors, which are produced by the adrenal gland and the brain and through partial metabolism to testosterone, characterized by an androgenic nature. [3,4,7]

Because the liver partially metabolizes oral DHEA into Testosterone[3-5,] it could, in principle, be incorporated as a prodrug into a COC pill, thereby maintaining T levels in women who use these COCs. [3]

Adding Dehydroepiandrosterone (DHEA) to COCs may maintain physiological levels of androgens and ameliorate adverse effects associated with androgen deficiency. [3-7]

OBJECTIVE

To evaluate the effects of adding oral Dehydroepiandrosterone (DHEA) to Combined Oral Contraceptives (COCs) on the hormonal profile in Caucasian women.

METHODS AND MATERIALS

We conducted a rigorous randomized, double-blind, placebo-controlled trial involving 35 healthy Caucasian women (age range: 21-35 years; body mass index (BMI) range: 18.5-25 kg/m2) who used a Combined Oral Contraceptive containing 30µg ethinyl estradiol (EE) with 150µg Levo-

norgestrel (LNG) for at least three months. Levonorgestrel was chosen because of its intrinsic androgenic effects (4).

Study participants discontinued OC use for at least three menstrual cycles, after which they were randomized by random selection to a study group to receive 30 µg EE/150 µg LNG containing COCs together with oral DHEA 50 mg/Daily dose (n=18) or placebo group (n=17) for the following six cycles.

The study protocol was approved by the local ethical committee of Tbilisi State Medical University, and informed consent was obtained from each participant before the study began.

The exclusion criteria included estrogen-dependent neoplasia—current or previous—endocrine conditions, thromboembolic disease, and liver, pancreatic, or renal diseases.

Before the study, we performed a gynecological examination, pelvic transvaginal ultrasound scan (TVUS) before and after 1, 3, and 6 months of treatment, and blood tests to evaluate coagulation parameters and mammography.

We determined serum levels of Free testosterone (FT), Total Testosterone (TT), Sex Hormone Binding Globulin (SHBG), Dehydroepiandrosterone (DHEA), Dehydroepiandrosterone Sulfate (DHEAs), Androstenedione (AD), Estradiol (E2), Estrone (E1) and Albumin during the screening period, at baseline and after 1, 3 and 6 months of treatment.

RESULTS

Taking Combined Oral Contraceptives reduced the levels of all determined androgens - for Total Testosterone by 56.2%, for Free Testosterone by 65.7% (P<0.001), and also reduced the levels of Dehydroepiandrosterone, Dehydroepiandrosterone Sulfate, and Androstenedione, while increasing the concentration of Sex Hormone Binding Globulin (P<0.001).

Adding DHEA to COCs significantly increased the levels of all measured androgens compared to placebo: both Free Testosterone (FT) and Total Testosterone (TT) levels were restored to their baseline levels (Fig 1 - A, B). (P<0.001).

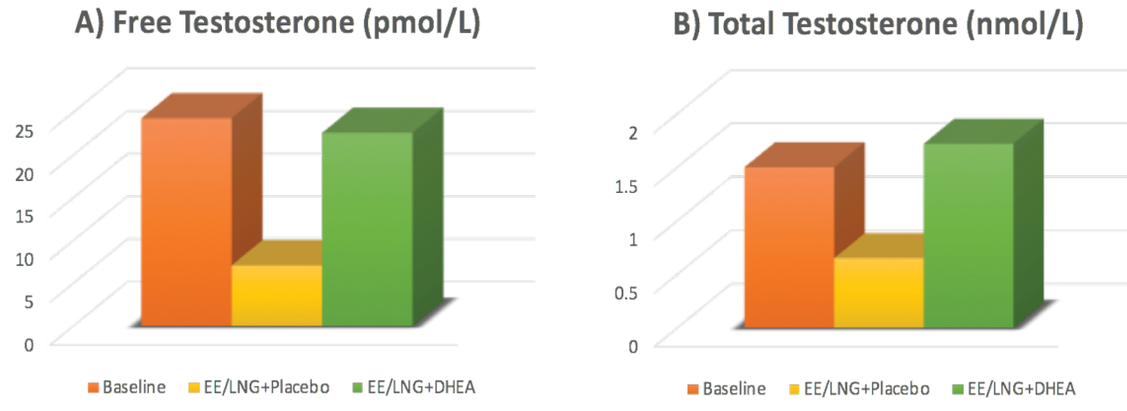

Figure 1.

Adding DHEA to Combined Oral Contraceptives did not affect Sex Hormone Binding Globulin (SHBG) levels (Fig 2). (P<0.001)

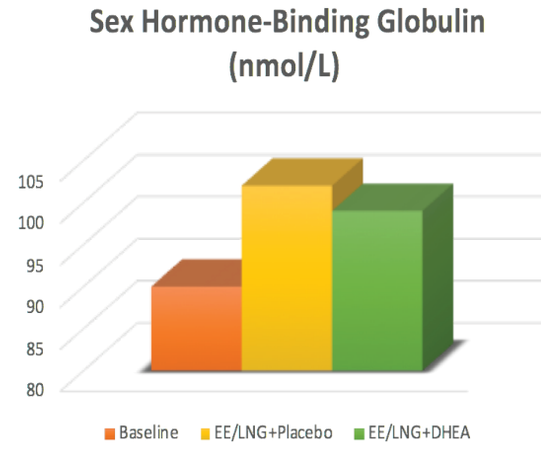

Figure 2.

Moreover, adding DHEA significantly increased the levels of Dehydroepiandrosterone (DHEA) and Dehydroepiandrosterone Sulfate (DHEAs) (Fig 3). (P<0.001)

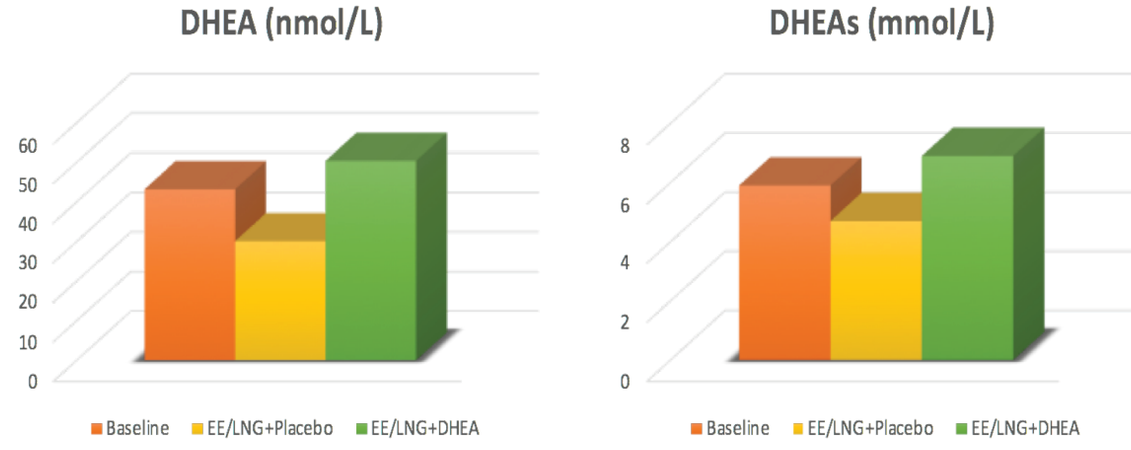

Figure 3.

Adding DHEA significantly increased the levels of Estrone (E1) and decreased the levels of Estradiol (E2) (Fig 4 - A, B). (P<0.001)

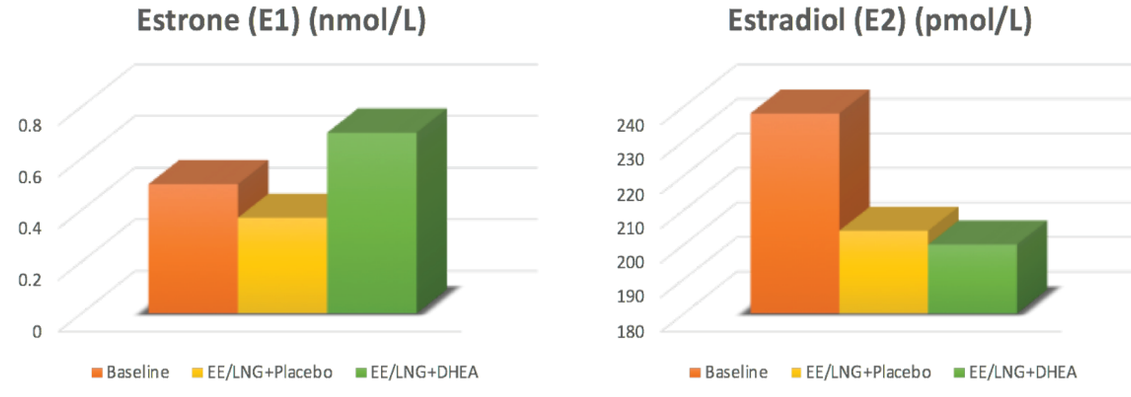

Figure 4: A

Figure 4: B

DISCUSSION

The use of combined oral contraceptives (COCs) is associated with decreased androgen levels and increased Sex Hormone Binding Globulin (SHBG) concentrations. Adding DHEA to Ethinyl Estradiol/Levonorgestrel-containing COC restored both Free and Total testosterone levels to their physiological values.

Conclusion

Based on the results of our study, we can conclude that the addition of 50 mg/daily dose of oral DHEA to an EE/LNG-containing COC maintains physiological levels of Free and Total Testosterone in Caucasian women and thus may potentially ameliorate the adverse effects of COCs on female sexual function.

The results of our study provide the basis for planning and conducting more extensive, comprehensive, and modeled clinical trials to evaluate clinical effects.

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