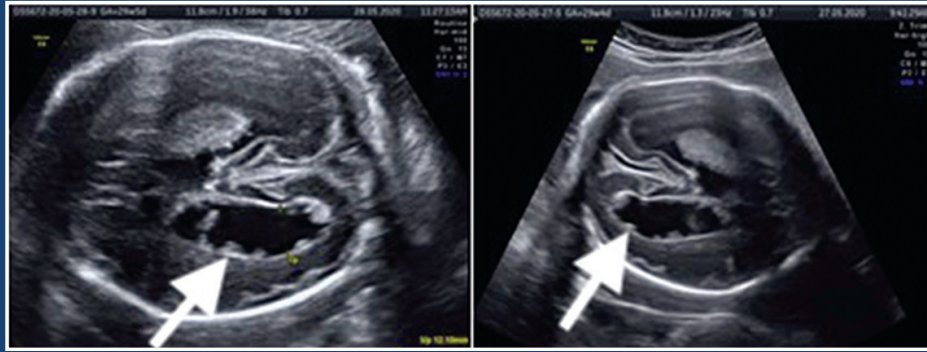




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Book;

Kohler G; Egelkraut H. In Kohler G and Egelkraut H (eds). *Munchener Funktionelle Entwicklungsdiagnostik im zweitem und drittem Lebensjahr. Handanweisung*. Munchen: Uni Munchen, Institut fur Soziale Paediatric und Jugendmedizin; 1984.

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Editorial



Dear Colleagues,

It is my great pleasure to introduce the second issue of the “Journal of the Turkish-German Gynecological Association (J Turk Ger Gynecol Assoc)” in the publishing year of 2023. This issue is consisted of seven articles and one review that we hope you will read with interest. Also you may have the opportunity to read the quiz. Here we share some of our favorite articles that were published in this issue of the journal.

High-risk HPV is considered a major risk factor for the development of cervical cancer. The relationship between multiple high risk HPV infections and high-grade dysplasia has been investigated but, but the results are controversial. You will read an article evaluating the association between multiple high risk HPV infections and high-grade dysplasia by analyzing colposcopic punch biopsies.

Preterm birth is a major cause of neonatal morbidity and mortality. Cervical length measured by transvaginal ultrasound best identifies for patients at high risk for preterm birth. You will also read an article which determines the standard mid-trimester cervical lengths of singleton and twin pregnancies.

You will also have the opportunity to read a review about the effects of diet and nutritional habits on reproductive cells.

I would also like to invite you to join us for our “Congress on the Synthesis of Holistic and Modern Approaches in Gynaecology”, which will be held in İstanbul on 2-3 June 2023. In our congress, holistic medicine practices in the field of obstetrics and gynecology will be presented, and it will be enriched with the opinions, suggestions and experiences of all of the faculty.

Dear Esteemed Readers, Authors and Reviewers

Our journal applies the continuous publication model. In other words, the articles are published online as soon as they are ready for publication, without having to wait for a certain number of papers to complete an issue. This targets to increase the efficiency of the publication process and enable readers to access important research findings in obstetrics and gynecology more quickly. We will keep the high standards of quality.

Please visit us online at www.jtgga.org and keep in touch with us by following us on Twitter @JtggaOfficial.

We are looking forward to receiving your valuable submissions, thank you in advance for your contributions.

Sincerely,

Prof. Cihat Ünlü, M.D.
Editor in Chief of *J Turk Ger Gynecol Assoc*
President of TGGF

Maternal and neonatal outcomes in adolescent pregnant women with one prior Cesarean section in Baghdad

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Abstract

Objective: Repeat adolescent pregnancy is a hot topic worldwide and imposes a challenge on the health system, especially when faced with limited resources. We aimed to assess maternal and neonatal outcomes in adolescent pregnant women with one prior cesarean section (CS).

Material and Methods: Singleton adolescent pregnant women with one prior CS scar were recruited and divided into two groups based on the obstetric decision for delivery and/or mother's wish, either trial of labor (TL) or elective cesarean section (ECS). If TL failed, an emergency CS was performed.

Results: Out of the total 109 involved women, TL and ECS groups included 78 (71.6%) and 31 (28.4%) women, respectively. Emergency CS was done for 57 (52.3%) women from the TL group, leaving only 21 (19.3%) women with successful TL who had statistically significant (non-recurrent) indications of the prior CS [12 (57.1%)]. Malpresentation (n=24; 77.4%) was the major indication in the ECS group, while fetal distress (n=29; 50.9%) was the main cause of failed TL. Total maternal morbidities in the TL group were significantly higher for adjusted [1.5 (1.1-4.2)] and non-adjusted odds ratios (OR) [2.4 (1.6-5.6)]. Neonatal complications, such as admission to neonatal intensive care unit, were higher in the TL group without reaching significance. However, the adjusted OR [1.9 (1.1-3.3)] for perinatal asphyxia was significantly increased in TL group.

Conclusion: Maternal morbidities and perinatal asphyxia were significantly higher in the TL group of adolescent women compared with the ECS group in this study. (J Turk Ger Gynecol Assoc 2023; 24: 86-91)

Keywords: Adolescent, complications, pregnancy, previous cesarean section

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Introduction

The rate of surgical delivery by cesarean section (CS) has increased globally, and this is especially true in our part of the world in the Middle East (1).

The term adolescent pregnancy is used when the pregnant woman is aged ≤ 19 years. The rate of adolescent pregnancy is increasing now throughout the world including the United States and Europe, and not limited to the third world and developing populations (2,3). In general, there are increasing

rates of adolescent pregnancy reaching as high as 27%, while different cultures and different countries may report some variation in the incidence (4).

It may be clinically challenging to manage the delivery of an adolescent woman having a prior CS scar. Trial of labor (TL) is an apparent good option, but there are many limitations related to the wide range of preferences of obstetricians and adolescent women. Although it is well known that TL in a previously scarred women may reduce future CS rates, the situation in adolescents this is not clear due to a lack of



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published data. It is estimated that more than half of women with a single previous scar of CS have successful TL rates. Again, limited data is available in adolescents (5,6).

Risk of comorbidities in TL, especially uterine rupture, still constitutes a major concern. Contrasting opinions are offered, either to encourage or discourage TL in resource-limited settings due to the expected complications of the pregnant women and their newborns (5,7,8).

Since we have a high local rate of first CS in adolescents (48.96%) (8), we planned to examine if concerns regarding TL were justified in adolescent pregnant women having a single prior delivery with CS in comparison to an elective cesarean section (ECS) approach in Baghdad city by evaluating maternal and neonatal outcomes.

Material and Methods

This work was conducted between the start of May, 2019 and the end of April, 2022 in an obstetric department of Baghdad Teaching Hospital at Medical City Complex. All full-term singleton para one pregnant women with one previous CS and aged ≤ 19 years old who presented for labor were included. This hospital acts as a tertiary referral center for complicated and high-risk deliveries from all other parts of the country.

Exclusion criteria were:

- 1) No, or more than one previous CS delivery;
- 2) former single CS delivery but with any one or more of: multiple pregnancy; antepartum hemorrhage; emergency CS before labor onset; chronic disease (such as hypertension and diabetes); and/or missing data.

Recruited women were divided into two categories, TL or ECS. The decision to apply either TL or ECS was made by the attending obstetrician according to the hospital guideline (like post-date pregnancy) and/or mother's wish. Intrauterine growth retardation, fetal presentation other than cephalic, multiple pregnancy, genital herpes disease, and former myomectomy were considered contraindications to TL. Newly delivered babies were examined by the attending neonatologist.

The partogram was used to look after involved women and a continuous monitoring of the fetal heart using a sonic aid was done with regular checking of uterine tenderness, vaginal hemorrhage, and liquor staining. Artificial rupture of membranes was the standard approach to augment labor without use of oxytocic medications due to uterine rupture risk. Labor was not induced. TL had to be terminated and an emergency CS was performed when tenderness was felt at the previous scar site, partogram went over the action line, and fetal distress was suspected (which was defined as meconium-stained liquor, and fetal heart rate <110 /minute, >160 /minute, or decelerated). Perinatal asphyxia was identified when 5 minutes Apgar score was <6 . Low birth weight (LBW) was considered

if the weight was within the range of 1.5-2.5 kilograms, and very LBW newborn was defined as <1.5 kilograms (9-11).

Women were monitored at the hospital for 24 hours after vaginal delivery and 72-96 hours after non-complicated CS delivery.

Detailed medical history was obtained by the attending obstetrician, including sociodemographic information, antenatal care visits (ANC), medical diseases (current or previous), previous CS, and perinatal outcomes.

Statistical analysis

Statistical Package for the Social Sciences version 25 was used to compare TL and ECS groups (IBM Inc., Armonk, NY, USA). Chi-square test and Student's t-test were utilized to analyze categorical and continuous data, respectively. The normality of distribution of the data was examined using the Shapiro-Wilk test. Maternal and gestational age, regular ANC, period of inter-delivery, and indication of prior CS delivery were evaluated as confounding and interacting parameters. No significant correlation was revealed among these covariates. Logistic regression analysis (multivariate) was used to investigate the covariates. Adjusted parameters including odds ratios (OR) and 95% confidence intervals were derived from the regression coefficients. A p-value ≤ 0.05 was assumed significant.

Results

The total number of adolescent pregnant women presenting during the study period was 586, and after exclusions 109 women with only one prior CS were recruited. Of these, 78 (71.6%) were given a TL (TL group), and 31 (28.4%) were chosen to have an ECS group. Out of TL group, 57/109 (52.3%) women had failure of TL and delivered by an emergency CS. The net result was 21 (19.3%) women delivered vaginally, and 88 (80.7%) had a cesarean delivery (Figure 1).

Women in the TL group were slightly younger at 18.12 years versus 18.42 years in the ECS group and also had later gestational age (37.4 vs 36.9 weeks) and more regular ANC. Furthermore, compared to ECS women, TL women were more likely to have consanguineous marriage and non-recurrent indication of previous CS, but less months of inter-delivery period (Table 1). Women with successful TL had significantly more (non-recurrent) indications for the prior CS than women with failure of TL [12 (57.1%) vs 20 (35.1%), $p=0.02$], while unknown indications were significantly more common in the failed TL group [19 (33.3%)], as shown in Table 2.

Table 3 shows the main causes of TL failure [fetal distress was the main cause "29 (50.9%)"], and ECS indications [malpresentation revealed to be the major one "24 (77.4%)"].

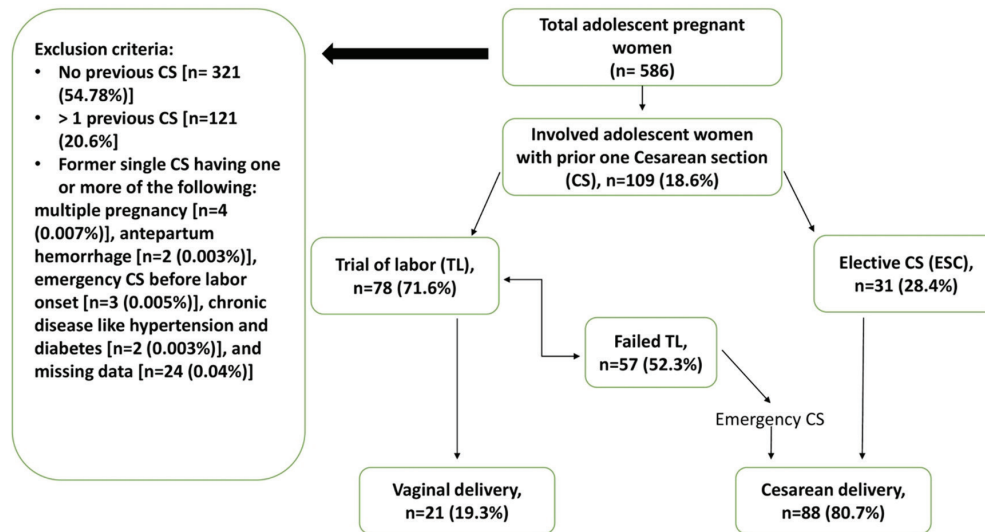


Figure 1. Scheme of participants
ECS: Elective cesarean section

Table 1. General characteristics of involved women

Maternal parameter	TL group, n=78	ECS group, n=31	OR (95% CI)
Age (years)*	18.12±3.16	18.42±1.76	0.6 (0.4-1.3)
Gestational age (weeks) on delivery*	37.4±3.1	36.9±2.9	1.3 (0.2-2.7)
Regular antenatal care	35 (44.9%)	11 (35.5%)	1.8 (1.4-3.2)
Educational level**			
Literate	62 (79.5%)	24 (77.4%)	1
Illiterate	16 (20.5%)	7 (22.6%)	0.9 (0.2-1.8)
Occupation**			
Housewife	68 (87.2%)	26 (83.9%)	1
Employed	10 (12.8%)	5 (16.1%)	0.8 (0.5-1.6)
Consanguinity	33 (42.3%)	12 (38.7%)	1.4 (0.7-2.1)
Parity**			
One	44 (56.4%)	16 (51.6%)	1
Two	22 (28.2%)	10 (32.3%)	0.7 (0.2-1.5)
≥3	12 (15.4%)	5 (16.1%)	0.9 (0.3-2.2)
Indication of prior Cesarean section**			
Recurrent	25 (32.1%)	17 (54.8%)	1
Non-recurrent	34 (43.6%)	6 (19.4%)	3.7 (2.5-6.3)
Unknown	19 (24.4%)	8 (25.8%)	0.9 (0.5-1.9)
Period of inter-delivery of previous Cesarean section (months)**			
≤12	21 (26.9%)	10 (32.3%)	1
12-24	31 (39.8%)	14 (45.1%)	0.8 (0.4-2.3)
≥24	26 (33.3%)	7 (22.6%)	2.8 (1.7-5.9)

*Data is expressed as mean ± standard deviation (Student's t-test), **Data is expressed as number (%) (chi-square test). TL: Trial of labor, ECS: Elective cesarean section, OR: Odds ratio, CI: Confidence interval

Compared to ECS group, TL group had significantly more morbidities in both adjusted and unadjusted OR [1.5 (1.1-4.2) vs 2.4 (1.6-5.6), respectively]. However, all counted maternal

outcomes were more frequently encountered in TL women without reaching significance, as illustrated in Table 4. Also, most neonatal adverse outcomes were non-significantly more

Table 2. Indication of prior CS in TL women

Indications, n (%)	Successful TL, n=21	Failed TL, n=57	p-value
Non-recurrent	12 (57.1)	20 (35.1)	0.002**
Malpresentation	6 (28.6)	6 (10.5)	<0.01**
Fetal distress	3 (23.8)	3 (5.3)	<0.01**
Others*	3 (14.3)	11 (19.3)	0.038**
Prolonged labor	7 (33.3)	17 (29.8)	0.095
Unknown	2 (9.5)	19 (33.3)	<0.01**

*Others for successful TL included 2 multiple pregnancies and 1 antepartum hemorrhage, while for failed TL included 6 antepartum hemorrhages, 3 multiple pregnancies, and 2 gestational hypertension cases. TL: Trial of labor, CS: Cesarean section

Table 3. Indications of failed TL and ECS women

Indications, n (%)	Failed TL women, (n=57)	ECS group, (n=31)
Fetal distress	29 (50.9)	Nil*
Prolonged labor	18 (31.6)	Nil*
Tender uterine scar	9 (15.8)	Nil*
Uterine rupture	1 (1.7)	Nil*
Malpresentation	Nil*	24 (77.4)
Intrauterine growth retardation	Nil*	3 (9.7)
Post term	Nil*	2 (6.5)
Prior myomectomy	Nil*	1 (3.2)
Genital herpes	Nil*	1 (3.2)

*Nil: means no reported cases for the specific mentioned indication. TL: Trial of labor, CS: Cesarean section

Table 4. Maternal and neonatal outcomes of labor

Complications	TL group, (n=78)	ECS group, (n=31)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)*
Maternal, n (%)				
Total morbidities	6 (7.7)	1 (3.2)	2.4 (1.6-5.6)	1.5 (1.1-4.2)
Puerperal sepsis	4 (5.1)	1 (3.2)	1.3 (0.5-1.8)	1.7 (0.8-6.4)
Postpartum hemorrhage	3 (3.8)	1 (3.2)	1.8 (0.3-7.5)	3.2 (0.4-12.3)
Ruptured uterus	1 (1.3)	0 (0.0)	Not applicable	Not applicable
Blood transfusion	1 (1.3)	0 (0.0)	Not applicable	Not applicable
Hysterectomy	1 (1.3)	0 (0.0)	Not applicable	Not applicable
Neonatal, n (%)				
LBW	25 (32.1)	9 (29.0%)	1.2 (0.2-2.4)	1.4 (0.4-2.1)
NICU admission	17 (21.8)	7 (22.6)	0.7 (0.6-1.5)	0.8 (0.5-2.2)
Perinatal asphyxia	7 (9.0)	1 (3.2)	1.6 (0.4-2.6)	1.9 (1.1-3.3)
VLBW	3 (3.8)	1 (3.2)	1.7 (0.5-2.7)	2.2 (0.6-5.3)
Stillbirth and early neonatal death	1 (1.3)	0 (0.0)	Not applicable	Not applicable

*Odds ratios have been adjusted for maternal and gestational age, regular antenatal care visits, period of inter-delivery, and indication of prior surgical delivery (CS). TL: Trial of labor, ECS: Elective cesarean section, OR: Odds ratio, CI: Confidence interval, LBW: Low birth weight, NICU: Neonatal intensive care unit, VLBW: Very low birth weight

common in the TL group compare to the ECS group, with the exception of NICU admission, which was slightly more frequent in the ECS group (Table 4).

Discussion

Adolescent pregnancy is associated with well-known adverse maternal and perinatal complications, which result in increased pressure on health services throughout the world with relatively more burden in resource-limited health systems (12).

On the other hand, TL is also associated with well-known related complications that affect the pregnant mother and her newborn, which again are relatively more problematic when combined with restricted infrastructures of health settings (13). The rate of CS in our adolescent study sample was very high (80.7%) and is much higher than local and international figures (8,14). This may be in part due to the tertiary referrals received in our center, including risky adolescent obstetric cases and repeated requests of ECS in parallel with the beliefs of our population who prefer CS over TL to avoid labor pain (8,15).

Our rate of TL success (19.3%) is apparently less than that reported previously from Turkey (83%) although health services are better resourced in the latter country (6).

In the present study, adolescents who had TL tended to be slightly younger, having regular ANC, and were unemployed. This could be because these women at a younger age may have had less financial security, thus providing less power to select ECS and discuss personal preferences with the obstetrician (16). TL had been shown to be significantly more successful in non-recurrent indication for prior CS compared to recurrent causes such as failure of progress, while unknown indications have a

significant negative effect on TL (7,17). This highlights the need to carefully examine the medical records of women presenting with adolescent pregnancy.

The total morbidities in TL women were significantly more common than in the ECS group, although being attached to the hospital guidelines avoiding the use of oxytocic agents to induce labor in TL women. It was hypothesized that many women with prior single CS scar visiting tertiary centers for labor present late after failed TL, and this would increase the risk of obstetric complications when those women try to avoid another CS, especially in the adolescent age group or due to delay in seeking medical help which is linked directly to poor availability of transportation and the background culture of the community in rural and peripheral areas where home-based labor may be the choice rather than going to the hospital unless complications occur. This community may opt for labor outside appropriate health settings and even may encourage the use of non-conventional medications given by non-health professionals. A significant factor during the study period was the travel restriction and fear of infection associated with the COVID-19 pandemic, which occurred during the study period (18-20).

Perinatal asphyxia in neonates was significantly associated with TL compared to ECS. Indeed, most of asphyxiated cases belonged to women who had a failed TL followed by emergency CS, or those women who had started labor outside our hospital and presented late (21-23).

Although LBW was more common in TL compared to ECS women, this was not significantly different and was high in both groups, as birth weight is commonly affected in adolescent pregnancy (8,16).

Study Limitations

A strength of the present study was the prospective monitoring of TL and ECS outcomes in adolescent pregnant women. However, there are some limitations, including a single center study design. Even though the center was the largest tertiary center in Iraq, health care resources were still restricted. Nevertheless, we have no justified reasons to think that other centers in the country have different circumstances which would contradict the results presented. Another limitation is that we did not follow up involved women after discharge to monitor possible long-term adverse outcomes. Although we tried our best to adjust our results, residual factors that we could not measure might have a confounding effect. Moreover, the study was performed in the best tertiary center in the capital, Baghdad. However, the results will need to be supported by larger multi-centric prospective studies.

Conclusion

Significant higher rates of maternal morbidities and perinatal asphyxia were associated with TL compared to ECS in

adolescent pregnant women with prior single CS in this study, conducted in Baghdad city during the COVID-19 pandemic.

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Ethics Committee Approval: *The University of Baghdad, College of Medicine, and Al-Kindy College of Medicine approved the study (approval number: 173, date: 11.02.2019).*

Informed Consent: *Helsinki Declaration guidelines were applied, and an instructed consent was gathered from all participants.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Surgical and Medical Practices: S.K.J., H.A.M., M.A.W., R.A.; Concept: S.K.J., H.A.M., R.A.; Design: S.K.J., H.A.M.; Data Collection or Processing: S.K.J., M.A.W.; Analysis or Interpretation: S.K.J., H.A.M., R.A.; Literature Search: H.A.M., M.A.W., R.A.; Writing: S.K.J., H.A.M., M.A.W., R.A.*

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Comparing maternal and neonatal prooxidant-antioxidant balance during delivery

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Abstract

Objective: Oxidative stress (OS) is due to a disturbance in the balance between the production of free radicals and antioxidant defense, resulting in a predominance of free radicals over endogenous anti-oxidant defenses. OS may have many causes. Pregnancy, and especially delivery, are associated with increased OS. The relationship between maternal and infant prooxidant-antioxidant balance (PAB) is unclear. Therefore, the aim of the present study was to compare PAB in mother and baby pairs.

Material and Methods: This cross-sectional study was conducted in 104 mothers and normal term infants during 2017-2020. PAB was measured in healthy mothers before delivery and in umbilical cord samples after delivery. Data on the infant characteristics including age, gestational age, birth weight, Apgar score, and maternal history including the duration of mother's education, weight of the last month, and gravidity were collected using a researcher-made questionnaire. The cord and maternal PAB were compared by statistical methods.

Results: In this study, the mean PAB of the neonates and mothers was 30.76 and 214.87 HK, respectively. The results revealed a moderate association between the PAB neonate and maternal PAB before delivery but it was not significant.

Conclusion: Overall, the level of oxidants and antioxidants reduced during pregnancy and before delivery, and it was found that the relative incidence of neonatal PAB increases by increasing maternal PAB. (J Turk Ger Gynecol Assoc 2023; 24: 92-6)

Keywords: Neonatal PAB, prooxidant-antioxidant balance, mother, delivery, umbilical cord

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Introduction

Free radicals are atoms or molecules that have one or more unpaired electrons and are thus highly reactive. Oxidation is a chemical reaction in which an electron or hydrogen ion is transferred from one molecule to another, leading to unstable products called free radicals that can trigger chain reactions at a cellular level and cause death or cell damage (1,2).

The human body has developed protective systems to ameliorate free radical damage through the "antioxidant defense system". This system includes enzymatic antioxidants such as superoxide dismutase, catalase, and glutathione peroxidase (3,4).

Oxidative stress (OS) is defined as a disturbance in the balance between the production of free radicals and antioxidant defenses, and cellular damage occurs when these defenses



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cannot neutralize active oxygen species. OS may damage any biological molecule, including proteins, lipids, and DNA, and may also disrupt cellular signaling systems (5,6).

Pregnancy is associated with biochemical changes in the body and leads to the absorption of oxygen by increasing energy demands and thus tends to increase OS through the production of oxygen-free species (2,7). During the labor phase, severe maternal pain, muscle contractions, and high oxygen uptake can increase the production of free radicals (2,8). In addition, the release of inflammatory mediators, such as arachidonic acid metabolites and interleukin 6, which are involved in the onset of natural delivery, can increase the production of free radicals (9). It should be noted that deficiency of antioxidant vitamins (vitamins C and E, and beta-carotene) may increase the level of oxygen-free radicals (10). Increased OS during pregnancy can endanger the health of the fetus and the mother (1,11). In certain conditions, the production of free radicals exceeds the antioxidant capacity and may damage fetal tissues and organs, including lungs, brain, and retina, and thus threaten the life or affect quality of life of the baby (5,12).

It has been suggested that the antioxidant capacity of the umbilical cord blood and a placental barrier is sufficient for protecting the fetus from oxidative damage caused by the increased OS in pregnant women with preeclampsia (13). OS impairs placental vascular function and adverse outcomes in prenatal periods (14). It has been reported that increased OS may occur following the contraction of skeletal and smooth uterine muscles during normal vaginal delivery compared to cesarean delivery (15). However, there is no relationship between the prooxidant-antioxidant balance (PAB) and the type of delivery (16). It is critical to maintain a balance between the production of oxygen-free radicals and the functional failure of the fetal and neonatal antioxidant system during, before, and after delivery (17).

Although the antioxidant activity during natural pregnancy is determined by many factors including genetics, levels of antioxidant vitamins (maternal diet), maternal body habitus and co-existent pathologies, antioxidant concentrations may be insufficient to respond to the increased production of free radicals. Many pregnancy-related complications, such as fetal death, intrauterine growth retardation, and preeclampsia, occur due to insufficient maternal antioxidant protection to balance the high production of oxygen-free radicals and fat peroxidation in the placenta (18). All tissues, especially the placenta and fetus, require greater oxygenation during pregnancy. Although free oxygen species produced by the mother and the fetus promote the growth and development of fetal cells, its increase and imbalance with the creation of OS causes changes in the structure of cells, and thus causes damage with harmful effects on fetal and maternal cells (1,19). Neonatal antioxidant

status indicates a decrease in the concentration of glutathione peroxidase, superoxide dismutase, beta-carotene, riboflavin, alpha proteinase, vitamin E, copper, zinc, transferrin, and other plasma factors (20).

To the best of our knowledge, no previous study has compared maternal and neonatal PAB. Given the importance of this balance in maternal, infant, and delivery health, the current study compared PAB in the mother and the infant.

Material and Methods

This cross-sectional study was performed in 104 healthy mothers and neonates during 2017-2020 after receiving informed consent from participants. In this study, PAB in healthy mothers and newborns, via cord blood sampling was evaluated. Exclusion criteria were mothers with diabetes, preeclampsia, hypertension, and other complication during pregnancy and newborns with congenital anomalies.

A checklist was compiled to obtain the required data, including newborn characteristics (birth weight, length, age, head circumference, gestational age, and first minute Apgar score) and maternal history (maternal duration of education, maternal weight in the last month and gravidity). PAB was measured in umbilical cord blood samples from neonates and mothers before delivery. At least 0.2 mL of serum was taken from the umbilical cord blood and was sent to Bu-Ali Research Institute to measure PAB through a cold chain.

The amount of antioxidants was investigated by 3.3-3.5 tetramethylbenzidine (TMB) staining and oxidation of colored cations. The standard solution consisted of 250 μmol of hydrogen peroxide and 3 millimoles of uric acid per 10 millimoles of NaOH.

To make the TMB cation, 60 mg of TMB powder was dissolved in a ratio of 10 mL to 20 mL of the solution and mixed well. Then, it was placed in a dark and dry container for two hours. Afterward, 25 units of peroxidase enzyme were added to 20 mL of the solution which is distributed in each milliliter of the solution and kept at 20 °C.

Subsequently for reagent preparation, 200 mL of TMB was added to 10 mL of acetate (0.05 mol, pH=5.8). The prepared solution was mixed with 1 mL of TMB cation and 10 mL of TMB solution, respectively. Next, it was kept in a dark and dry place for two minutes. In addition, 10 μL of each sample was mixed as the standard with 200 μL of the working solution and placed in 96 plates in a dark environment at 37 °C for 12 minutes. Then, 100 μL of 2N hydrochloric acid was added to each well. Color development was measured at 450 and 620 nm wavelengths. From standard samples, a standard curve was created representing the value of PAB in HK units and the role of hydrogen peroxide in the standard solution.

This work was approved by the ethical and scientific committees of the School of Medicine, Mashhad University of Medical Sciences (approval number: IR.MUMS.MEDICAL.REC.1398.375).

Statistical analysis

PAB values of samples were read from the standard curve created for each assay. The data were analyzed using SPSS, version 22 (IBM Inc., Armonk, NY, USA). Further, parametric and non-parametric variables were measured by the correlation method and the correlation coefficient with statistical tests appropriate for the type of the variable. Finally, the central index and the paired samples test and Pearson paired samples correlation test were used. A p-value less than 0.05 was considered to indicate significance.

Results

In the present study, 16 neonates were excluded from the evaluation due to lysis of the laboratory sample. The mean gestational age and 1-minute Apgar score were 39.65 ± 0.8 weeks and 9.4 ± 0.7 , respectively. The other characteristics of neonates and mothers are presented in Table 1.

Neonatal PAB increased as maternal PAB increased (Figure 1). In this study, the mean PAB of neonates was 30/76 HK while that of mothers was 214/87 HK. According to Pearson's correlation test between neonatal and maternal PAB before delivery, there was a moderate association although it was not significant (Figure 1, $r=0.135$, $p>0.05$).

Discussion

In the present study, the mean maternal PAB during delivery was 215 HK while in another study, the means of maternal PAB in non-pregnant and pregnant women in the first trimester and the labor phase were reported 129, 168, and 221 HK, respectively

Table 1. Mean comparison of the characteristics of mothers and neonates

Variable	Mean \pm SD
Birth weight (gram)	3239.06 ± 417.245
Height (cm)	50.83 ± 1.822
Head circumference (cm)	34.67 ± 0.944
Gestational age (week)	39.65 ± 0.813
First minute Apgar score	9.43 ± 0.720
Maternal length of education (year)	9.3 ± 1.542
Maternal weight of the last month (kg)	68.78 ± 4.785
Gravidity	2.35 ± 1.147
Neonatal PAB (HK)	31.276 ± 52.975
Maternal PAB before delivery (HK)	214.87 ± 39.96
SD: Standard deviation, PAB: Prooxidant-antioxidant balance	

(2). In the first trimester of pregnancy, placental tissue contains low concentrations and activities of antioxidant enzymes. Therefore, trophoblastic cells are poorly protected from oxygen-related injuries (21). In the second trimester, when the oxygen pressure in the intervillous space increases three-fold, the OS in the placenta increases significantly. Thus, oxidative damage changes the function of the placenta, affecting the later stages of pregnancy (22). Abnormalities of the placenta may lead to OS, and endothelial dysfunction plays a key role in the development of pregnancy complications such as recurrent miscarriage and preeclampsia (21). The consumption of antioxidant supplements by at-risk women for preeclampsia is associated with improved antioxidant activity (23). The results of previous research demonstrated a significant reduction in vitamin E levels during labor compared with the postpartum period in mothers and neonates, indicating an increase in OS during delivery (24). Therefore, there appears to be an increase in PAB in pregnancy and a peak before delivery. The PAB balance is easily disrupted during labor and practitioners should ensure that mothers maintain a proper balance during pregnancy, especially during labor. In the present study, the mean PAB of neonates was equal to 31 HK. The findings of a study comparing PAB levels in neonates with and without asphyxia indicated mean PAB levels were 20 and 40 HK in healthy neonates and neonates with asphyxia, respectively (5). In two other studies, the mean PAB of neonates was reported to be about 40 HK after jaundice recovery (25,26). A study investigated the effects of selenium consumption, critical to the function of some anti-oxidant enzymes, during pregnancy on the PAB levels of neonates. The mean PAB level in the neonates of the control group was 31 HK (27). Based on the results of the present study, the mean

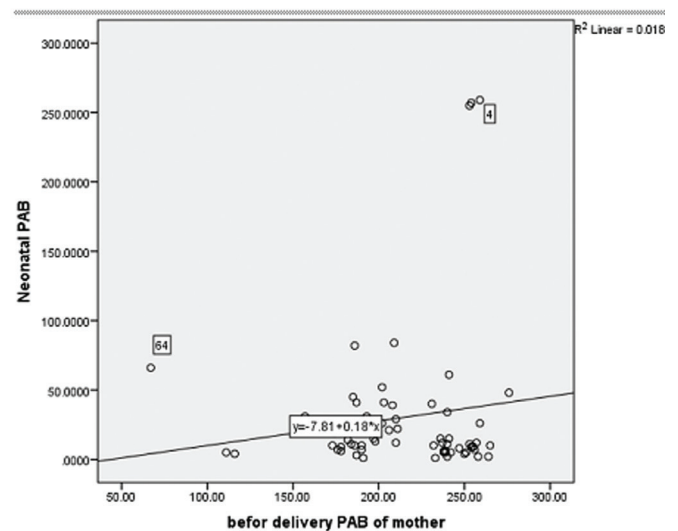


Figure 1. The correlation between maternal PAB before delivery and neonatal PAB
PAB: Prooxidant-antioxidant balance

neonatal PAB was one-sixth of maternal PAB and there was a moderate association between neonatal and maternal PAB before delivery although it was not significant.

Levels of vitamins A, E, and beta-carotene have been reported to be lower in the umbilical cord plasma of neonates compared to maternal plasma. This is due to low levels of lipophilic molecules in the umbilical cord which limits the transmission capacity of the umbilical cord plasma for tocopherols and carotenoids (28). OS is found to play a role in the pathogenesis of many neonatal conditions, including asphyxia, retinopathy of prematurity (ROP), and bronchopulmonary dysplasia. However, PAB may be disturbed and OS increased due to the immaturity of the fetal antioxidant system. Premature neonates frequently need extra oxygen, and rapid changes in tissue oxygen levels from low intrauterine to high levels, especially if sudden, can lead to the decreased vascular endothelial growth factor. This results in impaired vascularization of the retinal vessels and may lead to ROP (29).

There is a critical and sensitive balance in the cell between free radical formation and antioxidant defense and repair systems, as there is a normal physiological balance between antioxidants and peroxides in healthy cells, when free radicals are produced by normal cellular function but neutralized by the antioxidant system. This system can pass through the placenta, and the highest amounts of antioxidants are transferred in the third trimester of pregnancy (2,5).

In the present study, the mean value of PAB (measured in HK units) in neonates was about one-sixth of the level in the mothers. Thus this system appears to be inadequate in neonates. However, neonates are exposed to increased OS in high-risk pregnancies and deliveries including premature rupture of membrane and preeclampsia, and in these cases, the lower levels of natural PAB may be inadequate to protect fetal tissues.

Conclusion

Pregnancy and labor change the PAB by increasing oxygen consumption and energy demands. We suggest that more care should be provided for mothers in order to maintain a proper balance during pregnancy, especially during delivery when PAB increases and reaches its peak before labor. The mean PAB of neonates was found to be one-sixth of its level in mothers. The low levels of PAB in neonates predispose them to greater vulnerability compared to their mothers.

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Ethics Committee Approval: This work was approved by the ethical and scientific committees of the School of Medicine, Mashhad University of Medical Sciences (approval number: IR.MUMS.MEDICAL.REC.1398.375).

Informed Consent: It was obtained.

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Increased bladder injury rate during emergency and repeat cesarean section

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Abstract

Objective: Bladder injury is one of the complications of cesarean section (CS). It is reported that the overall incidence of bladder injury is 0.22-0.44% of CS. It is, however, unclear what factors influence this rate. The aim of this study was to determine if there is a difference in bladder injury rate between scheduled and emergency CS, as well as in primary and repeat CS at a large metropolitan hospital that serves a population at high risk for obstetric complications. In addition, the use of urology consultation following bladder injury and whether demographic factors and labor characteristics affect the rate of bladder injury were investigated.

Material and Methods: A total of 8,488 records were reviewed (4,292 primary CS and 4,196 repeat CS) from January 1, 2013 to December 31, 2020. The incidence of bladder injury was calculated and the rate of intraoperative urology/urogynecology consultation was recorded. Then the association between bladder injury and intraoperative urology/urogynecology consultation and between bladder injury and maternal age, body mass index (BMI), and gestational age were compared.

Results: There was a significant increase in risk of bladder injury in repeat CS versus primary CS ($p=0.01$). There was also a significant increase in risk of bladder injury in emergency CS versus scheduled CS ($p=0.04$). Intraoperative urology/urogynecology consultations were significantly higher in the bladder injury versus no bladder injury groups ($p<0.0001$). Both emergency CS and repeat CS are predictors of bladder injury with odd ratios of 5.7 and 7.4, respectively.

Conclusion: These results add to the existing evidence that bladder injury is a rare complication in CS that may occur more often in women undergoing repeat or emergency CS than primary or scheduled CS. Given that the risk increases with repeat or emergency CS, patients should be made aware of such risks and surgeons should make careful intraoperative considerations with close postoperative follow-ups. (J Turk Ger Gynecol Assoc 2023; 24: 97-100)

Keywords: Bladder, cesarean, urogynecology

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Introduction

According to the National Center for Health Statistics, the cesarean section (CS) delivery rate in the United States was 31.9% for 2020, representing a steady increase since 1996 with a brief decline in 2008 and 2009 (1). As the CS rate has increased, maternal mortality and morbidity have also risen steadily over the last three decades (2). In 2004 it was reported that the overall complication rate from CS was 21.4% (3). In

recent literature these complications are widely discussed. Uterine rupture, abnormal placentation, ectopic pregnancy, and preterm birth are most common (4). In a meta-analysis, emergency CS was associated with significantly more maternal and fetal complications and mortality than elective CS (5). During a CS, the most common approach is to make a low transverse incision across the lower uterine segment. Anterior to this location is the bladder. Creating a bladder flap depends on the physician's preference and the time available for the



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procedure. Most physicians avoid creating a bladder flap since there is no evidence that supports the bladder flap as a method to reduce bladder injury (6). On the contrary, there is evidence showing that bladder flap formation is associated with short-term urinary complaints, such as urinary retention and dysuria (7-9). There is, however, an increased operative time in CS deliveries when a bladder flap was created versus those when bladder flap was omitted (9,10).

The overall bladder injury incidence is reported to be 0.22-0.44%. Furthermore, these rates are 0.11-0.42% for primary CS and 0.27-0.81% for repeat CS (11-14). Repeat CS is the primary risk factor for bladder injury (12). The reason is attributed to the formation of adhesions postoperatively and it is shown that the incidence of adhesive disease following a primary CS ranges from 46-65% (15). In patients with repeat CS, the bladder may be adherent to the lower uterine segment where the hysterotomy is commonly made. Bladder injuries can occur at the time of bladder flap creation, hysterotomy and even closure of the hysterotomy because of the proximity of the bladder to the inferior aspect of the hysterotomy. Unfortunately, in emergency CS, diligent and careful dissection is not the most important priority. Failed vaginal delivery continues to be the major cause of emergency CS, primarily due to fetal distress and abnormal fetal position (16). Emergency CS was reported to be accompanied by significantly more maternal and fetal complications and mortality than elective CS in a meta-analysis (5). Although there are case reports of bladder injury during emergency CS, there is no clear data showing whether there is an association or not.

It has been reported that repeat CS is a risk factor for bladder injury (13). In this study, the aim was to determine if there is a difference in bladder injury rates between scheduled and emergency CS, and between primary and repeat CS at a large metropolitan hospital that serves a population at high risk for obstetric complications. In addition, the use of urology consultation following bladder injury and whether demographic factors and labor characteristics affect the rate of bladder injury were investigated. Recommendations will be made, based on the findings of this study.

In a major study, the overall incidence of bladder injury was 0.28%, with an incidence of 0.56% during repeat CS and 0.14% during primary CS (13). To show such an effect, at least 3,103 cases per group needed to be reviewed. Despite fast-paced developments in this medical field, there is no large and recent study investigating bladder injury in repeat and emergency CS in the last two decades. In the present study, 8,488 records were reviewed, consisting of 4,292 primary CS and 4,196 repeat CS from January 1, 2013 to December 31, 2020. This study is the first to investigate the association between bladder injury and intraoperative urology/urogynecology consultation and between bladder injury and maternal age, body mass index (BMI), and gestational age.

Material and Methods

This was a retrospective cohort study that received Ascension St. John Hospital Institutional Review Board exemption (approval number: 1790154) and exemption for informed consent. A chart review was performed of women who underwent either a scheduled or emergency CS delivery at a large urban metropolitan hospital. The inclusion criterion was women (18 years and older) who underwent a scheduled or emergency CS delivery from January 1, 2013, to December 31, 2020. The billing data was queried for cases using the ICD-9 codes of 740, 741, 742, and 744 as well as ICD-10 codes of 10D00Z1, 10D00Z2 and 10D00Z0. Once the cases were identified, the operative reports were reviewed for surgical details, as well as the anesthesia and nursing reports, to identify the pertinent variables. The following variables were collected from these charts: patient demographics, BMI, gestational age at delivery, primary or repeat CS, reason for CS, bladder injury noted at the time of surgery, if the patient had been in labor, and the type of anesthesia. The primary outcome was the incidence of bladder injury. The secondary outcomes were intraoperative urology/urogynecology consultation, demographic characteristics, and the nature of the CS (repeat or emergency).

Statistical analysis

Statistical analysis was done using SPSS version 25.0 (IBM Inc., Armonk, NY, USA) and a p-value of less than 0.05 was considered to indicate statistical significance.

Comparisons were made between the group that had no bladder injury and the group that had bladder injury. Continuous variables were analyzed using the mean with standard deviation or median with range or interquartile range. Categorical variables were analyzed using frequency distributions and chi-squared test. Univariable analysis of factors associated with bladder injury were assessed using Student's t-test and the chi-squared analysis. Non-parametric tests were used for data that are non-normally distributed. A Mann-Whitney U test was used for analysis of the cervical dilation due to the skew and variability. Multivariable analysis of bladder injury was performed using logistic regression.

Results

A total of 8,488 CS reports were reviewed, dating from January 1, 2013, to December 31, 2020. There were 3,838 emergency CS (45%) and 4,650 (55%) scheduled CS. There were also 4,292 primary CS (51%) and 4,196 repeat CS (49%). There was a total of 28 cases of bladder injury identified including 18 (64%) during emergency CS versus ten (36%) during scheduled CS ($p=0.04$). Furthermore, seven (25%) of bladder injury cases were primary CS versus 21 (75%) in repeat CS ($p=0.01$). Intraoperative urology/urogynecology consultation

was made in 24/28 (86%) of bladder injury cases versus 0% in the “No Bladder Injury” group ($p < 0.0001$) (Table 1). The mean gestational age in weeks in the No Bladder Injury group was 37.7 ± 3.1 versus 37.4 ± 3.3 in the “Bladder Injury” group ($p = 0.68$) (Table 2). There was a significant difference in the proportion of women in “No Bladder Injury” group (32%) being in labor versus 57% in “Bladder Injury” group ($p = 0.004$).

A significant increase was found in the risk of bladder injury in repeat CS versus primary CS ($p = 0.01$). There was also a significant increase in the risk of bladder injury in emergency CS versus scheduled CS ($p = 0.04$). Intraoperative urogynecology/urology consultations were significantly more common in the bladder injury group versus no bladder injury group ($p < 0.0001$). Both emergency CS and repeat CS were predictors of bladder injury with odd ratios of 5.7 and 7.4, respectively (Table 3).

Discussion

This study shows an increased risk of bladder injury in the setting of emergency CS versus scheduled CS with a relative

Table 1. Cesarean section delivery characteristics in the study sample

Delivery and post-delivery			
	No bladder injury, (n=8460)	Bladder injury, (n=28)	p-value
	n (%)	n (%)	
Failed operative delivery	71 (1)	0 (0)	-
Cesarean delivery			0.04
Emergent	3820 (45)	18 (64)	
Scheduled	4640 (55)	10 (36)	
Cesarean delivery			0.01
Primary	4285 (51)	7 (25)	
Repeat	4175 (49)	21 (75)	
Foley catheter	8457 (100)	28 (100)	-
Urogynecology consultation	1 (0)	24 (86)	<0.0001

Table 2. Patients’ background characteristics

Background			
	No bladder injury, (n=8460)	Bladder injury, (n=28)	p-value
	Mean ± SD	Mean ± SD	
Age (years)	30.0 ± 5.8	31.1 ± 5.0	0.30
Body mass index at admission	34.6 ± 7.8	32.9 ± 6.9	0.28
Gestational age (wks.)	37.7 ± 3.1	37.4 ± 3.3	0.68

SD: Standard deviation, BMI: Body mass index

Table 3. Logistic regression analysis for the risk of bladder injury

Characteristics	Odd ratio	p-value	95% CI
Emergency CS	6.68	<0.0001	2.5, 13.0
Repeat CS	7.42	<0.0001	3.0, 18.6

CS: Cesarean section, CI: Confidence interval

risk (RR) of 100%. In addition, a higher risk of bladder injury was associated with repeat CS versus scheduled CS (RR: 68%). In cases where bladder injury was suspected, urogynecology/urology was most likely to be consulted intraoperatively. There was no association between having a bladder injury and maternal age, BMI, and gestational age at the time of delivery. CS increases the risk of postoperative adhesions at the surgical site (17). Since the bladder is in close proximity to the anterior uterus, it can be affected by nearby anatomical changes. Adhesions were present in 37% of patients with prior CS while these were only found in 10% of patients with no prior CS in a study that evaluated over 15 thousand patients (17). It was also reported that adhesions were found in 32% of women who had one CS, in 42% after two CSs, and 59% after three or more CSs (17). Tulandi et al. (18) studied the site and extent of adhesion post CS. They reported that dense adhesions between the uterus and the bladder were found in 29.8% of one-repeat CS patients group versus 46.3% in two-repeat CS patients (18). These authors also reported dense adhesions between the uterus and the abdomen in 25.6% of one-repeat CS versus 48.2% in two-repeat CS (18). In a study that presented transvaginal ultrasound findings following CS, there was obliteration of the anterior cul-de-sac in multiple cases and fibrosis between lower uterine segment and cervix with the bladder in others (19). These findings correlate to limited mobility of the bladder and therefore increase the risk of bladder injury.

There is no consensus on the most favorable method for opening or closure of CS. However, some studies evaluated the impact of different uterine closure techniques on lower uterine segment anatomy and formation of adhesions. Double-layer closure was associated with better but thicker uterine scar healing than single layer closure (20,21). In addition, increased uterine scar thickness was found to increase adhesions and compression effect on the bladder, seen as a bulging mass on cystoscopy and therefore, increase risk of bladder injury (22). In addition to the formation of adhesions, changes to the natural position of the uterus can occur, thus increasing the risk of bladder injury in subsequent surgery. There is a statistically significant increase in antepartum and postpartum flexion angle of the uterus between cesarean and vaginal delivery (23).

Study Limitations

There is no agreement in the literature on why bladder injury risk increases with emergency CS. It is likely that the emergency itself and the anatomical changes mentioned above increase the risk of errors and therefore, iatrogenic injuries in many different ways.

Conclusion

These results build on the existing evidence that bladder injury is a rare complication of CS that more often occurs in women undergoing repeat or emergency CS compared to primary or scheduled CS. Our study has several strengths, including the large sample volume and diverse demography. Also, the study data came from an urban institution that serves a diverse population making it applicable to a wider profile of cases. However, the generalizability of our study is limited by its retrospective nature and the single institution data source could be non-inclusive to other centers that practice different techniques. Additional analysis is needed to understand the association between different CS techniques and bladder injury and the additive effect of repeat and emergency CS on the risk of bladder injury.

Due to the rapid increase in the prevalence of CS deliveries, the risk of bladder injury during primary versus repeat CS and scheduled versus emergency CS was assessed. Given that the risk increases with repeat and emergency CS, patients should be made aware of such risk and surgeons should make careful intraoperative considerations with close postoperative follow-ups.

Ethics Committee Approval: *This was a retrospective cohort study that received Ascension St. John Hospital Institutional Review Board exemption (approval number: 1790154).*

Informed Consent: *This was a retrospective cohort study that received exemption for informed consent.*

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The role of multiple high-risk human papillomavirus infections for cervical biopsies and findings in colposcopic procedures

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Abstract

Objective: The clinical outcome of high-risk HPV (hr-HPV) infection varies according to genotype(s). Patients may harbor either one single hr-HPV (s-HPV) or multiple HPV (m-HPV) genotypes. Recently, the relationship between m-HPV infections and high-grade dysplasia has been investigated, and controversial results have been obtained. Therefore, the clinical significance of m-HPV is not clear. This study aimed to evaluate which group is associated with higher grade dysplasia by analyzing colposcopic punch biopsies.

Material and Methods: A total of 690 patients who were scheduled for a diagnostic excisional procedure between April 2016 and January 2019 due to the detection of high-grade cervical intraepithelial neoplasia (CIN 2/3) in colposcopy were included. Patients who were not scheduled for colposcopic examination or cervical punch biopsy, or who were scheduled for an excisional procedure due to smear-biopsy incompatibility or persistent low-grade dysplasia were excluded. Patients with a negative HPV test and an unknown HPV genotype were also excluded.

Results: Among the patients scheduled for excision (n=404), 74.5% had a s-HPV and 25.5% had a m-HPV infection. The proportion of CIN 1, 2 and 3 per patient in the m-HPV group was significantly higher than the s-HPV group (p=0.017). When this analysis was made for the number of CIN 2+3 per patient in the s-HPV and m-HPV groups, it was 1.29 (389/301) and 1.36 (140/103), respectively, and no difference was found (p=0.491).

Conclusion: Patients in the m-HPV group, who underwent more colposcopic cervical biopsies, had higher numbers of CIN lesions, regardless of age and cytology results. (J Turk Ger Gynecol Assoc 2023; 24: 101-8)

Keywords: Cervical dysplasia, cervical intraepithelial neoplasia, colposcopy, HPV, multiple HPV infection

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Introduction

Every year, 570,000 patients are diagnosed with cervical cancer worldwide, and approximately 310,000 patients die (1). Although human papillomavirus (HPV), especially high-risk HPV (hr-HPV), infection is a prerequisite for cervical cancer, additional risk factors leading to viral persistence play an important role in the oncopathogenesis (2-4). More than 200 HPV genotypes have been identified and the relationship

between some genotypes and cervical cancer has been well elucidated (5,6). Based on cervical cancer cases and control group studies, “the International Agency of Research on Cancer (IARC)” has reported that different HPV genotypes have different oncogenic risks.

The IARC divides HPV genotypes for all cancers that may be associated with HPV into four risk groups: carcinogens (group 1), probably carcinogenic (group 2A), possibly carcinogenic (group 2B), and unclassifiable (group 3) (7). Furthermore,



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the genotypes are classified with either sufficient and limited evidence. Twenty different hr-HPV genotypes were identified in these two groups: HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 as having sufficient evidence, and HPV genotypes 26, 53, 66, 67, 68, 70, 73, and 82 as having limited evidence (8). Other HPV genotypes were defined as low or undetermined risk. Due to these risk differences, patients can be referred for colposcopy according to their HPV genotypes and cervical cytology results. Additionally, patients may harbor either one or multiple HPV genotypes. Recently, the relationship between multiple hr-HPV (m-HPV) infections and high-grade dysplasia has been investigated but, controversial results have been reported. Therefore, the clinical significance remains unclear. In a few studies, m-HPV infections are associated with cervical cancer, high-grade dysplasia, and larger cervical lesions (9-14). In contrast, Muñoz et al. (15) found no difference between the single hr-HPV (s-HPV) and m-HPV groups in terms of cervical cancer risk. However, the number of cervical punch biopsies performed in these groups was not compared.

The aim of the present study was to evaluate which group was associated with more diffuse or higher-grade dysplasia by analyzing the number of colposcopic cervical punch biopsies performed in the s-HPV and m-HPV groups.

Material and Methods

Patients included in the study had attended the gynecological oncology department due to abnormal Pap smear and/or hr-HPV genotype(s) and were scheduled for a diagnostic excisional procedure between April 2016 and January 2019 due to the detection of high-grade cervical intraepithelial neoplasia (CIN 2/3) in colposcopy. If available, patient age, cervical cytology results, HPV test results, number of colposcopic cervical punch biopsies and histopathology results, endocervical curettage (ECC) results and diagnostic excisional procedure type were retrieved from patient files and computer records.

Written and verbal consent was obtained for a standardized colposcopic examination and punch biopsy \pm ECC to be performed when necessary. All colposcopies were performed by the same team in the gynecological oncology department using the Olympus OCS 500 and Leisegang colposcopy devices. A biopsy was not performed in the cervical quadrant which had no abnormal findings on colposcopy. Colposcopic cervical sterile punch biopsies were performed under local anesthesia or sedation. The biopsies were then sent to the pathology department in formaldehyde, with a label indicating the patient name, file number, and biopsy clock dial. All punch biopsies and curettage materials were evaluated by gynecopathologists, and all histopathological results were reported according to the American Society for Colposcopy and Cervical Pathology guidelines.

Patients who were not scheduled for colposcopic examination or cervical punch biopsy, or who were scheduled for an excisional procedure due to smear-biopsy incompatibility or persistent low-grade dysplasia were excluded. Patients with microinvasive or invasive cancers detected on cervical cytology or colposcopic punch biopsy were also excluded. Pap smear results, reported using Bethesda (2014), were evaluated in five groups: benign (no dysplasia or cervicitis); atypical squamous cells-undetermined significance (ASC-US); atypical squamous cells-cannot exclude high-grade squamous intraepithelial lesions (ASC-H); low-grade squamous intraepithelial lesions (LSIL); and high-grade squamous intraepithelial lesions (HSIL). In addition, no patients had cervical cytology reported as atypical glandular cells or adenocarcinoma in situ. Cytology was characterized into two groups; Cytology group A and B. Cytology group A consisted of "ASC-H" and "HSIL" while Cytology group B consisted of "ASC-US", "LSIL" or "no dysplasia". The results of HPV-DNA (Qiagen HC2) tests conducted in the community-based national HPV screening program conducted by the Ministry of Health, General Directorate of Public Health, Cancer Department, were evaluated. Patients with negative HPV test results or unknown HPV genotype, and those infected with HPV genotypes with low or undetermined risk for cervical cancer were excluded. Only patients infected with IARC hr-HPV genotypes with sufficient and limited evidence of cervical cancer were evaluated. According to the patient's HPV genotypes, those with HPV-16 and/or HPV-18 positivity were classified as HPV group A, those with other hr-HPV genotype positivity were classified as group B, and those with HPV-16 and/or 18 and other hr-HPV genotype positivity were classified as group C. Patients infected with only one hr-HPV type were included in the s-HPV group, and those infected with at least two different hr-HPV genotypes were included in the m-HPV group. ECC results were characterized into two groups; ECC group A and B. ECC group A consisted of "CIN 2" or "CIN 3" while ECC group B consisted of "CIN 1" or "no dysplasia". Written and oral consent was obtained from all patients before surgery. The study was reviewed by the University of Health Sciences Turkey, Zeynep Kamil Women and Children Diseases Training and Research Hospital Local Ethics Committee and was performed under the ethical standards described in an appropriate version of the 1975 Declaration of Helsinki, as revised in 2000 (approval number: 28, date: 05.02.2020).

Statistical analysis

IBM SPSS, version 25.0 (IBM Corp., Armonk, N.Y., USA) was used for the statistical analysis. The mean, median, and standard deviation (SD) were used in the descriptive statistics of the data. Student's t-test was used to compare the mean values. Non-parametric tests were used to analyze categorical and

dichotomous variables, whereas parametric tests were used to analyze continuous variables with a normal distribution. Significance was set at $p < 0.05$.

Results

Of the 690 patients included at baseline, 203 patients did not meet the HPV criteria (167 patients with HPV-negative or unknown HPV genotype and 36 patients with low or unclassified risk), 42 patients with persistent low-grade dysplasia, 24 patients without colposcopy (gross cervical lesion, cervical cancer), and 17 patients scheduled for excision due to smear-biopsy incompatibility were excluded. In total, 404 patients with high-grade dysplasia (CIN 2/3) were included (Figure 1). The HPV genotypes, cervical cytology results, colposcopic punch biopsy counts, ECC results, and excisional procedure types are shown in Table 1. Among the patients scheduled for excision, 74.5% (301/404) had a s-HPV and 25.5% (103/404) had a m-HPV infection. The mean age of the patients was 40.5 ± 0.4 years and the mean age of the m-HPV group was significantly lower than that of the s-HPV group ($p = 0.032$). There was no significant difference between the percentage distribution of

Table 1. Pathological data and HPV genotype related characteristics (n=404)

	%	n
HPV genotyping		
↗ HPV 16 positivity	54.7 ↘	221
HPV group A → HPV 18 positivity	4.2 → 60.6	17
↘ HPV 16 and 18 positivity	1.7 ↗	7
HPV group B → HPV others high risk (not including 16 or 18)	19.1	77
HPV group C → HPV others high risk and HPV 16 and/or 18	20.3	82
Cervical cytology		
No lesion or cervicitis	54.2	219
ASC-US	12.6	51
ASC-H	6.2	25
LSIL	12.4	50
HSIL	5.4	22
Inadequate sampling	9.2	37
Single or multiple HPV status		
s-HPV group	74.5	301
m-HPV group	25.5	103
Colposcopic cervical biopsy count		
One biopsy	25.0	101
Two biopsies	35.2	142
Three biopsies	27.7	112
At least four biopsies	12.1	49
ECC results		
No lesion or cervicitis	54.2	219
CIN 1	6.4	26
CIN 2	11.9	48
CIN 3	24.0	97
Inadequate sampling	2.5	10
Not performed	1.0	4
Excisional procedure type		
LEEP	10.6	43
CKC	74.0	299
Other center or refuse the treatment	15.4	62

HPV: Human papillomavirus, ASC-US: Atypical squamous cells-undetermined significance, ASC-H: Atypical squamous cells-cannot exclude high grade squamous intraepithelial lesions, LSIL: Low grade squamous intraepithelial lesion, HSIL: High grade squamous intraepithelial lesion, LEEP: Loop electrosurgical excision procedure, CKC: Cold knife conization, s-HPV: Single HPV, m-HPV: Multiple HPV, ECC: Endocervical Curettage, CIN: Cervical intraepithelial neoplasia

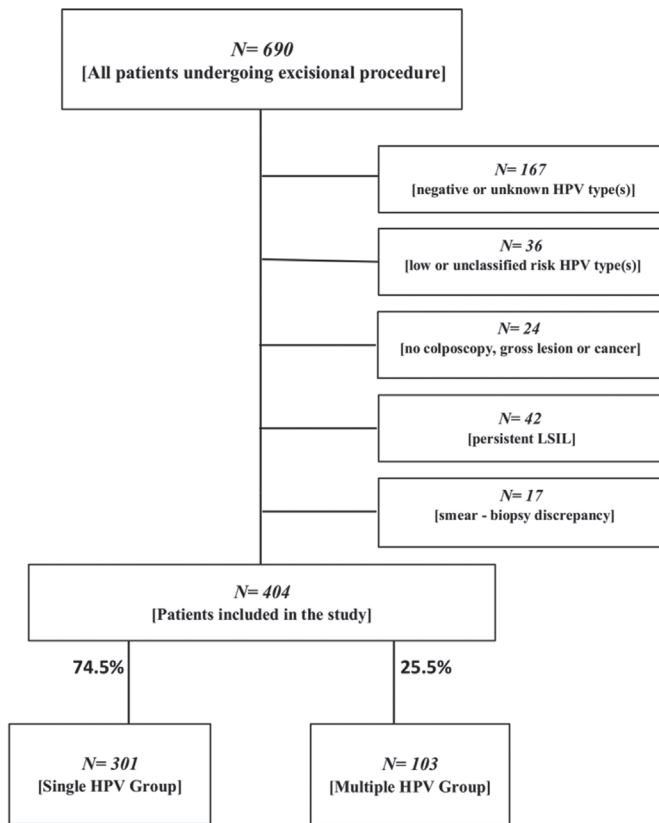


Figure 1. Inclusion and exclusion criteria are shown in the flow chart of this study
HPV: Human papillomavirus, LSIL: Low-grade squamous intraepithelial lesion

the ECC groups ($p=0.214$) and cytology groups ($p=0.710$) in the s-HPV and m-HPV groups. All statistical analyses are presented in Table 2.

A total of 17 different genotypes were available (except for HPV-26, -67, and -73) from 20 different HPV genotypes that were reported as high risk. In the s-HPV (73.4%) and m-HPV (68.9%) groups, there was no difference in the percentage of existing HPV-16 infection, which is the most common genotype ($p=0.380$). In the s-HPV group, the second most common genotype was HPV-31 (6%) and the third was HPV-18 (5.6%). In the m-HPV group, the most common types, excluding HPV-16, were HPV-18 (31.1%), HPV-51 (20.4%), HPV-31 (16.5%), and HPV-39 (16.5%). In the m-HPV group, 62.1% (64/103) of the patients were infected with two, 22.3% (23/103) with three, 10.7% (11/103) with four, and 4.9% (5/103) with five HPV

Table 2. Pathological data and HPV genotype related characteristics in single (s) or multiple (m) high-risk HPV groups

	HPV groups		
	s-HPV	m-HPV	p-value
Ages (n=404)			
Mean of age	41.06	38.97	$p^a=0.032$
Referral cytologies (n=367) (%)			
No dysplasia	158 (57.9)	61 (64.9)	$p=0.725$
ASC-US	41 (15.0)	10 (10.6)	
ASC-H	20 (7.3)	5 (5.3)	
LSIL	38 (13.9)	12 (12.8)	
HSIL	16 (5.9)	6 (6.4)	
Cytology group A	36 (13.2)	11 (11.7)	$p=0.710$
Cytology group B	237 (86.8)	83 (88.3)	
ECC results (n**=390) (%)			
ECC group A	113 (39.0)	32 (32.0)	$p=0.214$
ECC group B	177 (61.0)	68 (68.0)	
Number of colposcopic biopsies (n=404) (%)			
One biopsy	84 (27.9)	17 (16.5)	$p=0.003$
Two biopsies	110 (36.5)	32 (31.0)	
Three biopsies	73 (24.3)	39 (37.9)	
Four biopsies	28 (9.3)	12 (11.7)	
Five biopsies	6 (2.0)	3 (2.9)	
One or two biopsies	194 (64.5)	49 (47.6)	
At least three biopsies	107 (35.5)	54 (52.4)	
Mean number of biopsies	2.21	2.53	$p^a=0.005$
Cytology group A: Consist of ASC-H and HSIL, Cytology group B: Consist of no dysplasia, ASC-US or LSIL, ECC group A: Consist of CIN 2 or CIN 3, ECC group B: Consist of CIN 1 or no dysplasia. N: Number of patients, N*: Inadequate sampling excluded, N**: Unknown ECC results excluded, %: Percent, p: Obtained by Pearson χ^2 , p^a : Obtained by Independent Samples t-test			

genotypes. All hr-HPV genotypes in this study, except for HPV-16, were present at a higher rate in the m-HPV group than in the s-HPV group (Table 3).

At least one and at most five cervical biopsies were performed for all patients. When the rates of having ≥ 3 biopsies and having ≤ 2 biopsies were analyzed, the rate of having ≥ 3 biopsies was higher in the m-HPV group ($p=0.003$). In the m-HPV group, 52.5% of the patients had ≥ 3 biopsies (Figure 2). Furthermore, the mean number of biopsies was significantly higher in the m-HPV group than the s-HPV group (2.53 vs 2.21, respectively; $p=0.005$). The calculated mean biopsy numbers of HPV groups A, B and C were 2.27, 2.14, and 2.50, respectively, with no statistical differences between the groups ($p=0.061$).

The total number of punch biopsies was 665 for 301 patients in the s-HPV group and 261 for 103 patients in the m-HPV group. The detection rates of CIN 1, 2 and 3 in punch biopsies taken in the s-HPV and m-HPV groups were calculated as 72.3% (481/665) and 73.2% (191/261), respectively and in terms of detecting dysplasia, there was no statistical difference between the groups ($p=0.794$) (Figure 3). The detection rates of CIN 1, 2, and 3 in the cytology A and B groups were calculated as 75.4% (101/134) and 72.1% (505/700), respectively, and again there was no difference between the groups ($p=0.442$). However, the

Table 3. Percentages of high-risk HPV genotypes found in single (s) or multiple (m) groups

HPV Genotypes	s-HPV	%	m-HPV	%
16	221	73.4	71	68.9
18	17	5.6	32	31.1
26	-	-	-	-
31	18	6.0	17	16.5
33	9	3.0	10	9.7
35	6	2.0	12	11.7
39	2	0.7	17	16.5
45	3	1.0	15	14.6
51	5	1.7	21	20.4
52	3	1.0	13	12.6
53	-	-	8	7.8
56	3	1.0	7	6.8
58	6	2.0	8	7.8
59	1	0.3	14	13.6
66	2	0.7	8	7.8
67	-	-	-	-
68	3	1.0	8	7.8
70	-	-	3	2.9
73	-	-	-	-
82	2	0.7	2	1.9
HPV: Human papillomavirus, s-HPV: Single HPV, m-HPV: Multiple HPV				

number of CIN 1, 2, and 3 per patient in the m-HPV group was significantly higher than in the s-HPV group ($p=0.017$). All the statistical analyses are detailed in Table 4. The age distribution of the HPV groups is shown in Figure 4.

The power analysis of the study was calculated using OpenEpiPower to compare the two means calculators at www.openepi.com. The mean number of biopsies in the s-HPV and m-HPV groups was 79.8% at a 95% confidence interval with \pm SD values.

Discussion

The prevalence of m-HPV in infected patients is reported to range between 18.5% and 46% (9,14,16). In the present study the m-HPV rate was 25.5% and significantly more punch biopsies were performed in this group. m-HPV infection is associated with larger lesions and more severe dysplasia (10,12,16,17). It has also been discussed in microdissection studies that s-HPV infection may cause dysplasia, and m-HPV infection may be associated with a greater number of cervical dysplasias (18,19). The findings of the present study support these previous reports that infection with more than one HPV genotype causes lesions that are more common and can involve more than one quadrant. However, Li et al. (20) reported that high-grade dysplasia and

cancer lesions are more common in s-HPV infections. In the present study, the counts of CIN 2 and 3 lesions per patient did not differ between the s-HPV and m-HPV groups.

Lesion size is related to cervical cytology severity (21,22). However, Nam et al. (23) found no relationship between cytology results and quadrant involvement. Cytology and ECC results can be seen as confounding factors in these three previous studies. However, we believe that because these possible confounding factors did not show a significant distribution difference in the s-HPV and m-HPV groups, it makes our findings more robust. In addition, the present study provided an opportunity to examine age-related changes in a patient with high grade dysplasia and hr-HPV positivity. When the distribution of the population by age was examined, both the m-HPV and s-HPV groups showed a single peak between the ages of 30 and 40. Since the mean age of the m-HPV group was younger, it appears that infections with hr-HPV of more than one genotype decrease as patient age increases. This may be associated with an individual's immunity or a decreasing number of sexual partners with age.

Statistical analysis also allowed us to examine the HPV hierarchy in the group to be excised. Exception for HPV-16, 16 different hr-HPV genotypes had higher rates in the m-HPV group than in the s-HPV group. Furthermore, the similar distribution of the HPV-16 infection in the s-HPV and m-HPV groups was at least as valuable as the other confounding factors such as the ECC and cytology groups. The close relationship of the HPV-18 genotype with CIN 2, 3, and cancer lesions has been shown in previous studies (24-26). In our results, HPV-18 infection was found more frequently in the m-HPV group, suggesting that the mechanisms of oncopathogenesis in m-HPV infections may work differently than what has previously been suggested. Moreover, the fact that 54.2% of the patients had normal cytology results emphasizes the importance of cervical cancer screening using this co-test.

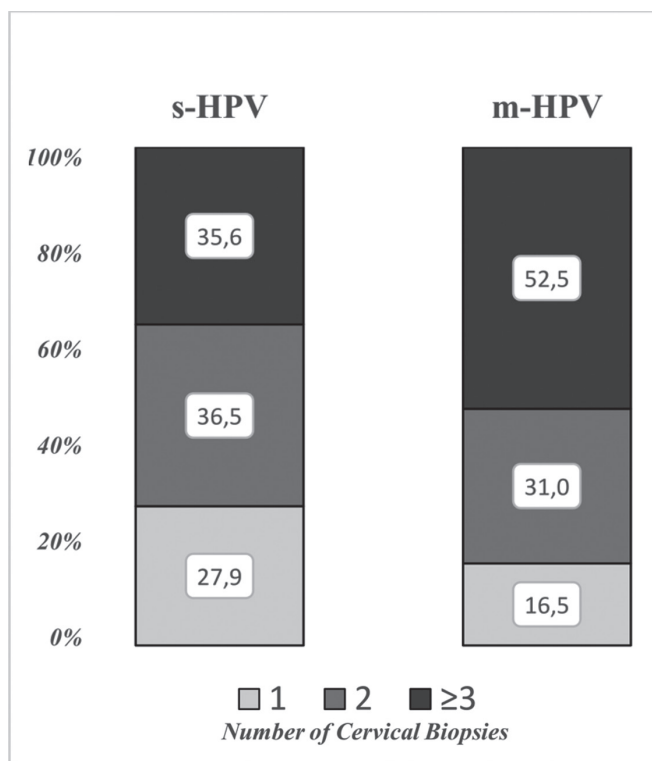


Figure 2. Percentage distributions of cervical punch biopsy count according to single or multiple high-risk HPV groups
m-HPV: Multiple HPV, s-HPV: Single HPV, HPV: Human papillomavirus

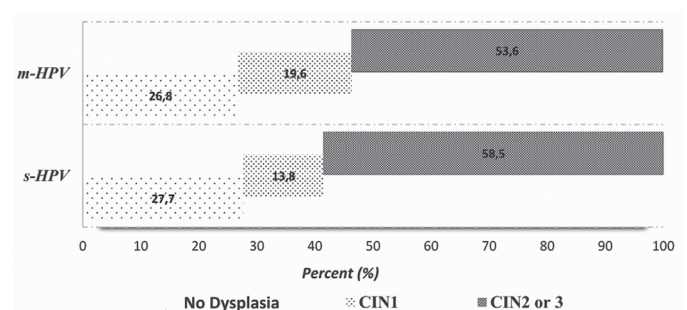


Figure 3. Percents of high-risk s-HPV and m-HPV groups according to no dysplasia, CIN 1 or CIN 2-3
m-HPV: Multiple HPV, s-HPV: Single HPV, CIN: Cervical intraepithelial neoplasia, HPV: Human papillomavirus

Table 4. Distribution of biopsy results in high-risk single (s) or multiple (m) HPV groups

	Punch biopsy count	s-HPV				m-HPV				p-value
		Patient (n)	Punch biopsies (Total)	Punch biopsies (DD)	%	Patient (n)	Punch biopsies (total)	Punch biopsies (DD)	%	
Normal/cervicitis	Total	301	665	184	27.7	103	261	70	26.8	p=0.794
CIN 1	Total	301	665	92	13.8	103	261	51	19.6	p=0.031
CIN 2, 3	Total	301	665	389	58.5	103	261	140	53.6	p=0.179
CIN 1, 2, 3	DD Punch Biopsy per patient (DD/N) (CIN 2-3)									
	1.29				1.36				p ^b =0.491	
	1	84	84	77	91.7	17	17	16	94.1	p ^a =1.000
	2	110	220	164	74.5	32	64	50	78.1	p=0.559
	3	73	219	144	65.8	39	117	78	66.7	p=0.866
	≥4	34	142	96	67.6	15	63	47	74.6	p=0.314
	Total	301	665	481	72.3	103	261	191	73.2	p=0.794
CIN 1-2-3	DD Punch biopsy per patient (DD/N) (CIN 1-2-3)									
	1.60				1.85				p ^b =0.017	

P: Obtained by Pearson χ^2 , P^a: A obtained by Fisher's exact test, p^b= Obtained by Independent samples t-test, n: Number, DD: Dysplasia detected, CIN: Cervical intraepithelial neoplasia

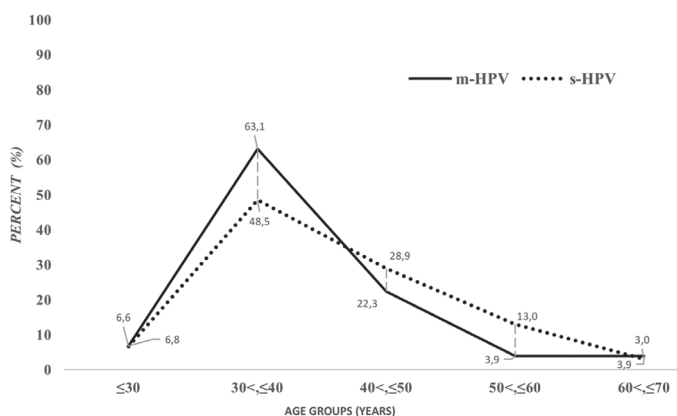


Figure 4. Proportional distribution of single and multiple high-risk HPV genotypes according to age groups
m-HPV: Multiple HPV, s-HPV: Single HPV, CIN: Cervical intraepithelial neoplasia, HPV: Human papillomavirus

The possibility of detecting lesions increases with a higher number of biopsies (21,27). In another study, the benefit of obtaining multiple biopsies, independent of cytology and HPV-16 status, was reported (28). In the current study, the number of dysplasias per patient in the s-HPV and m-HPV groups was 1.60 and 1.85, respectively, with a significant difference between these groups. These results support the hypothesis that because there are more colposcopic findings in the m-HPV group, more biopsies are performed.

This study is valuable because of the number of biopsies and the prevalence of dysplasia in light of HPV genotypes in a large group of patients with high-grade lesions, planned excision, and infection with hr-HPV genotypes. The possible limitations

of this study include retrospective design, HPV and cervical cytology results were known before colposcopic procedures, two different colposcopes were used, and colposcopy was performed by four different experts. However, even if physicians feel the need to perform more biopsies in cytology reports, such as HSIL or ASC-H, the distribution of these cytologies in the s-HPV and m-HPV groups did not differ. By excluding IARC defined low-risk HPV genotypes from the study, patient groups were more homogeneous leading to more robust findings. Owing to the high rate of patients needing ECC, we were also able to analyze the ECC results in the s-HPV and m-HPV groups. The most important parameter that distinguishes this study from others is the examination of the colposcopy punch biopsy counts and HPV genotypes of patients who were scheduled for excision due to the detection of CIN 2/3. Therefore, we evaluated patients with persistent hr-HPV genotypes infections, that is, those diagnosed with CIN 2 or 3 in the colposcopic biopsy. As this study was not a colposcopy accuracy study, evaluating all patients who underwent colposcopy would lead to an increase in confounding factors. All patients had hr-HPV genotype(s) specified by the IARC and high-grade pre-invasive disease. In this specific population, if the relationship between colposcopic punch biopsies and dysplasia detected in these biopsies with HPV genotypes is analyzed, the most valuable and accurate result can be achieved. If we evaluated a newly infected population (insufficient virus persistence), it would be expected that there would be fewer abnormal findings on colposcopic examination. Therefore, the mean number of punch biopsies would be lower. This could lead to

misinterpretation of HPV genotypes found in newly infected patients as low-risk. We also had the opportunity to compare the distribution of hr-HPV genotypes and their hierarchies in this specific group.

Conclusion

Patients in the m-HPV group, who underwent more colposcopic cervical biopsies, had higher numbers of CIN lesions, regardless of age and cytology results. However, there was no relationship between the increase in the number of biopsies and the detection of high-grade lesions. The mechanisms by which m-HPV infections cause dysplasia, how they spread to different quadrants, and why they show more CIN lesions should be investigated.

Ethics Committee Approval: The University of Health Sciences Turkey, Zeynep Kamil Women and Children Diseases Training and Research Hospital Institutional Review Board approved the study (approval number: 28, date: 05.02.2020).

Informed Consent: Written and oral informed consents were obtained from all patients.

Peer-review: Externally peer-reviewed.

Author Contributions: Surgical and Medical Practices: S.A., M.A., C.K.; Concept: S.A., U.K.Ö., M.A.; Design: S.A., E.K., M.A., C.K.; Data Collection or Processing: S.A., U.K.Ö., C.M.A.; Analysis or Interpretation: S.A., U.K.Ö., C.M.A.; Literature Search: S.A., U.K.Ö., C.M.A.; Writing: S.A., U.K.Ö., E.K., C.M.A., C.K., M.A.

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Neurovascular bundle-sparing ventral clitoroplasty in adult patients: description of the technique and long-term outcome on clitoral functions

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Abstract

Objective: To describe the technique and assess long term effects of neurovascular bundle-sparing adult clitoroplasty on clitoral functions in patients.

Material and Methods: A case series study enrolling three patients diagnosed with adult clitoromegaly who underwent neurovascular bundle-sparing ventral clitoroplasty operation. All of the patients were examined at the first, third, sixth, twelfth and twenty-fourth months post-operatively to evaluate clitoral functions.

Results: Three patients diagnosed with adult clitoromegaly, aged 17, 21 and 24 years, were enrolled in the study. The primary complaint of all patients was unpleasant enlarged appearance and hypersensitive clitoris. Mean calculated clitoral index was 143 mm², 150 mm², and 120 mm². Operation time was 90, 140 and 120 minutes, respectively. No major complication occurred during the operation but moderate ecchymosis and edema of the vulva occurred in all patients, lasting up to three weeks. On follow up examination, partial sensorial loss was noted at the first month in one patient, which completely resolved by the third month and beyond. Two patients who were sexually active reported that they were very comfortable with intercourse and cosmetic appearance. No clitoral enlargement or pain were reported by patients through the 24-month follow up period.

Conclusion: Neurovascular bundle-sparing ventral clitoroplasty is a safe and cosmetically acceptable procedure, which effectively preserves the neurovascular bundle and long-term clitoral functions. (J Turk Ger Gynecol Assoc 2023; 24: 109-13)

Keywords: Clitoromegaly, nerve sparing clitoroplasty, clitoral function

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Introduction

Compared to penile anatomy, clinical interest and focus on clitoral anatomy has long been underestimated by clinicians. The clitoris is a multiplanar structure, deep in the labia minora fat and vasculature, with a broad attachment to the pubic arch and via its supporting tissue to the mons pubis and labia. The clitoris has a paramount functional role in female sexual pleasure and orgasm, and is composed of paired bulbs, and corpora that are continuous with the crura, and glans. It is attached to the urethra and vagina centrally (O'Connell et al.).

The clitoris and the penis are homologous in much of their anatomy, and they share similar embryological origins from the genital tubercle. However, since there is no fusion of the urogenital folds the urethra does not pass through the body of the clitoral gland. Another difference from the penis is that the glans of the clitoris is encircled by a fibrous tunica albuginea which is not anatomically observed in glans penis (1). Clitoromegaly is defined as abnormal enlargement of the clitoris and may present either congenitally or can be acquired due to increased androgen exposure. There is a limited number of clinical entities that result in clitoromegaly. The



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most prevailing etiology is congenital adrenal hyperplasia which is mainly caused by 21 hydroxylase or and 11 beta hydroxylase enzyme deficiencies. Virilizing ovarian or adrenal neoplasms and inadvertent excessive androgen exposure during pregnancy are other etiological factors responsible for congenital clitoromegaly. High dose long-term use of androgen-bearing drugs or injections, polycystic ovary syndrome, and excessive clitoral stimulation secondary to masturbation can also cause acquired clitoromegaly. In rare circumstances, neurofibromatosis, epidermoid cysts, hemangiomas and some nevi can cause clitoromegaly.

The leading complaints in patients with clitoromegaly are unpleasant appearance, shape, excessive stimulation and irritation, and sexual dysfunction causing psychological consequences. The primary surgical aim is to reconstruct a pleasant clitoris without damaging neurovascular functions. In this study we assessed the long-term outcome of neurovascular bundle-sparing clitoroplasty in three patients diagnosed with adult clitoromegaly.

Material and Methods

In this retrospective case series study, 24-month follow up data of three patients diagnosed with adult clitoromegaly who underwent neurovascular bundle-sparing ventral clitoroplasty was evaluated. All of the patients were examined at the first, third, sixth, twelfth and twenty-fourth months post-operatively to evaluate clitoral function and cosmetic healing. The impact of operation on sexual outcome was assessed in two patients who were sexually active. Clitoral index was calculated by multiplying the length and width of the clitoral glans, and a diagnosis of clitoromegaly was established when the clitoral index was $>35 \text{ mm}^2$.

Description of the procedure: First, the excess skin of the clitoral hood was marked, a foley catheter placed in the bladder, and a circumferential incision was made 3-5 mm behind the corona on the inner prepuceal layer. Second, by means of parallel incisions made on the skin covering the body of clitoris, Byars' flaps were formed, and clitoral shaft was circumferentially denuded of the overlying skin cover to the clitoral body roots. Third, Buck's fascia of the clitoral body was mobilized, and neurovascular bundle coursing on the dorsolateral aspects was identified. Then, this was dissected, starting as ventral as possible up to the level of bifurcation and then suspended (Figure 1). During this step special care was taken to avoid any inadvertent injury to the neurovascular package. Fourth, following entering the avascular plane that exists between tunica albuginea and the first layer of Buck's fascia, the clitoral shaft was separated from the neurovascular package, and removed as much as possible 1 cm distal to their proximal bifurcation. Fifth, while the glans clitoris was pulled

forward using a 4/0 polyglactin suture (Figure 2), a triangle shaped incision was made on the glans clitoris, glans size was reduced and reconnected using 5/0 absorbable polyglactin. Sixth, the reduced clitoral glans, which was connected with a thin Bucks fascia, was repositioned on the remaining end of the erectile bodies, close to the crural convergence, using 2/0 absorbable polyglactin suture material. Seventh, subcutaneous tissue along the clitoral hood was reapproximated, leftover skin of the labia minora were trimmed away and reconstructed appropriately next to the glans clitoris.

The surgical procedures were performed under general anesthesia in the high dorsal lithotomy position. All of the patients were fully informed regarding the possible risks and benefits of surgery, especially a possible risk of loss of clitoral sensation was emphasized, and a written informed consent was provided from all patients. All of the procedures were performed using microsurgical instruments, when required with the use of magnification. A Foley catheter was left in place overnight and all the patients were discharged healthy, on the first postoperative day.

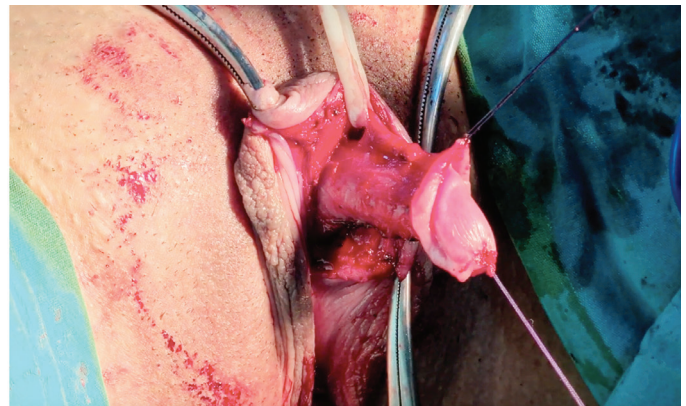


Figure 1. Neurovascular bundle coursing on the dorsolateral aspects was dissected, and suspended

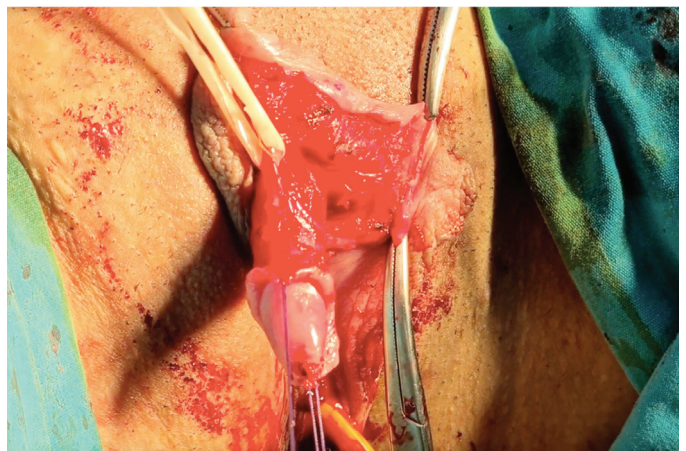


Figure 2. Glans clitoris is pulled forward using a 4/0 polyglactin suture

Results

Three patients, aged 17, 21 and 24 years were enrolled in the study (Table 1). The main underlying etiology was late onset adrenal hyperplasia in two patients and anabolic steroid use in one patient. The primary complaints of the patients were hypersensitive clitoris, unpleasant appearance, and clitoral embarrassment. Mean calculated clitoral index was 143 mm², 150 mm², and 120 mm² (Figure 3a-c). Operation times were 90, 140 and 120 minutes. No major complication occurred during the operation. However, moderate ecchymosis and edema of the vulva occurred in all patients, lasting up to three weeks (Figure 4a,b). No major blood loss, hematoma formation or ischemic tissue loss occurred. Clitoral index calculated at the sixth month post-operatively was <30 mm² in all patients (Figure 5a-c). A complete healing was observed in all patients by the third month of follow-up. However, partial clitoral sensorial loss was noted at the first month in one patient, which completely resolved by the third month and did not recur during follow-up. All patients were satisfied with the new appearance of the clitoral glans throughout follow up, and the two patients who were sexually active reported that they were quite comfortable during intercourse. Moreover, none of the patients reported clitoral pain or enlargement during follow-up. To prevent any further clitoral enlargement appropriate steroid suppression, titrated against serum 17-alpha-hydroxyprogesterone levels, was implemented in two patients diagnosed with late onset adrenal hyperplasia.

Discussion

In this case series study, it was demonstrated that neurovascular bundle-sparing ventral clitoroplasty was an effective and safe surgical technique, both in terms of cosmetic appearance and long-term sexual function.

The most crucial aspect of clitoral surgery is to preserve neurovascular functions, which entails a thorough knowledge of clitoral anatomy and its relevant structures. The clitoris is a multiplanar structure with a broad attachment to the pubic arch, mons pubis and labia (2). Centrally it is attached to the urethra and vagina. It includes erectile bodies which are

composed of paired bulbs and paired corpora, and continuous with the crura and the glans clitoris. Although it has clearly been demonstrated that clitoral body and crura have erectile tissue components, some studies argued the existence of erectile tissues in glans clitoris, vestibular bulbs and labia minora (2).

An enlarged clitoris that appears as a small penis is extremely disturbing, causing both sexual and psychological problems that have profound impacts on self-esteem. When establishing a diagnosis of clitoromegaly, the most commonly used criteria is the clitoral index, which is calculated by multiplying the length and width of the clitoral glans. It was also reported that the clitoral index increases with advancing age. The length and the width of the clitoral body can only be calculated following a through dissection. In a detailed anatomical dissection study, the mean clitoral index was 30 mm² and ranged between 16 and 100 mm², which is consistent with the classical definition of clitoromegaly as a clitoral index of >35 mm² (3). In their study, the mean measured length and width of the glans, body and crus were 8 mm and 4 mm, 29 mm and 8 mm, and 50 mm and 9 mm, respectively. In our case series, the calculated clitoral index was >35 mm² in all three and this reduced below 30 mm² following the operation in all patients.

Historically, there have been three main types of operation performed for enlarged clitoris; partial or total clitorrectomy, recession clitoroplasty, and ventral neurovascular bundle-sparing clitoroplasty. Proper anatomic reconstitution of all clitoral structures is of paramount importance for an optimal surgical outcome. Since irreversible loss of clitoral sensation, decreased ability to achieve orgasm or painful erections are among the most fearful complications of clitoral surgery, partial or total clitorrectomy has mostly been abandoned. A search for a more conservative approach has led to novel "function preserving" surgical techniques. Neurovascular bundle-preserving clitoroplasty has become the most widely accepted surgical technique for the treatment of clitoromegaly. To preserve clitoral functions, a thorough knowledge regarding the anatomical course of the neurovascular bundle is of paramount importance. The main innervation pathway of the clitoris, dubbed as the dorsal nerve of the clitoris, stems from the pudental nerve. Jackson et al. (3) eloquently demonstrated

Table 1. Characteristics of the patients

Patient no	Age	Clitoral index (mm ²)	Operation time (min)	Etiology	Complaints	Follow-up
A	17	143	90	Late onset adrenal hyperplasia	Unpleasant appearance, clitoral embarrassment	Edema, ecchymosis
B	21	150	140	Synthetic anabolic steroid use	Unpleasant appearance and hypersensitive clitoris	Edema, ecchymosis, sensorial loss at 1 st month
C	24	120	120	Late onset adrenal hyperplasia	Unpleasant appearance and hypersensitive clitoris	Edema, ecchymosis

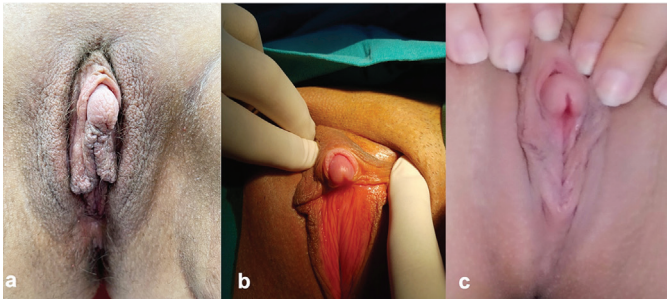


Figure 3. Preoperative clitoral images of the patients (a-c)

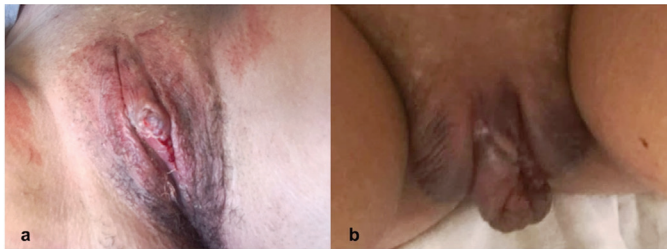


Figure 4. Postoperative ecchymosis and edema (a,b)

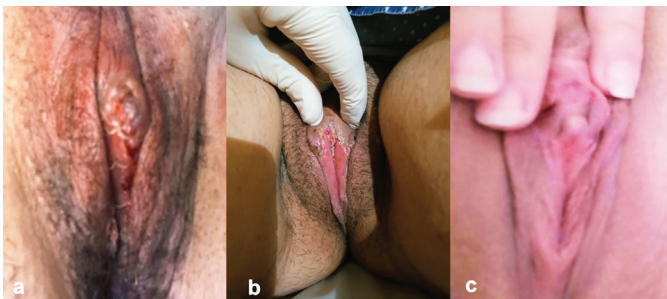


Figure 5. Postoperative clitoral images of the patients: patient (a) at 1st week, patient (b) in 1st month, and patient (c) in 3rd month

that the dorsal nerve of the clitoris emerged onto the superficial pouch of the anterior perineal triangle by piercing the pubic ramus adjacent to the medial surface of ischiopubic ramus.

Pippi Salle et al. (4) described a corporal-sparing technique dubbed “corporeal sparing dismembered clitoroplasty” in eight consecutive patients. In this technique, initially the glans and its neurovascular bundles are degloved from the corpora. Instead of resecting and reducing corporal tissue, following complete dividing of corpora starting at the bifurcation, each separated hemicorpus is rotated inferiorly and laterally, and placed inside the labial scrotal folds. These authors reported that all patients were well, without early complications during the follow up performed at six and twelve months. Additionally, “Tunica Albugenia-sparing clitoroplasty” techniques were described by some others, arguing that despite neurovascular bundles being well preserved, stripping these structures of the Tunica Albugenia leaves them unsupported and vulnerable to damage in the long term (5). However, some surgeons may

find these techniques complicated and that they can prolong the duration of surgery. Notably, the duration of the operation decreased from 140 minutes in the first case to 90 minutes in the third case, with increased experience. Moreover, none of our patients experienced irreversible loss of clitoral sensation, mostly due to applying microsurgical dissection techniques and using 4/0 and 5/0 absorbable sutures.

Another crucial step, as was performed in all our cases, is starting the separation of neurovascular bundle as ventrally as possible to prevent any possible injury to dorsal arteries and nerves of the clitoris (6). Only one patient reported partial loss of clitoral sensation at the first postoperative follow-up and normal sensation returned completely thereafter. A major remark to discuss here is that many of surgery related complaints are associated with vaginoplasty rather than clitoral surgery, since labia minora and majora are profoundly innervated structures (3). Another essential part of clitoral surgery is to reconstitute a pleasant appearing clitoris, especially the glans, provided that all anatomical structures are evenly redistributed in the perineum. Failure to do so would definitely have a profound impact on long-term psychological well-being of the patient. It has also been reported that clitoral pain or enlargement can occur following clitoral recession or incomplete reduction of erectile bodies, mostly due to entrapment of erectile tissues underneath the pubis (7). Since most of the erectile corporal tissues were removed, no episode of painful erection or clitoral enlargement was reported by any of the patients through two years of follow-up in our series. All of our patients were quite satisfied with the reconstructed new appearance of the clitoris.

Conclusion

In conclusion, neurovascular bundle-sparing ventral clitoroplasty is a feasible, safe, and cosmetically acceptable operation that improves engagement in sexual activity in the medium term. Starting dissection as ventral as possible to minimize the risk of injury and using microsurgical instruments under magnification when required, can help performance of an optimal surgery that ensures favorable outcome in terms of clitoral function. Even though various surgical techniques have been described for surgical treatment of enlarged clitoris, the optimal mode of surgery should be selected according to patients' wishes and surgical experience with the applied technique through an individualized approach.

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Ethics Committee Approval: *This current study was approved by the Institutional Review Board (approval number: E.2943, date: 15/02/2023).*

Informed Consent: *The patients have given their informed consent to be published.*

Peer-review: *Externally peer-reviewed.*

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Centile charts of cervical length in singleton and twin pregnancies between 16 and 24 weeks of gestation

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Abstract

Objective: The aim of this study was to determine the standard mid-trimester cervical lengths of singleton and twin pregnancies.

Material and Methods: This study was conducted by retrospective analysis of mid-trimester transvaginal cervical measurements of women with singleton and twin pregnancies that were examined by a single perinatologist in a single center.

Results: A total of 4621 consecutive asymptomatic pregnant women admitting for advanced obstetric ultrasound screening were evaluated. Of these 4340 (93.9%) were second trimester singleton pregnancies and 281 (6.1%) were twin pregnancies and were included. Mean cervical length measurements of singleton and twin pregnancies were 6.5 ± 38.2 mm and 7.2 ± 37.6 mm respectively ($p=0.17$). Overall, the 5th percentile of cervical length measurement after analysing singleton and twin pregnancies together was 29.4 mm at 16 weeks, 30 mm at 17 weeks, 30 mm at 18 weeks, 30 mm at 19 weeks, 30 mm at 20 weeks, 30 mm at 21 weeks, 30 mm at 22 weeks, 31 mm at 23 weeks, 29 mm at 24 weeks.

Conclusion: In our population the 5th percentile value of cervical length which is 30 mm in singletons and 10th percentile cervical length which is 31 mm in twins can be used to follow-up and treat pregnant women at risk for preterm deliveries. (J Turk Ger Gynecol Assoc 2023; 24: 114-9)

Keywords: Normogram, centile charts, cervical length, preterm birth

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Introduction

In the last few decades, developments in obstetric care has led to a considerable decrease in both maternal and perinatal mortality and morbidity. Among these obstetric complications, preterm deliveries create an increased level of burden on the individual pregnant woman, on family and on even on society as a whole, by causing long-term consequences, such as growth retardation, mental retardation, chronic diseases and cognitive impairments (1-3). Nevertheless, preterm delivery

constitutes between 7-11% of all deliveries and so remains one of the leading causes of neonatal morbidity and mortality in developed countries (4,5).

Short cervix is defined as a cervical measurement lesser than 25 mm before 24th week of pregnancy (2nd-3rd percentile) (6,7). A short cervix diagnosed before the 24th gestational week could predict preterm deliveries in later weeks of pregnancy. Risk of preterm delivery significantly increases in presence of a short cervix in women with a history of a previous preterm delivery (8-11). Therapeutic interventions, such as cervical



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cerclage placement, or medical measures, such as progesterone administration, initiated at early phases of pregnancy have been demonstrated to prevent preterm delivery in 30% to 40% of high risk patients (6,7,12). Globally, various values of cervical lengths have been proposed as a threshold level to initiate these therapeutic or prophylactic interventions throughout. Different clinical guidelines suggest cervical length shorter than 15 mm (0.5 percentile) or shorter than 20 mm (1st percentile) as threshold levels. Nonetheless, the American College of Gynecology recommends initiation of therapeutic or prophylactic interventions in case of a cervical length measurement shorter than 20 mm women without a history of preterm birth. Moreover, initiation of therapy was suggested for patients with a cervical length shorter than 25 mm in women with a previous history of preterm delivery before 34th week of pregnancy (13). These variations in optimal cut-off values of cervical lengths in predicting preterm delivery were primarily dependant on methodological discrepancies and different population of patients in the various studies. The level of evidence about models involving prediction of preterm delivery depending on cervical length in twin pregnancies is low. Therefore various modalities of management are applied among clinicians. Cervical length is routinely evaluated trans-abdominally in every pregnant women as a part of detailed fetal anomaly screening sonography that is usually performed between the 18th-22nd gestational week. Inadequate imaging of cervix or a short cervical measurement in this examination warrants transvaginal cervical length measurement that would be repeated once for every two weeks between 16th and 24th weeks of pregnancy (14).

The aim of this study was to evaluate the cervical length of a high number of asymptomatic, consecutive, pregnant women between 16 and 24 weeks of gestation and produce a histogram and percentile charts of cervical length in this patient population.

Material and Methods

In this study, trans-vaginal cervical measurement of women, consecutively admitted to a single clinic, were retrospectively evaluated. A single perinatologist (EC) carried out all of the measurements between 2016 and 2021 using transvaginal ultrasound (Voluson E8 4Mhz probe, GE company). Cervical measurements were performed in the lithotomy position following the emptying of the maternal bladder by urinary catheterization to standardize all patients. The cervix was visualized in sagittal axis and endocervical length, appearing as a weak linear echodensity between the internal and external cervical ostia, was measured. All of the measurements were performed without applying excess pressure to cervix and a mean of three measurements obtained from a single patient was recorded as cervical length.

Independent risk factors such as age, body mass index, ethnicity, parity, conception via assisted reproduction, history of previous surgeries, chronic diseases, and secondary obstetrical complications were not taken into account. Mean \pm standard deviation (SD), median (range) and percentile values of cervical lengths were reported, by gestational age in weeks. This study was following approval by Alanya Alaaddin Keykubat University Faculty of Medicine Clinical Research Ethics Committee (approval number: 2022/12, date: 25.05.2022). Informed consents were obtained from all participants.

Statistical analysis

Statistical analysis was performed using IBM SPSS, version 23.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics are expressed as mean \pm SD for normally distributed data and as median (minimum-maximum) for non-normally distributed data. Categorical variables are expressed as numbers and percentages (%).

Results

Overall 4,647 patient records were evaluated. Twenty-one triplet pregnancies and five women with a history of cervical conization or loop electrosurgical excision procedure were excluded from the study. Thus, 4340 singleton pregnancies and 281 twin pregnancies that were eligible were included in the study. The demographic data of the study population is given in Table 1. Women with twin pregnancies were significantly younger, more frequently nulliparous and had in vitro fertilization treatment for the present pregnancy compared with the singleton pregnancy group.

Overall cervical length measurements of singleton pregnancies were 38.3 ± 6.5 (0-67) mm and 37.6 ± 7.3 (9-59) mm respectively ($p=0.17$). Comparison of cervical length measurement between singleton and twin pregnancies across 16-24 weeks are presented in Table 2. The percentiles of cervical length in singleton pregnancies is given in Table 3. The 5th percentile of cervical length was between 29-31 mm throughout the period 16-24 weeks of gestation. The percentiles of cervical length in twin pregnancies is given in Table 4. The 5th percentile of cervical length was 27-30 mm between 16-18 weeks and 21-26 mm between 18-24 weeks. Chart analysis and histogram of

Table 1. The demographic data of the study population

Variable	Singleton, (n=4340)	Twin, (n=281)	P
Age (years)	28.2 \pm 4.7	27.5 \pm 5.1	0.01
Nulliparous	2690 (62%)	221 (78.7%)	<0.001
Multiparous	1650 (38%)	60 (21.3%)	<0.001
In vitro fertilisation	303 (7%)	236 (84%)	<0.001

cervical length in singleton and twin pregnancies are given in Figure 1. Overall cervical length percentiles, including singleton and twin pregnancies together, is given in Figure 2. The 5th percentile was 29.4 mm at 16 weeks, 30 mm at 17 weeks, 30 mm at 18 weeks, 30 mm at 19 weeks, 30 mm at 20 weeks, 30

mm at 21 weeks, 30 mm at 22 weeks, 31 mm at 23 weeks, 29 mm at 24 weeks. When all 4340 singleton pregnancies in the period 16-24 gestational weeks was considered, the 2.5th percentile was 28 mm, the 5th percentile was 30 mm and the 10th percentile was 31 mm. When 281 twin pregnancies during

Table 2. Comparison of cervical length measurements between singleton and twin pregnancies across 16-24 weeks of gestation

GA (week)	Singleton (n)	Mean (SD)	Median (range)	Twin (n)	Mean (SD)	Median (range)	p
16	78	38.2 (6.1)	37.0 (19-55)	10	40.9 (10.6)	38.0 (27-59)	0.46
17	158	38.7 (6.8)	38.0 (23-59)	19	39.2 (4.8)	37.0 (30-51)	0.73
18	331	37.8 (7.2)	37.0 (2.9-62)	34	38.02 (6.6)	35.5 (30-54)	0.89
19	490	38.4 (7.2)	38.0 (3.3-65)	29	38.5 (7.5)	38.0 (25-54)	0.96
20	907	38.3 (6.3)	38.0 (4.2-60)	62	36.6 (6.7)	35.5 (16.5-58)	0.05
21	914	38.1 (6.3)	38.0 (3-67)	54	37.3 (8.4)	37.0 (9-55)	0.48
22	683	38.5 (5.9)	38.0 (9-60)	42	38.0 (6.7)	38.5 (23-55)	0.58
23	363	38.3 (6.4)	38.0 (0-59)	12	38.5 (7.2)	39.0 (23-51)	0.95
24	416	37.9 (6.5)	37.0 (9-60)	19	35.7 (7.7)	36.0 (24-50)	0.15
Total	4340	38.2(6.5)	37.6 (0-67)	281	37.6 (7.2)	37.1 (9-59)	

GA: Gestational age, n: number of observations. Lengths are given in mm. SD: Standard deviation

Table 3. Percentile values according to gestational week in singleton pregnant

GA (weeks)	n	Percentile (mm)								
		1 st	5 th	10 th	25 th	50 th	75 th	90 th	95 th	99 th
16	78	19	30	31.9	34.7	37	42	46.1	49.2	
17	158	24.1	29.9	30.9	34	38	42	48	52	59
18	331	22	30	30.2	33	37	42	47.8	52.4	58.6
19	490	22.2	30	31	34	38	42	47	52	60
20	907	20.3	30	32	34	38	42	46	49	56
21	914	22.1	30	32	34	38	42	46	49	58
22	683	26	31	32	35	38	43	47	49	54
23	363	17.7	31	32	35	38	42	47	49	55
24	416	21.6	29	30.7	33	37	42	47	49	58

GA: Gestational age

Table 4. Percentile values according to gestational week in twin pregnant

GA (weeks)	n	Percentile (mm)								
		1 st	5 th	10 th	25 th	50 th	75 th	90 th	95 th	99 th
16	10	27	27	27	34.5	38	47.7	58.7		
17	19	30	30	35	36	37	42	46		
18	34	30	30	31	32	35.5	45.2	47	48.7	
19	29	25	25	31	32.5	38	42.5	51	52.5	
20	62	16.5	26.3	31	33.7	35.5	40	45	52.4	
21	54	9	21	31	33	37	44	46.5	49.7	
22	42	23	25	28.2	34.5	38.5	43	44.7	53.5	
23	12	23	23	25.4	33.7	39	44	49.2		
24	19	24	24	24	32	36	39	49		

GA: Gestational age

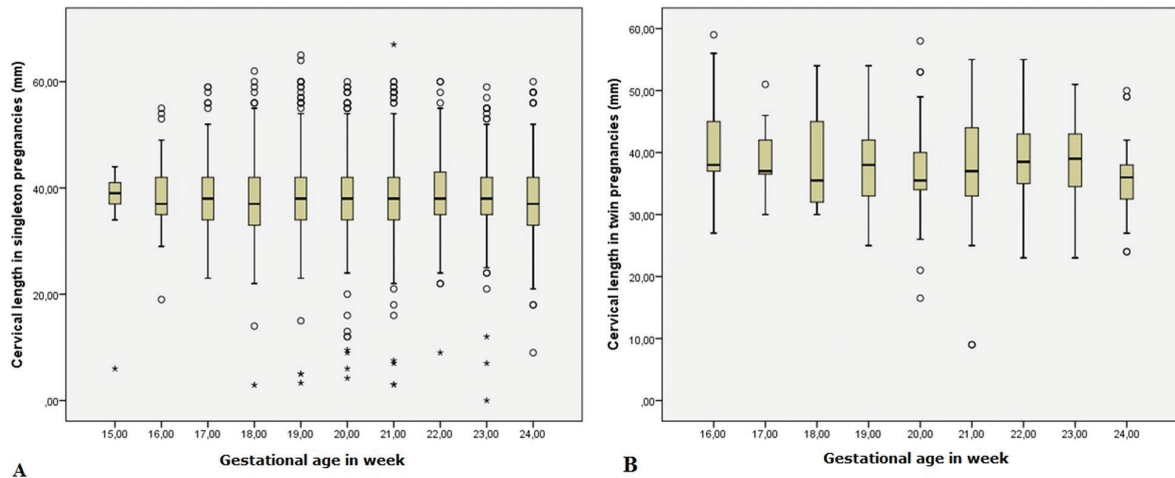


Figure 1. Longitudinal box plots of observed cervical lengths across gestational age. (A) Single pregnancy, (B) Twin pregnancy

****Boxes range from the 25th to the 75th percentile, with the horizontal line inside the box indicating the median**

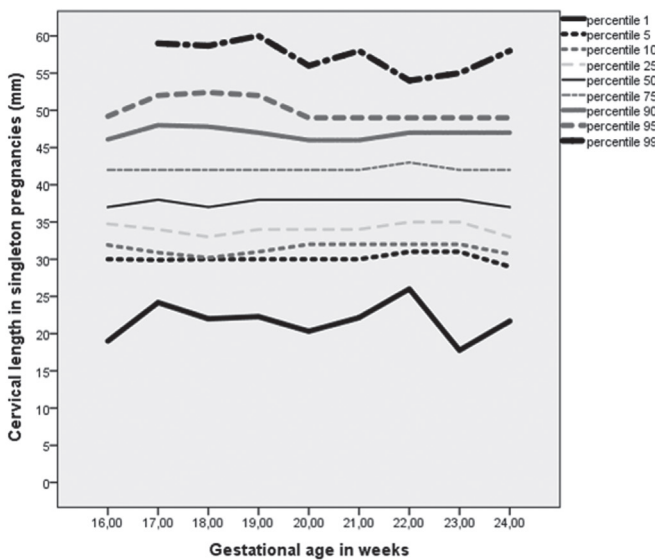


Figure 2. Reference ranges for cervical length across gestation. First to 99th percentiles are indicated

the same period were evaluated, the 2.5th percentile was 24 mm, the 5th percentile was 26 mm and the 10th percentile was 31 mm. Distribution of cervical lengths in singleton and twin pregnancies are shown in Figure 3. Incidence of short cervix increased as the gestation progressed.

Discussion

Contemporary predictive models for preterm delivery includes a comparison of each patients cervical measurement with standard normograms. Reliability of these models partially depends on accurate determination of cervical length percentile and, as normal values for cervical length could be expected to vary in different populations, in this study we

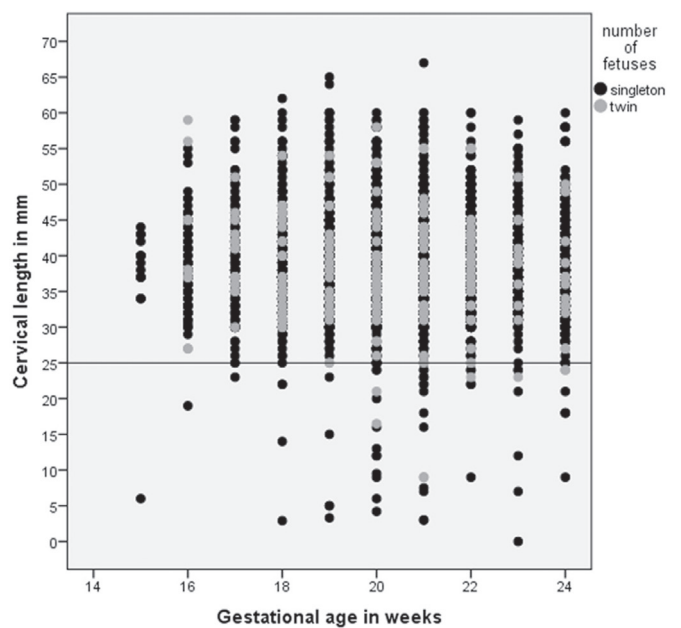


Figure 3. Scatter plot of cervical length measurements obtained from 4340 singleton and 281 twin pregnancy patients

****Solid line: 25 mm with short cervix border**

aimed to evaluate the normal ranges of cervical lengths in a Turkish population and establish a standard cervical length normogram for this specific population.

To avoid the limitations of a trans-abdominal approach in cervical length measurement, such as difficulty in cervical visualization and lengthening of cervix due to a filled bladder, in this study we preferred to assess records of patients that were evaluated solely by a trans-vaginal approach. Although cervical measurements could be performed from the 14th week of pregnancy, measurements made between 16th and 18th

gestational weeks at the time that the cervix separates from the lower uterine segment are considered more consistent and accurate (15). In normal conditions cervical length remains stable between the 14th and 28th gestational weeks and thus changes in cervical length in this period of pregnancy are described with a bell-curve.

The published definitions of short cervix are somewhat obscure. A wide variety of cervical lengths, from 10-35 mm, were studied as empirical cut-off values for prediction of spontaneous preterm birth in singleton pregnancies (16). The same cut-off values were empirically used for twin pregnancies, while a limited number of studies have reported the value of cervical shortening as an important predictor of spontaneous preterm delivery in mathematical models.

Normograms prepared by using specific populations may demonstrate variations and might have a negative impact on prediction of preterm delivery. For instance, in a study conducted in North America, mean cervical length of women in the 24th gestational week was estimated to be 34.0±7.8 mm (17). In contrast, a similar study conducted in Iran demonstrated that mean cervical length was 38.3±5.61 mm and another conducted in Switzerland was found mean cervical length in the 24th week to be 39.1±5.6 (18,19). Considering the discrepancies in these data, normograms prepared based on North American data could be expected to have higher false negativity in prediction of preterm delivery for a patient from Switzerland or Iran. In the present study, the mean cervical length for Turkish women in the 24th gestational week was 37.9±6.5 mm.

Singleton normograms enable comparison of 5th percentile values between the different populations. The 5th percentile for cervical length between 16-24 weeks of gestation was found to be 27 mm in Chicago, 30 mm in an American Hispanic population, 30.6 mm in a Thai population, 28 to 29 mm in South Africa, 25 mm in Switzerland, 32-25 mm in Paris, 30 mm in Iran, and between 30-33 mm in Brasil (9,20-25). In our large cohort, the overall 5th percentile of cervical length was 30 mm between 16-24 weeks of gestation, including both singleton and twin pregnancies. Small variations in different populations result from the sample sizes and the weight of pregnant women between 16-19 weeks or 20-24 weeks. The cervical length tend to be a few millimeters shorter as the pregnancy approaches the 24th week of gestation.

Data involving normal range of cervical length in twin pregnancies is scarce, in comparison to that for singleton pregnancies. A study conducted on 172 patients in Brazil found median cervical length of women with twin gestation to be 39 mm (37-40) (26). In the present study, the estimated median value of cervical length in twin pregnancies was 36 mm (24-50). Normogram data for cervical length in twin gestations are also

rare. As preterm delivery rate of twins are higher, studies tend to take the 10th percentile of cervical length as a cut-off for short cervix (27). The 10th percentile of cervical length was reported to be 30 mm in Canada and 25 mm in Poland (27,28). The 5th percentile of cervical length was 21-25 mm at 18-22 weeks in Brasil (29). In the present study in a Turkish population these values were 26 mm and 31 mm for cervical length at the 5th and 10th percentile in one of the largest twin cohorts so far reported. Cervical length is dynamic throughout the pregnancy. Studies have shown that multiple variable can affect cervical length dynamics and affect preterm delivery (30). Both 25 mm and 30 mm cut-off values may be used in our population to test interventions for preventing preterm births in twin pregnancies.

Study Limitations

The limitation of this study is that not all patients were followed-up until delivery, so the predictive value of the present data was not calculated. However, in two prior studies from our center an empirical 30 mm cervical length cut-off value was used to prevent early preterm delivery <32 weeks of gestation (31,32). In our population weekly follow-up of singleton pregnant women by cervical length, and emergency cerclage when indicated, prevented 62.5% of preterm deliveries earlier than 32 weeks (31). Vaginal progesterone treatment in singleton pregnant women with cervical length less than 30 mm and emergency or ultrasound-indicated cerclage was used when indicated postponed 98.7% of the deliveries beyond 34 weeks of gestation (32). In this context, comparing an individual patients' cervical length with the population-appropriate normal values may assist in increasing the accuracy of preterm delivery prediction.

Conclusion

The 5th percentile value of cervical length was 30 mm in singletons, while the 10th percentile cervical length was 31 mm in twin pregnancies in this Turkish population. These values can be used to follow-up and treat pregnant women at risk for preterm delivers.

Ethics Committee Approval: *This study was following approval by Alanya Alaaddin Keykubat University Faculty of Medicine Clinical Research Ethics Committee (approval number: 2022/12, date: 25.05.2022).*

Informed Consent: *Informed consents were obtained from all participants.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Surgical and Medical Practices: E.Ç.; Concept: B.A., Y.C.; Design: B.A., Y.C., E.K.; Data Collection or*

Processing: A.K., E.K.; Analysis or Interpretation: E.Ç., B.A.; Literature Search: Y.C., E.K., A.K.; Writing: B.A., Y.C., A.K., E.Ç.

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

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Maternal and perinatal outcomes of COVID-19 vaccination during pregnancy

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Abstract

Objective: To investigate maternal adverse effects and perinatal and neonatal outcomes of women receiving coronavirus disease-2019 (COVID-19) vaccination during pregnancy.

Material and Methods: Seven hundred and sixty pregnant women who were followed up in obstetrics outpatients were included in this prospective cohort study. COVID-19 vaccination and infection histories of the patients were recorded. Demographic data, including age, parity, and presence of systemic disease and adverse events following COVID-19 vaccination were recorded. Vaccinated pregnant women were compared with unvaccinated women in terms of adverse perinatal and neonatal outcomes.

Results: Among the 760 pregnant women who met study criteria, the data of 425 pregnant women were analyzed. Among these, 55 (13%) were unvaccinated, 134 (31%) were vaccinated before pregnancy, and 236 (56%) pregnant women were vaccinated during pregnancy. Of those who were vaccinated, 307 patients (83%) received BioNTech, 52 patients (14%) received CoronaVac, and 11 patients (3%) received both CoronaVac and BioNTech. The local and systemic adverse effect profiles of patients who received COVID-19 vaccination either before or during pregnancy were similar ($p=0.159$), and the most common adverse effect was injection site pain. COVID-19 vaccination during pregnancy did not increase the ratio of abortion (<14 wk), stillbirth (>24 wk), preeclampsia, gestational diabetes mellitus, fetal growth restriction, second-trimester soft marker incidence, time of delivery, birth weight, preterm birth (<37 wk) or admission to the neonatal intensive care unit compared to the women who were not vaccinated during pregnancy.

Conclusion: COVID-19 vaccination during pregnancy did not increase maternal local and systemic adverse effects or poor perinatal and neonatal outcomes. Therefore, regarding the increased risk of morbidity and mortality related to COVID-19 in pregnant women, the authors propose that COVID-19 vaccination should be offered to all pregnant women. (J Turk Ger Gynecol Assoc 2023; 24: 120-4)

Keywords: COVID-19, vaccination, pregnancy outcomes, neonatal

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Introduction

Coronavirus disease-2019 (COVID-19) remains a global public health issue, more than two years after COVID-19 was declared a pandemic by the World Health Organization (WHO) on 11th March 2020. Pregnant women are considered a high-risk group for serious complications if they develop COVID-19. Studies have shown that pregnancies with COVID-19 are more likely to need hospitalization, intensive care unit admission, and invasive ventilation than non-pregnant patients (1).

Furthermore, pregnant women with COVID-19 are reported to experience a high ratio of perinatal complications, such as preeclampsia, preterm birth, abortion, and stillbirth (2,3). Despite the increased risk, pregnant women were not included in the initial vaccination schedule (4). Up to the last quarter of 2021, the WHO recommended that pregnant women be vaccinated if they work in departments at risk of COVID-19 or have a serious chronic disease. Subsequently, the WHO recommended vaccinating pregnant women due to the serious effects of COVID-19 in pregnancy and in the light of more



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complete knowledge about vaccine safety in November 2021 (5). However, pregnant women may exhibit great hesitation about vaccination despite the updated recommendations based on both the COVID-19 infection and vaccines. At the time of writing, when there are efforts to normalise daily life, COVID-19 vaccine programs should continue to control the number of COVID-19 cases but also avoid associated morbidity and mortality in people with high risk, such as pregnant women. Therefore COVID-19 vaccination studies in pregnant women still have significant value. Thus, the aim of this study was to investigate maternal adverse effects and perinatal and neonatal outcomes associated with COVID-19 vaccination during pregnancy.

Material and Methods

The data for this prospective cohort study was collected at a University Medical Faculty, Obstetrics and Gynaecology Clinic between January 2022 and April 2022. A total of 760 pregnant women were seen in the outpatient clinic during the study data collection period. Of these, 22 multiple pregnancies, 55 pregnant women who were lost to follow-up, eight pregnant women with a history of chromosomal anomalies (four patients) or recurrent abortions (four patients), and 250 pregnant women who did not want to share their information were excluded from the study. The data of the remaining 425 pregnant women were included in the analysis. Written informed consent was signed by all participants. The study was approved by the Ankara University Ethical Committee (2021/472, date 31.12.2021). All pregnant women who agreed to participate in the study were questioned about their vaccination status and history of COVID-19 infection, regardless of their gestational wk. At the same time, the information of pregnant women who had the COVID-19 vaccination or got COVID-19 infection during the pregnancy was updated. Vaccination and infection status was confirmed via the e-NABIZ system, which is the patient registration database of the Turkish Health Ministry. The types and doses of COVID-19 vaccines were recorded. Patients who received any COVID-19 vaccine during pregnancy were divided into three groups depending on the stage of pregnancy at the time of vaccination: first trimester (<14 wk); second trimester (between ≥ 14 and <28 wk); and third trimester (≥ 28 wk). In addition, the participants were evaluated in three groups based on their medical history of COVID-19: those who have never been exposed to COVID-19 infection; those who had COVID-19 infection before pregnancy; and those who had COVID-19 infection during pregnancy.

Demographic data, including age, parity, and pre-existing systemic diseases and adverse effects associated with vaccination were documented. In addition, patients who did or did not receive vaccination during pregnancy were compared

in terms of adverse pregnancy outcomes [abortion <14 wk, stillbirth >24 wk, preeclampsia, and gestational diabetes mellitus (GDM)], and neonatal outcomes [preterm birth <37 wk, congenital anomaly, fetal growth restriction, admission to neonatal intensive care unit (NICU) and neonatal death]. The pregnancy and neonatal outcomes of the COVID-19 infection history in pregnancy were also recorded.

Statistical analysis

IBM SPSS, version 26, was used for all statistical analyses (IBM Inc., Armonk, NY, USA). For assessment of normal distribution of data sets, the Kolmogorov-Smirnov test and histograms were used. Mean \pm standard deviation was used to summarise normally distributed continuous variables, while the median (minimum-maximum) was used for non-normally distributed values. Categorical variables are given as n (%). Comparisons between the groups were evaluated using the Kruskal-Wallis H and Mann-Whitney U test for continuous variables and the chi-square test or Fisher's exact test for the categorical variables. A $p < 0.05$ was considered to indicate statistical significance, and 95% confidence intervals were computed.

Results

Of the 425 participants, 55 (13%) were unvaccinated, 134 (31%) were vaccinated before pregnancy and 236 (56%) were vaccinated during pregnancy. In the latter group, 102 patients were vaccinated in the first trimester, 109 patients in the second trimester, and 24 patients in the third trimester. Demographic data of pregnant women, including age, gravidity, and accompanying comorbidities are given in Table 1.

Two types of vaccines, CoronaVac and BioNTech, were administered to a total of 370 pregnant women. Of those, 307 patients (83%) received BioNTech, 52 patients (14%) received CoronaVac, and 11 patients (3%) received both CoronaVac and BioNTech. Local and systemic adverse effects of pregnant women with a history of vaccination before or during pregnancy were recorded (Table 2). The most common adverse effect was injection-site pain. There was a statistically significant difference between those who received BioNTech and CoronaVac in terms of adverse effects ($p < 0.001$). More adverse effects were observed in the group receiving BioNTech but there was no statistically significant difference between the group vaccinated before pregnancy and the group vaccinated during pregnancy in terms of adverse effects after the vaccination ($p = 0.159$).

Total number of pregnancies resulting in a live birth was 395 (93%). The group vaccinated during pregnancy was compared to the group not vaccinated during pregnancy in terms of abortion (<14 wk), stillbirth (>24 wk), preeclampsia, GDM,

Table 1. Demographics variables of patients

	Unvaccinated	Vaccination before pregnancy	Vaccination during pregnancy		
			The first trimester	The second trimester	The third trimester
Total (n)	55	134	102	109	25
Maternal age (years) [†]	27.5±4.7	29.4±6.0	29.7±5.2	29.1±5.6	30.2±4.3
Nulliparity (n)	30 (54%)	54 (40%)	27 (26%)	27 (24%)	7 (28%)
Maternal comorbidity (n)	15 (27%)	27 (20%)	27 (26%)	28 (25%)	4 (16%)
The types of COVID-19 vaccines (n)	Pfizer-BioNTech	109 (81.3%)	90 (88.2%)	95 (87.2%)	24 (96%)
	CoronaVac	25 (18.7%)	12 (11.8%)	14 (12.8%)	1 (4%)
Vaccination time during pregnancy [‡]			4 (1-13)	21 (14-27)	31 (28-37)

[†]Mean ± standard deviation, y: years, [‡]median (minimum-maximum) weeks of gestation

Table 2. Local and systemic reactions of COVID-19 vaccines

	Vaccination during pregnancy (n=236)	Vaccination before pregnancy (n=134)
No side effect	84 (36%)	58 (43%)
Injection-site pain	109 (46%)	63 (47%)
Fatigue	35 (15%)	26 (19%)
Headache	27 (11%)	14 (10%)
Myalgia	19 (8%)	14 (10%)
Injection-site swelling	14 (6%)	1 (1%)
Fever (>38 °C) or felt feverish	10 (4%)	16 (12%)
Joint pain	7 (3%)	3 (2%)
Nausea	6 (3%)	1 (1%)
Injection-site redness	5 (2%)	-
Vomiting	5 (2%)	1 (1%)
Chills	4 (2%)	1 (1%)
Diarrhea	2 (1%)	3 (2%)
Injection-site itching	1 (1%)	1 (1%)

COVID-19: Coronavirus disease-2019

fetal growth restriction, and presence of second-trimester soft markers. There was no statistically significant difference between the groups, except for abortion rates. The abortion rate in the group who did not receive the vaccine during pregnancy (20/189, 10%) was significantly higher than the group vaccinated during pregnancy (2/236, 1%), ($p<0.001$). The abortion rate was 3.6% (2/55) in the unvaccinated group and was 13.4% (18/134) in the group vaccinated before pregnancy. In addition, time of delivery, birth weight, preterm birth (<37 wk) and admission to the NICU did not statistically differ between the groups (Table 3). There was no neonatal mortality throughout the study.

Table 3. Pregnancy and neonatal outcomes in the pregnant women that received and did not receive the vaccine during pregnancy

	COVID-19 vaccination during pregnancy		
	Yes (n=236)	No (n=189)	p
Maternal age (y) [†]	29.5±5.0	28.9±5.7	0.594
Gestational age at delivery (wk) [†]	38.6±1.7	38.6±1.1	0.813
Birthweight (g) [†]	3,212±512	3,085±442	0.272
COVID-19 infection during pregnancy	26/236 (11%)	14/189 (7%)	0.205
Abortion (<14 wk)	2/236 (1%)	20/189 (10%)	<0.001
Stillbirth (>24 wk)	0	0	-
Eclampsia or preeclampsia	5/230 (2%)	1/165 (1%)	0.587
Gestational DM	9/230 (4%)	7/165 (4%)	1.0
Preterm birth (<37 wk)	13/230 (6%)	13/165 (8%)	0.753
Fetal growth restriction	7/230 (3%)	10/165 (6%)	0.421
Second-trimester soft marker	35/230 (15%)	31/165 (19%)	0.273
NICU	23/230 (10%)	7/165 (4%)	0.185
Neonatal death	0	0	-

NICU: Neonatal intensive care unit, DM: Diabetes mellitus, wk: Weeks, y: Years, g: Gram, [†]values are shown as mean ± standard deviation, COVID-19: Coronavirus disease-2019

Among the study participants, one fetus was terminated at the 16th gestational week due to Down syndrome, and another fetus was terminated at the 19th gestational week due to premature rupture of membranes. Both pregnant women had been vaccinated with CoronaVac before pregnancy. In the BioNTech vaccinated group, a cystic hygroma was detected in a pregnancy vaccinated in the first trimester, chorionic villus sampling was performed and routine follow-up was continued after a normal karyotype was detected. A healthy baby was born in the 39th week. Except for these, no other major foetal structural or chromosomal anomalies were detected.

COVID-19 infection during pregnancy occurred in 40 pregnant women. Ten of them were unvaccinated during the COVID-19 infection, and two pregnant women had a COVID-19 infection within 14 days after the COVID-19 vaccination. Moreover, COVID-19 infection was detected at the time of delivery in six patients. COVID-19 infection in those were either asymptomatic or with mild symptoms. Only one unvaccinated pregnant woman was admitted to the intensive care unit for seven days due to COVID-19 infection at the 13th weeks of gestation. The patient was vaccinated with two doses of BioNTech in the second trimester and no perinatal morbidity was observed during pregnancy. In terms of COVID-19 infection, 110 (26%) patients had COVID-19 before pregnancy, while 275 (65%) patients did not have a known history of COVID-19 infection.

Discussion

In addition to personal protective measures (washing hands, wearing masks, and keeping social distance) to prevent the spread of COVID-19 infection, the most powerful weapon has been vaccination, as in previous pandemics (6). Vaccination not only reduces the risk of having COVID-19 infection but also provides a milder course of symptoms in case of infection. Symptomatic COVID-19 infection during pregnancy increases the risk of maternal morbidity and mortality (7). Therefore, vaccination should not be avoided due to pregnancy. In our country, COVID-19 vaccination was begun in January 2021. Until November 2021, pregnant women could be vaccinated at their own discretion. Pregnant women were generally advised to get vaccinated after the first trimester, although there was scarce evidence. Since November 2021, vaccination has been recommended for all pregnant women, regardless of the gestational week (5).

In a recent study comparing the adverse effects of mRNA vaccines between pregnant and non-pregnant women, injection-site pain was more common in the pregnant women, while other adverse effects were more common in the non-pregnant group, but on the whole reactogenicity in these two groups was similar (8). Gray et al. (9) and Kachikis et al. (10) published similar results. In the present study, patients were vaccinated with an mRNA vaccine (BioNTech) or an inactivated vaccine (CoronaVac). Headache, injection site swelling and redness, joint pain, nausea, vomiting, and chills were more common in the group vaccinated during pregnancy while injection site pain, fatigue, myalgia, fever (>38 °C) or feeling feverish and diarrhea were more common in the group vaccinated before pregnancy. The proportion of patients without adverse effects tended to be higher in the group vaccinated before pregnancy than those vaccinated during pregnancy (43-36%, respectively; $p=0.159$). This difference might be attributed to the shorter time interval after vaccination in women vaccinated during pregnancy.

In a study, 140 pregnant women who received at least one dose of vaccine during pregnancy (85.7% of patients were vaccinated in the second trimester and 14.3% of patients were vaccinated in the third trimester) and 1188 unvaccinated pregnant women were compared in terms of poor pregnancy outcomes (stillbirth, fetal abnormalities, small gestational age, and admission to the NICU), and results were similar between the groups (11). In the present study, pregnant women who were vaccinated during pregnancy and who did not receive the vaccine during pregnancy were compared in terms of poor pregnancy outcomes and there was no significant difference between the groups, except for abortion rates. It is possible that pregnant women at high risk of abortion or with symptoms may have avoided the vaccination during pregnancy. In a study, Kharbanda et al. (12) examined 13160 abortions, and they found that the COVID-19 vaccine during pregnancy did not increase the risk of abortion. Furthermore, in a recently published meta-analysis of recent studies, it was demonstrated that COVID-19 vaccination during pregnancy did not increase the risk of adverse perinatal and neonatal outcomes (11,13-18).

Study Limitations

Our study had some strengths. The first was that it was a prospective study and variables such as COVID-19 vaccination and COVID-19 infection were recorded throughout the pregnancy period, since these patients were followed up and delivered in our hospital. Second, COVID-19 vaccination rates in pregnancy were high, especially in the first trimester. However, there were also some limitations. The number of patients in the study is not enough to compare perinatal outcomes. Parallel to the high rates of COVID-19 vaccination of at least one dose (93%) in our country (19), the patients in our study had a high rate of COVID-19 vaccination (87%). Therefore, since our unvaccinated pregnant rate (13%) was low, we formed two groups of those who received the COVID-19 vaccination during pregnancy and the other pregnant women, and we made all comparisons between these groups. Since BioNTech was preferred more frequently, the number of pregnant women who underwent CoronaVac was low. Again, there was no significant difference between the vaccine types in terms of maternal and neonatal adverse outcomes. We did not administer the vaccines, the patients who were vaccinated before pregnancy were not evaluated in the prospective study, and the fact that these patients may not remember the side effects of the vaccine is a limitation. Finally, COVID-19 infection screening might have resulted in low COVID-19 infection rates in our study group, since it was only symptom-based and based on personal attendance at a screening center.

Conclusion

In conclusion, pregnant women who received COVID-19 vaccination during pregnancy did not have more adverse local and systemic effects compared to the non-vaccinated women. Moreover, poor perinatal and neonatal outcomes were similar in pregnant women who received or did not receive COVID-19 vaccination during pregnancy. We propose, in light of these results and in parallel with the current literature, that COVID-19 vaccination should be offered to all pregnant women. However, long-term maternal and perinatal outcomes of vaccines containing mRNA are not known yet.

Ethics Committee Approval: *The study was approved by the Ankara University Ethical Committee (2021/472, date 31.12.2021).*

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The effect of dietary habits on oocyte/sperm quality

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Abstract

The effects of diet and nutritional habits on reproductive cells can be categorized in a variety of ways. In this review, the literature is divided, based on the dietary consumption effects on oocytes and sperm. Topics on dietary patterns and the intrauterine effect of maternal nutrition are covered. In general fruits, vegetables, whole greens, fish, legumes, and also dietary sources containing unsaturated fats can improve reproductive germ cell quality. In epidemiological studies, the food intake frequency questionnaire is one of the most common methods to assess diet. Due to methodological heterogeneity in dietary assessment and inadequacy in the measurement of dietary intake in the questionnaires used, several unreliable results may be reported. Thus, the quality of evidence needs to be improved, since nutritional diets may not be so simply objective and they are inadequate to explain obvious underlining mechanisms. In addition, various compounds that may be ingested can affect molecular mechanisms, influenced by other external factors (drugs, pesticides, smoking, alcohol) and changes in human nutritional parameters. Artificial Intelligence has recently gained widespread interest and may have a role in accurate analysis of dietary patterns for optimal nutritional benefit. Therefore, future prospective randomized studies and objective measurements, consisting of molecular level analysis of the impact on cells and clear-cut methods are needed for accurate assessment of the effect of dietary habits on reproductive treatment.

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Introduction

Diet in particular can affect oocyte and sperm quality in three ways. First, maternal nutrition is one of the most important causes affecting the gametes in utero (1). Second, an increase in body mass index (BMI) resulting from excessive dietary intake affects associated systemic and inflammatory processes (2). Finally, abrupt changes in the metabolic environment in the sperm/follicular fluid due to a variety of nutritional factors has also been shown to affect the quality of the gametes (3). Eating patterns, BMI, and nutritional consumption are among the modifiable factors known to affect infertility (4). Dietary patterns have also a substantial influence on metabolism

through various regulatory mechanisms that lead to local and systemic hormonal changes.

Global diets have been classified into four types based on different compositions, which include omnivorous, mediterranean, pescatarian (prudent), and vegetarian diets (5). A vegetarian diet excludes all meat, poultry, and seafood and includes a higher intake of fiber and usually a lower consumption of total and saturated fat, protein, vitamin B12, vitamin D, and calcium (6). However, the vegetarian diet may be sub-categorized into the Lacto-vegetarian diet, consisting of vegetarian diets and dairy products (milk, cheese, yogurt, and butter), Ovo-vegetarian diets allowing eggs but excluding dairy products, and Lacto-Ovo vegetarian diets that allow dairy



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products and eggs. Furthermore, vegan diets exclude all animal products, thus excludes meat, poultry, fish, eggs, and dairy products — and foods that contain these products. A pescatarian diet is largely vegetarian but includes seafood. A pescatarian diet may also completely or partially avoid meat and poultry, dairy and eggs in which case it is also known as a prudent diet. The Mediterranean diet is notable for increased consumption of fruits and vegetables, whole grains, sugars, oils, eggs, milk products, seafood, nuts, and moderate amounts of meat foods (poultry, pork, beef, lamb). Finally, the omnivorous diet includes all types of foods (7). The results of diet on oocyte and sperm quality, considering the amounts of macronutrients and vitamins and the role of essential fatty acids, proteins, vitamins, and minerals have been investigated. Studies have mostly compared Mediterranean and Western diets, whether healthy diet typified by the prudent or Mediterranean diet pattern is beneficial compared with the Western diet pattern that consists of richer saturated fats, red meat, junk foods, sweets, and excessive amounts of refined and processed foods (Table 1).

Nutrients, including carbohydrates, fats, and proteins, provide energy for growth and repair of tissues and the production of

enzymes, hormones, and fat-soluble vitamins (7). A usual diet consists of 55-65% carbohydrates, 30% fat, and 10-20% protein. All macronutrients obtained from energy and nutrients source rates show diversity (7).

Proteins

According to the European Food Safety Authority, for nitrogen balance, the recommended average daily protein intake is 0.66 g/kg body weight (8). Animal protein sources, such as red meat, poultry (usually chicken and turkey), various types of eggs, and dairy products (cheese, milk, whey) contain significant amounts of protein, micronutrients, and vitamins. Meat products are one of the most widely consumed and heavily investigated sources of protein. Several potential mechanisms could be mentioned to explain the reason behind meat consumption. In addition, a high intake of red meat may raise dietary iron intake. Studies of esophageal, stomach, and colon cancers have reported that excessive iron intake can increase oxidative stress that can promote chronic inflammatory processes and affect the immune system and may also damage tumor suppressor genes (9). Processed meat, such as salami and sausage, are

Table 1. Characteristics of different diet types

	Omnivorous diet (7)	Mediterranean diet (5)	Pescatarian (prudent) diet (5)	Vegetarian diet (6)	Western diet (5)
Red meat	√√	√	-	-	√√√√
Poultry meat	√√√	√√√	-	-	√
Sea food	√√√	√√√√	√√√	-	√
Dairy products	√√√	√√	-	- Lactovegetarian√√√	√√√√ High fat dairy products
Eggs	√	√	-	- Ovo vegetarian√√√	Fried
Nuts	√√√	√√√√	√√√√	√√√√	√
Vegetables	√√√	√√√√√	√√√√√	√√√√√	√
Fruits	√√√	√√√√√	√√√√√	√√√√√	√
Trans-unsaturated fat (French fries, muffins, cookies, biscuits, chocolates, margarine, fried chicken, and crackers)	√√	√	√	-	√√√√√
Mono-unsaturated fat (olive oil)	√√√	√√√√√	√√√√	√√√√	√
Simple carbonhydrates (sugar)	√√√	√	√	√	√√√√√
Complex carbohydrates (whole grains milk and dairy products bread, cereals, legumes, vegetables, and fruits)	√√√	√√√√√	√√√	√√√	√
Junk foods	√√	-	-	-	√√√√√
Refined and processed foods	√√	-	-	-	√√√√√

*Representing √ very low, √√ low, √√√ moderate, √√√√ high, √√√√√ very high intake

modified by several methods, some of which involve the use of preservatives and flavourings that are used to extend shelf life and alter the flavor, respectively. Large epidemiological studies have shown that processed red meat consumption is associated with negative effects on sperm and oocyte quality by increased oxidative stress and production of reactive oxygen species (ROS) radicals (10).

Fatty acids

Fatty acids have a significant role in nutrition as they are high energy sources, contain fat-soluble vitamins and essential fatty acids, and are important for oxidative stability (11). Saturated fats account for approximately 10% of the energy in the American diet. The main sources of saturated fatty acids (SFA) in foods of animal origin are butter, cow's milk, meat, salmon, egg yolk, etc. Examples of plant product sources include chocolate and cocoa butter, coconut, and palm kernel oils. Transunsaturated oil, contributing about 1-2% of the energy in the American diet, is produced commercially using a metal catalyst to partially hydrogenate vegetable oils in the presence of vacuum and high heat or may occur naturally in meat and dairy products where ruminants hydrogenize unsaturated fatty acids via bacterial enzymes (11).

Olive oil is a major source of monounsaturated fats and contains a wide range of valuable antioxidants and including superoxide and other reactive species (12). A major benefit of dietary fat intake is to increase the amount of long-chain omega-3 fatty acid (OM-3FAs) because they cannot be synthesized directly by the human body and must be obtained through diet. OM-3FAs are also potential precursors of lipid mediators that impact the inflammatory process, such as prostaglandin E3 (PGE3) and leukotriene B5 (LTB5) (12). Major dietary sources of OM-3FAs cold-water fishes, mussels, oysters, shrimps, oilseeds (almonds, walnuts, and hazelnuts), sesame, flaxseed, and canola (13). Studies have shown that increasing the ratio of long-chain omega-6 fatty acid (OM-6FA) to OM-3FA in the diet promotes chronic inflammatory diseases, including inflammatory bowel disease, asthma, rheumatoid arthritis and atherosclerosis. It has been suggested that the optimal ratio of omega-6 polyunsaturated fatty acid (6-PUFA) to omega-3 polyunsaturated fatty acid (3-PUFA) was 1:1, while in Western diets this ratio may be 16:1 (13). It has been demonstrated that consuming the Western diet for long periods promotes the production of ROS that may lead to insulin resistance and inflammation in various tissues, as well as overproduction of eicosanoids and proinflammatory cytokines (14).

Carbohydrates

Carbohydrates are the primary source of energy for the body, which include dietary fiber (soluble and insoluble),

polysaccharides (starch found in plant food, such as bread and rice), monosaccharides (fructose, galactose, and glucose), and disaccharides (lactose, maltose, and sucrose) (15). Carbohydrate quality indicators are glycemic index (an index that shows the effect of carbohydrates on blood sugar), glycemic load (glycemic index times carbohydrate amount), and the extent of refined carbohydrate (whole grains versus refined grains), and the amount of dietary fiber (16).

Food products containing white flour increase the risk of obesity; therefore, it is important to not eat too many refined carbohydrates or too much food with a high glycemic index (17). It has been reported that very high sugar intake is related to elevated levels of estrogen in both animal and human models in males (18). Dietary carbohydrate intake can also have an important effect on inflammatory markers (19). Chronic exposure to postprandial hyperglycemia may induce oxidative damage by reducing plasma antioxidant defenses and increasing inflammation through free radical production (19).

Monoglutamate is the sole absorbable form of polyglutamate is converted from dietary folate with g-glutamyl hydrolase in the jejunum, and the activity of this enzyme is zinc-dependent (20). Moreover, zinc is a cofactor for homocysteine synthetase, which converts homocysteine to methionine and reduces homocysteine levels. The beneficial effects of zinc in interventional diet research are supported by randomized, placebo-controlled studies, where the administration of zinc and/or folic acid to sub-fertile patients has been shown to lead to a significant increase in semen concentration, from 18% to 74% (20). In addition, zinc, manganese, and selenium, all sourced from the diet, are cofactors for enzymes, including glutathione peroxidase or superoxide dismutase which are important protective enzymes against oxidative stress in reproductive cells (21).

The etiology of infertility factors remains unclear. Infertility can be associated with many physiological, environmental, and genetic factors, including chronic inflammation and oxidative stress. As far as nutrition is concerned, various components and nutrients have been proposed as possible determinants of normal functioning reproductive system over the last two decades (22). Similarly, several cross-sectional, case-control, retrospective, and prospective observational studies with large samples have investigated the relationship between diet and ovarian/semen quality and/or reproductive system with controversial results. Although there is a systematic review with meta-analysis of cohort studies that has carefully evaluated the contribution of the Mediterranean diet in improving sperm and oocyte quality, to the best of our knowledge there is no study that compares the results of both diets with a detailed information on the diet content (23).

The aim of this article was to discuss the effects of diet types, especially the Mediterranean and Western diets, on sperm and oocyte characteristics in the reproductive system and their impact on reproductive success in Assisted Reproductive Technologies (ART) treatments.

1. In utero impact of dietary patterns

Nutrient patterns affect intracellular and extracellular messengers, which in turn affect epigenetic mechanisms that may alter gene expression in the future (24). Severe obstetric illnesses, such as preeclampsia and preterm birth, have a lower incidence in patients whose diets are rich in fruits, vegetables, and whole grains (25). In addition, increased intake of saturated fat and sugar-sweetened beverages increased the risk of fetal malformations (neural tube defects, congenital heart disease, cleft lip and/or palate) and adverse pregnancy outcomes (20). Furthermore, dietary patterns play an important role in the maturation of the fetal immune system and in the composition of the fetal gut microbiota, which is a metabolically and immunologically active system (24).

In a study by Swan et al. (26), weekly maternal beef consumption was inversely related to sperm concentration in the male offspring. High beef consumers (who consumed more than 7 grams of beef per week) were at greater risk of problems with sperm quality in their sons and that their sons had 24.3% lower sperm concentration in adulthood than those who consumed less beef (26). However, there are possible confounders for this so-called nutritional effect. It has been experimentally demonstrated that various environmental drugs and chemicals ingested with certain nutrients in foods during pregnancy and lactation may affect subsequent metabolic and reproductive function in the offspring (27-29). Swan et al. (26) also suggested that maternal beef consumption, possibly due to the effects of xenobiotics in red and processed meat, could transform fetal testicular development (26). They showed the effects of anabolic steroids (a xenobiotic agent) in red and processed meat, could transform fetal testicular development and negatively influence reproductive capacity. They also noted that regional variations in nutrition may alter the results, because most mothers in their study were living in North America and findings may not apply to other regions of the world where other agricultural methods may be used (26). Furthermore, environmental estrogens from polycarbonate plastic and metal cans containing antiandrogenic chemicals [bisphenol A (BPA) and PVC plastics in the resin coatings], and phytates used in many other products can cause abnormalities, including cryptorchidism and hypospadias, that affect sperm quality and reproductive ability in males (28). Another confounding factor for in utero impact of dietary patterns is recall error of maternal food consumption (Table 2).

2. The effect of dietary patterns on sperm quality

Human sperm quantitative and qualitative parameters decreased worldwide even though this situation caused a change in the normal values of WHO sperm parameters. A meta-analysis shows a global 32.5% deterioration in sperm concentration over a period of 50 years (30). Various and diverse environmental and dietary factors that have become a part of modern life may be responsible for this deterioration of sperm quality.

2.1. Dietary effects on hormonal parameters

Dietary patterns affect the hormonal axis in male reproductive function. Consumption of excess high-calorie foods, leading to increased adiposity and possibly obesity, results in increased aromatase enzyme activity and higher levels of systemic 17-oestradiol (E_2) in the body. Increased E_2 levels affect the hypothalamic-pituitary axis through negative feedback, which inhibits gonadotropin releasing hormone secretion and thus and pituitary gonadotropins secretions (31). Studies considering diet type show that high-energy foods (Western diet) not only reduce sperm quality but also lead to chronic illnesses, such as obesity and diabetes mellitus (32). Also, damage to sperm DNA mitochondrial activity and sperm quality, testosterone deficiency is associated with erectile dysfunction. Sex hormone-binding globulin also decreases due to obesity, increasing serum oestradiol levels, reducing the frequency of pulsatile luteinizing hormone levels. Increased adiposity also has some physical consequences, including increased scrotal temperatures which are known to decrease sperm quality (33). Furthermore, the disruption of antioxidant mechanisms seen in obesity because of high-calorie intake, impair sperm quality through impaired testosterone secretion and altered sperm DNA (4). Similarly, excessive trans fatty acids (TFA), saturated fat, cholesterol, or simply a high-calorie diet lead to gonadotoxicity and impair testicular physiology, disrupting spermatogenesis (31,32).

2.2. Dietary effects on sperm parameters

Various human diets can influence many biological pathways in a number of ways. Semen quality is affected by various antioxidants (coenzyme Q10, carnitine), homocysteine, vitamins B6 and B12, folic acid, zinc, and OM-3FA levels, the levels of all of which are at least partly dependent on diet. Assessment of food consumption as well as dietary patterns (the Mediterranean, prudent or Western diet) include modifiable factors (34,35) (Figure 1).

2.2.1. The effect of the Western diet on sperm parameters

Higher consumption of unhealthy dietary components, such as rich full-fat dairy food, sweets, and processed meat, that are

characteristic of the Western diet has been associated with decreased semen quality, evaluated by various parameters including volume of the ejaculate, sperm concentration, total sperm count, progressive motility, total non-motile and total motile sperm count, DNA fragmentation index, and poor fertilization rate or pregnancy rate (36). In addition, the Western diet can lead to mitochondrial dysfunction and can induce oxidant/antioxidant imbalance in testicular tissue, amongst others. This may lead to cellular destruction because of directly damaged lipid membranes, and damage to amino acids, and nucleic acids through sperm peroxidation. ROS occur as a consequence of natural spermatogenesis, which is qualified as a response to the acrosome formation field (4). Also, ROS can lead to insulin resistance, oxidative stress, and inflammation in viscous tissues as well as the overproduction of eicosanoids and proinflammatory cytokines (37). In addition, ROS reduces the presence of adenosine triphosphate in sperm while increasing the risk of agglutination and decreasing mobility. This also prevents sperm from fertilizing oocytes, leading to increased apoptosis, reduced sperm acrosomal function, and lower chances of fertilization by direct damage to the sperm DNA (4). In general, data from animal models suggest that the Western diet generally correlates with decreased sperm quality, especially in terms of diminished motility and impaired sperm capacity, along with reduced sperm binding capacity to oocytes (38,39). Western diet increases testicular fatty acid and cholesterol content while altering sperm plasma membrane composition and sperm membrane-related events (40). Remarkably, sperm quality depends on high percentage of SFA and TFA which are common in the Western diet (34,41). In particular, studies have shown that consumption of high-energy diets disrupts mitochondrial function in the testicular bioenergetic state (39). Since these fatty acids are not endogenously produced in the body, excessive consumption contributes to the accumulation of fat in various organs such as testicular tissue, and the amount is inversely related to sperm counts (41,42). In conclusion, research has shown that the Western diet is directly related to diminished sperm concentration, altered sperm morphology, and asthenozoospermia (43-46) (Figure 2).

2.2.2. The effect of a Mediterranean diet on sperm parameters

In a review of 23 studies from 502 articles, the results confirmed the relationship between improved semen parameters and maintaining a healthy diet, while diets rich in lipophilic foods, soy isoflavones, and sweets resulted in impaired semen quality. The same authors report that despite the lack of underlying mechanisms and randomized controlled trials, dietary quality, and assessment of nutrient intake are important in the diagnosis

and treatment of male factor infertility (47). Attaman et al. (48) showed a moderately significant association between dietary fatty acids and semen quality. They found that the total number and concentration of sperm depended on daily consumption of saturated fat and TFA (48). Although sperm membranes consist of cholesterol, rising ratios in sperm membranes disrupt membrane structure and fluidity. Moreover, minimal changes in dietary cholesterol levels can result in differences in membrane cholesterol concentrations that are reflected in acrosome reaction and a decrease in capacitance in an animal study (49). Several studies have shown that higher intake of components such as legumes, vegetables, especially dark green vegetables, cereals, fruits, and PUFAs from olive oil in a balanced diet are associated with high sperm concentration and progressive mobility (20,50-53). Additionally, folate-rich fruits and vegetables, along with antioxidants such as β -carotene, lutein, and lycopene, can improve sperm quality (21,45,54). A case study comparing dietary habits in normozoospermic and oligoasthenoteratospermic patients showed that the former group were more likely to regularly consume certain fruits and vegetables, such as lettuce, tomatoes, peaches, and apricots (45). Consumption of fruits and vegetables is important for males when considering the successful fertilization with in vitro fertilization (IVF) (52). In an observational study of 250 patients undergoing Intracytoplasmic Sperm Injection (ICSI), sperm motility was positively influenced by fruit and cereal consumption (50). In addition, it has been shown that pesticides, which may be present in fruits and vegetables, can lead to less morphologically normal spermatozoa and lower sperm concentration and motility (45,50).

Consumption of fresh fruits, vegetables, chicken, light dairy products, and seafood decreases the risk of asthenozoospermia, while red meat consumption and high blood glucose levels increase this risk (45,51,55). Jurewicz et al. (56) reported on 336 patients attending the infertility clinic with slight oligoasthenospermia (20 to 300 mln/mL sperm concentration) after sub-dividing the men into three groups based on the diet: Western; prudent; or mixed. In the prudent diet group, the relationship between serum testosterone levels and sperm concentration was significant. Additionally, they showed that a prudent diet prevents sperm DNA from fragmentation (34,56). However, such an association of better sperm parameters with dietary pattern was not found in various other studies (34,57,58). Some studies have reported that nutritional factors do not affect live birth rate, while others show data on improving sperm parameters. The evidence is not entirely consistent, and different findings from different studies may be due to a number of reasons including that various populations were studied, study designs were different, and variable quality

of the studies (33). The studies suggest that the relationship between diet and semen quality does not affect ART outcomes, such as fertilization rate, implantation, clinical pregnancy, and live birth rate (57,58). Li et al. (59) showed that vitamin C and β -carotene intake and ART treatment were positively related with fertilization rates in men attending infertility clinics, although no association was found with pregnancy or live birth rate (59). The relationship between lifestyle factors and semen quality remains unclear due to methodological differences (60).

3. The effect of dietary patterns on oocyte quality

Oocyte growth and maturation are affected by endocrine, nutritional, and chemical factors. There is a variety of dietary effects on oocyte function and ovulation, such as glycemic load, antioxidant and anti-inflammatory processes, pro-estrogenic and anti-estrogenic hormonal effects, and the impact of fatty acids (16). Reduced intracytoplasmic transport proteins, raised glucose/lipid ratio, and elevated ROS levels in oocytes can result in meiotic defects, organelle dysfunction, and epigenetic changes (3). Oxidative damage to gametes can lead to the rearrangement of DNA through various enzymatic changes.

3.1. Hormonal effects on oocyte quality and embryo production

At the cellular level, high BMI-related hyperinsulinemia, lipotoxicity, oxidative stress on the endoplasmic reticulum (ER) and mitochondria, leptin, and adiponectin reduce insulin sensitivity and activate related inflammatory systems in obese women (61). In addition, animal studies suggest that hyperinsulinemia (as a result of a high-fat diet) and high follicular ATP content affect oocyte quality, embryonic development, and the implantation process (22,62,63). In animal studies, maternal hyperglycemia, high-fat diet, and obesity have been shown to adversely affect the progression of the blastocyst (62,64). Furthermore, a high BMI is unlikely to affect endometrial receptivity and cause an implantation failure, compared to its effect on oocyte quality, which is diminished in people who have obesity (61,65). These findings suggest that exposure to abnormal metabolic conditions during oogenesis and fertilization causes morphological changes in the programming of fetal tissues (66). Also, some studies have shown that high-fat diets (Western diet) are related to elevated serum estradiol, estrone sulfate, and estrone levels in premenopausal women (67). For example, mice studies demonstrated that a Western diet reduces primordial follicle numbers and increased follicular atresia that may shorten the reproductive life span of women (68), and this has been associated with the development of inflammation (69).

3.2. Dietary effects on oocyte quality and ART parameters

3.2.1. The effect of a Mediterranean diet on oocyte quality and ART outcomes

The Nurses' Health Study 2 evaluated the effectiveness of an investigator-defined "fertility diet", which showed that consumption of monounsaturated fats, low-glycemic carbohydrates, high-fat dairy products, multivitamins, and plant-derived iron and "fertility diet" supplements was associated with a lower risk of infertility due to ovulatory dysfunction (relative risk: 0.34; 95% confidence interval: 0.23-0.48) (70). The effect of a Mediterranean diet on oocyte function was found to increase ROS production and COX2 expression by increasing prostaglandins in oocyte maturation (71). In a review of 62 articles regarding lifestyle and ART, it was concluded that the success of ART is dependent on the implementation of healthy lifestyle counseling programs, especially by implementing fruits, vegetables, and unsaturated fat in a healthy balanced diet (72). A recent prospective cohort study by Jahangirifar et al. (73) analyzed the connection between maternal nutritional patterns and reproductive status in infertile women. They analyzed 140 primary infertility patients who underwent gonadotropin antagonist protocol treatment. In the study, total oocyte and metaphase II oocyte count, fertilization rate, embryo quality, and biochemical and clinical pregnancy rates were compared between a healthy diet and a Western diet. The authors showed that healthy nutrition may increase the quality and quantity of oocytes through high levels of antioxidant in fresh vegetables, fruits, and nuts in a healthy diet. An unhealthy diet can adversely affect the chances of getting pregnant. However, the fertilization rate and quality of embryo were not affected by diet. Toledo et al. (74) report a prospective cohort study with 161 infertile couples who received IVF/ICSI therapy and showed that patients who followed the Mediterranean diet had a 40% higher pregnancy rate. The authors suggested that the reason for this was due to the fact that the Mediterranean diet is high in vegetable oils which are rich in linoleic acid (74). In a review of 351 couples undergoing IVF treatment, consumption of fruits and vegetables was examined. It was shown that healthy eating patterns that include whole grains, fish, fruits, vegetables, and olive oil, not only improve overall health but also fertility outcomes (75). Also, in their prospective cohort study, Ricci et al. (76) concluded that the Mediterranean diet affected oocyte numbers and clinical pregnancy rates in women over 35 years of age, but not live birth rates. On the other hand, a meta-analysis by Sanderman et al. (77) suggested not recommending a single dietary pattern to improve pregnancy or live birth rates in female infertility undergoing IVF treatment. Such dietary pattern associations and conclusions were not revealed in other studies (76,78,79).

3.2.2. The effect of Western diet on oocyte quality and ART outcomes

Graier et al. (80) showed that triglyceride droplets and free fatty acids accumulate when the oocyte was exposed to a high lipid environment, and this caused significant damage to the ER and mitochondria, leading to lipotoxicity. Furthermore, Nehra et al. (81) demonstrated that increased OM-3FA consumption improved maternal reproductive function in rats compared to OM-6FA, which was associated with very poor reproductive success in older ages. These data suggest that the Western diet may disrupt oocyte metabolism, in terms of energy production in oocytes through ROS. Furthermore, a high glycemic index diet induced an inflammatory process and increased the formation and persistence of bacterial vaginosis (82). Mtango et al. (83) showed that during the fertilization and implantation process, female mice following a normal diet for 4-5 days, with low-protein diets, had fewer embryos, lower birth weight, and compensatory post-natal weight gain, hypertension, and changes in organ/body weight ratios in the adulthood. This increased free glucose levels in mouse oocytes, hence the hyperglycaemic environment further increased glycogen content in immature oocytes and mitochondrial dysfunction occurred (Figure 2).

The limitations of nutritional studies

Epidemiologic and observational studies are considered the sole evidence for nutrition studies but are associated with various limitations, including methodological differences and design faults, tiny groups, short-term follow-ups, poorly conceived endpoints, confounding circumstances, understandably limited numbers of interventional studies, the absence of placebo, poor matching of subjects and non-standardized dosing (4). Recording of dietary habits of individuals include foods regularly consumed over a certain period of time (months or years) and usually do not include specific irregularities that are consumed only for a few days/weeks. In addition, dietary adherence (low, moderate, high) is modified when considering the dietary effect calculated in frequency questionnaire (FFQ). Sun et al. (84) found in a study of 590 patients that significant increase in embryo numbers with ART were achieved in patients who adhered more to the Mediterranean diet model (84). However, it cannot be generalized for all reproductive population or for those attending infertility clinics (84,85). A FFQ with a verified semi-quantitative 165-item was utilized to collect dietary data. The use of FFQs to measure nutritional habits in studies is a major confounder for study comparability, as FFQs do not record the consumption of

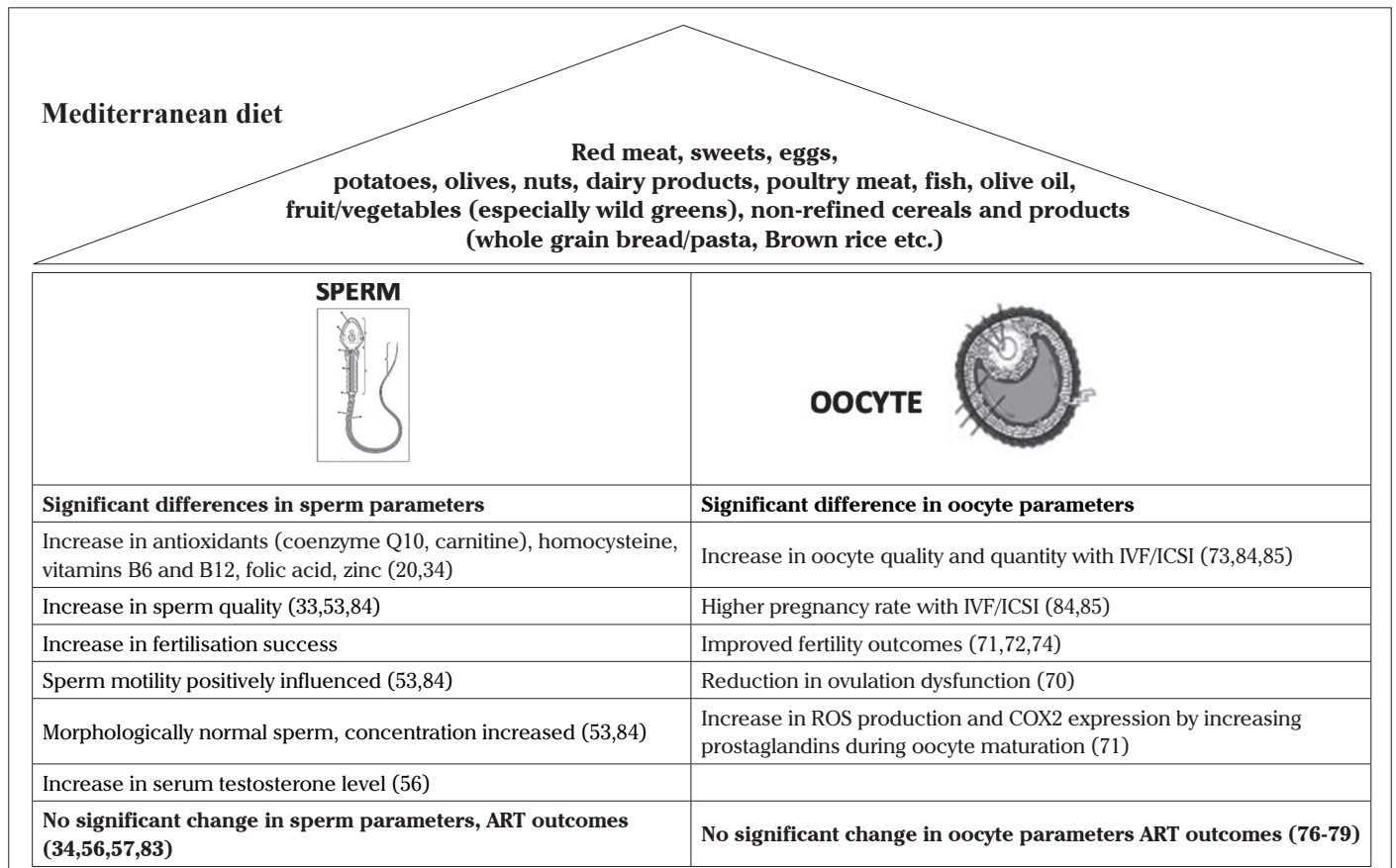


Figure 1. Impact of Mediterranean dietary patterns on female and male reproductive health

ART: Assisted Reproductive Technologies, IVF: In vitro fertilization, ICSI: Intracytoplasmic Sperm Injection, ROS: Reactive oxygen species

some specific but possibly key food items, variability in cooking skills, meal patterns, and differences in attitudes to food. Artificial intelligence (AI) may be adapted for dietary pattern analysis and detection of nutritional problems, image diagnosis, and personalized nutrition for solving this problem (86).

Confounding factors

In this industrial millennial age, uncontaminated food stuffs are an increasing rarity. One should always keep in mind that food, especially processed food, is under direct contamination by various intentional and unintentional factors, such as environmental supplemental chemicals, classified into different functional categories including pesticides, food packaging, food additives, hormones, polycarbonate plastic and metals (29). At present, one of the most common intentional but unnatural processes in meat production is the administration of estrogen-containing anabolic supplements to farm animals and animal feeds in order to increase animal weight (87). Hence, when we talk about the downside of meat consumption and its fertility effects, we can actually never talk about pure meat.

Some of the factors affecting human reproduction include air pollution, smoking, lack of exercise, obesity, drug use, additives

in beverages, phthalates, a group of chemicals used to make plastics more durable, organochlorine pollutants, and stress (33) but evidence linking chronic low-dose environmental exposures and fertility is weak. However, with the growing industry and lifestyle changes, new chemicals and compounds are emerging as threats to reproductive cells. Among these, compounds include pharmaceutical contamination of tap water, organochlorine pollutants in fruit and vegetables, BPA in plastic bottles, phthalates in skin care products, heavy metals and xenobiotics, such as antibiotics, herbicides, pesticides in various foods and food additives like flavor enhancers (monosodium glutamate) are all the focus of research into reproductive cells (88). Indeed, in the EARTH study conducted in 799 women and 487 men (89), some phthalate metabolites were associated with lower oocyte yield, lower clinical pregnancy likelihood, increased risk of pregnancy loss, and lower likelihood of live birth after infertility treatment in women. Certain urinary concentrations of phthalate metabolites have also been associated with lower implantation and live birth rates in men. Maternal soy and folic acid intake significantly altered the association between BPA and IVF outcomes in women (89). There are other harmful organic and

Table 2. Nutritional factors affecting in utero reproductive cells

References	Nutrients effect
(24)	- Effect intra and extracellular messengers capable of influencing the gene expression of epigenetic mechanisms and growth
(33)	- Contaminated foods of persistent organic pollutants - Raised mutagenicity, DNA damage, increase oxidative stress, impaired male fertility - Organochlorine pesticide contaminated foods (eggs, dairy products, meat and meat products, rice, fruit, vegetables, honey, oil, fish, mussel) impact on infertility and fetal malformation - Polybrominated diphenyl ethers (PBDEs) contaminated foods (fish, mussel) occur reproductive problems - Hexabromocyclododecanes (HBCDs) contaminated foods (eggs, oil, fish) impact on endocrine disruption, reproductive problems
(88)	- Biological, chemical, physical hazards can be introduced into the food supply at any time - Food contaminants/environmental contaminants which are metal fragments from equipment such as splinters, blades, needles, utensils, staples, glass food containers, stones incorporated in field peas and beans crops, plastic material used for packaging or used for cleaning equipment, splinters from wood structures and wooden pallets used to store or transport and heavy metal contamination include agricultural activities, such as pesticide and herbicide, irrigation water, mineral fertilizer are hazardous to individuals causing illness
(24)	- Nutrients affect gut microbiota and immune system
(87)	- Environmental endocrine disruptors (EED) such as pesticides, food packaging, food additives exposure during pregnancy on sperm quality in their offspring in adulthood
(20)	- High saturated fat, sugar beverages, reduced intake of fruits and vegetables raised the risk of fetal malformations (neural tube defects, congenital heart disease, cleft lip and palate) and occur adverse pregnancy outcomes
(25)	- High consumption of fruits, vegetables, whole grains have lower incidence of pre-eclampsia and premature birth
(89)	- Nanoparticles (NPs) in food may overcome certain biological barrier tissues (blood-testis barrier, placental barrier and epithelial barrier) and accumulate reproductive organs. - Dysfunction of the reproductive organs, negatively affect sperm quality, quantity, morphology and motility and follicular maturation. - Hormonally changes effect sexual behaviour
(26)	- Maternal beef consumption, and possibly xenobiotics in beef, may alter testicular development in utero and adversely affect reproductive capacity

Table 3. Confounding factors in nutrition studies

Heterogenous study population [demographic factors (e.g., race, ethnicity, age, and country of origin) methods, and findings, and remaining dietary patterns]
Confounding control and cohort selection
Lack of comprehensive control for confounding factors
Individual dietary items may have complex interactions
Individual recall error of food consumption
Heterogeneity ensuring adherence calculation can be difficult in adherence to the dietary pattern
Participants may need to remain in a trial for long periods of time to observe the effect
Exposure misclassification
Time period of exposure assessment
Study end points and followup variable (oocyte retrieval, oocyte number, biochemical pregnancy vs clinical pregnancy, live birth rate)
Methodological heterogeneities in dietary assessment
Inadequacies in the measurement of dietary intake in the questionnaires used
External and environmental factors such as pharmaceutical components in tap water, drugs, pesticides, harmful organic and inorganic pollutants (heavy metals such as lead, arsenic, cadmium, mercury), smoking, alcohol, xenobiotics, nanoscopic drug carriers and nanoimaging technologies
Environmental estrogens - polycarbonate plastic and metal cans containing chemicals, bisphenol A and PVC plastics, phytoestrogens
Individual detoxification capacity will differ between subjects because of external and environmental factors
FFQ problems with consumption of some foods' cooking skills, meal patterns, and attitudes difference
FFQ: Food frequency questionnaire

inorganic pollutants, such as metal parts in ground meat, bone fragments, product packaging pieces, stones, insects, or other personal items, and heavy metals (lead, arsenic, cadmium, mercury) can have detrimental effects on reproductive cells (88). Considering the fertility impact, various issues should be regarded as confounding factors, not only health related maternal problems but also the detoxification capacity of the mother and the offspring. Additionally, lifestyle factors, such as smoking and lack of exercise can alter the balance between ROS and antioxidant protection. Gene expression of epigenetic mechanisms and growth may also be altered.

In addition, the recent development of nanometre-sized materials in medicine is another point that needs to be addressed. Nanoparticles are used in all fields of life, including pharmacology, medicine, chemistry, physics, materials science, engineering and industry, cosmetics and clothing, and food. Nanoscopic drug carriers and nanoimaging technologies can overcome certain biological barriers, such as the blood-testis barrier, placental barrier and epithelial barrier, and accumulate in reproductive organs. This accumulation affects Sertoli and Leydig cell function, the epididymis, ovaries and uterus, and may lead to dysfunction of the reproductive organs, adversely affecting sperm quality, quantity, morphology and motility, and reducing the number of mature eggs and follicular development. Hormonally induced changes in sexual behavior may also be altered (90).

While some researchers have examined the effects of diet on sperm parameters in the general population (32,34,91), others evaluated the effects of diet only on infertile patients who are admitted to the fertility outpatient clinics (20,21,43,45,48,50,51, 54,56,92). The results showed that some discrepancies occur in the differential effects of diet in fertile and infertile men, but in short, diets containing fruits and vegetables, fish, or low-fat dairy products are associated with better semen quality. Another problem is that some studies compare the diet effects on IVF success rates that include biochemical pregnancies, that is pregnancy diagnosed only by detection of human β -choriongonadotropin in serum or urine (20,78,84,85), while others compare clinical pregnancies, defined as pregnancy diagnosed by ultrasound examination of one or more gestational sacs or by clear clinical signs of pregnancy or live births (after 22 completed weeks of gestation) (76,78,85). Many beneficial effects of a healthy diet, and recommendations can be provided with confidence, but more studies are currently needed to clarify the findings of underlying mechanisms (33) (Table 3).

Conclusion

Research in this field has shown that foods rich in fibers, folate, fruits and vegetables, seafood, poultry, nuts, whole grains, and PUFAs can increase sperm quality. Furthermore, animal proteins, unsaturated fats, and vegetable proteins can improve fertility and induce ovulation. Current studies are based mostly

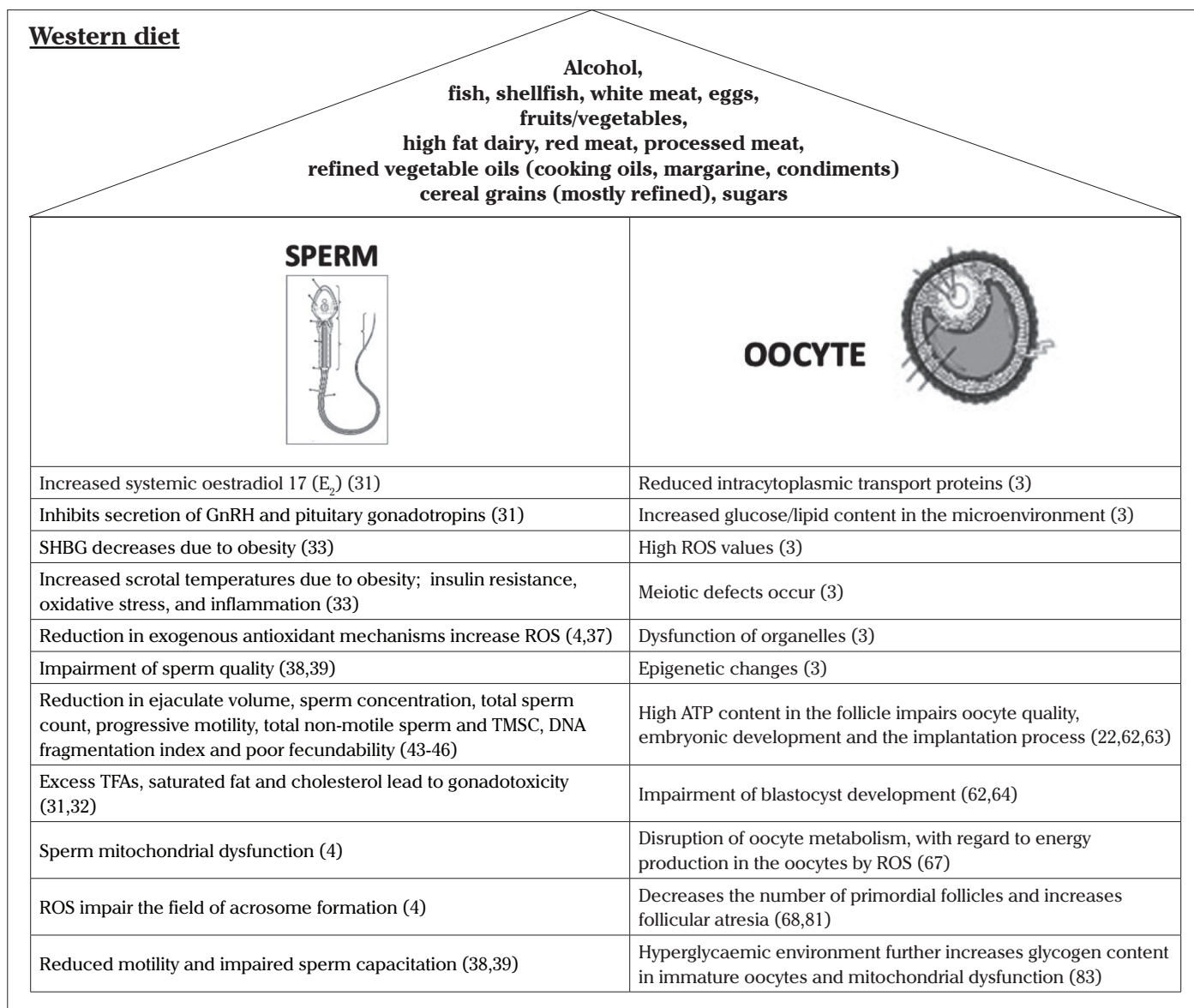


Figure 2. Impact of Western dietary patterns on female and male reproductive health

GnRH: Gonadotropin releasing hormone, SHBG: Sex hormone-binding globulin, ROS: Reactive oxygen species, TMSC: Total motile sperm count, TFA: Trans fatty acids

on animal models and the exact effects on humans are still yet to be discovered. Therefore, the dietary effects on human infertility are not conclusive. The evidence of the role of the diet on male and female fertility is weak or of very low quality. In addition, well-constructed, randomized controlled trials into diet models are needed to define the effect of a healthy diet on the quality of reproductive cells. As dietary habits vary from society to society and even from person to person obtaining clear evidence into this topic is challenging. Improved nutrition may be possible through more effective public health intervention programs that are tailored by specific nutrition characteristics and the health concerns closely associated with family planning.

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What is your diagnosis?

A 28-year-old Afro-Asian, 16-week twin pregnant woman attended our center due to fatigue and fever. At her first antenatal visit at 10 weeks, dichorionic twin pregnancy was present. One of the fetuses had negative fetal cardiac activity, the other did not have any abnormal ultrasonographic findings and findings were consistent with 10 weeks. The nuchal translucency and nasal bone were normal. She had a healthy pregnancy eight years previously, which delivered through a normal vaginal route. Fetal ultrasonographic findings were consistent with 16 weeks twin pregnancy with vanishing twin at hospital admission. The amniotic fluid of the live fetus was normal, the sac margins were regular, and the cervical length was 40 mm. The dead fetus was consistent with 9-10 weeks. The patient was hospitalized because of fatigue and fever. In the laboratory findings, the C-reactive protein (CRP) value was very high (105 mg/L) and D-dimer value was 2250 ng/mL. White blood cell count and international normalized ratio were in normal ranges. Hemoglobin value was only 7 mg/dL. She has febrile episodes, ranging 37.2-38 °C. No microorganisms were grown. No findings suggestive of choroamnionitis were found. We started empiric antibiotics (piperacillin-tazobactam) for suspicion of common microorganisms. In three days, there was no decline in the CRP values or procalcitonin levels were detected. COVID-19 polymerase chain reaction (PCR) tests were also negative. Chest X-ray revealed many micronodules scattered throughout both lungs (Figure 1). On the thorax computed tomogram miliary nodules are evident throughout the lungs (Figure 2). We switched antibiotics to meropenem. After three days of meropenem, there was again no change in CRP values, while liver enzymes started to increase. We referred the patient for definitive diagnosis and treatment to the chest diseases department.

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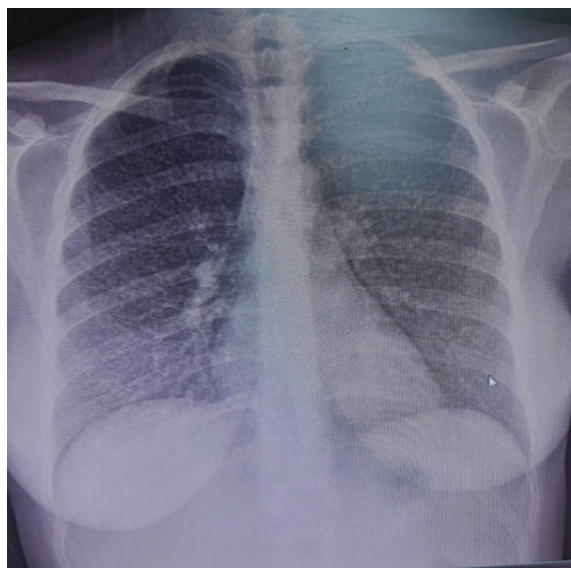


Figure 1. Chest X-ray image

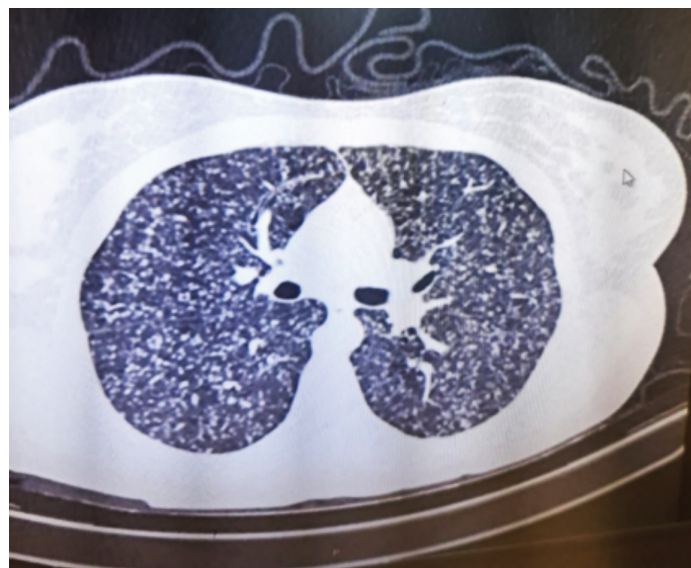


Figure 2. Thorax computed tomography image



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Answer

Miliary (disseminated) tuberculosis occurs as a result of the acute spread of tuberculosis bacilli through the blood, in numbers that can overcome the immunity of the host. The term miliary originates from diffuse micronodular pathological appearances with a diameter of 1-3 mm, and miliary tuberculosis affects many organs, such as liver, kidney, and brain as well as the lungs during the disease. Miliary tuberculosis can also develop from multiple sites where it was located during primary bacilli infection in the past, as a result of simultaneous activation due to a sudden decrease in immunity. In another mechanism, bacilli seen together with primary lung infection cause progressive disease in many organs at the same time (1,2).

It is a form of tuberculosis that is seen especially in people whose immune system is suppressed. The differential diagnosis includes pneumonia, sarcoidosis, lymphoma and lung malignancy. Quantiferon test resulted as inconclusive. She has no BCG vaccine scar. As sputum acid fast bacilli (AFB) investigation was suspicious, fiberoptic bronchoscopy was performed to take histopathological and microbiological specimens. Bronchial lavage was also negative for AFB. However, a Gen-Expert study revealed tuberculosis PCR positivity along with sensitivity to rifampicin. Radiometric culture results are still awaited at the time of writing. Biopsies did not show any specific results. This patient was started on four-drug initial regimen of antituberculous therapy once the diagnosis was established. The recommended duration of treatment varies between 6-24 months (3).

There are a limited number of cases in the literature. Although miliary tuberculosis is uncommon in pregnancy, it is difficult to

diagnose when present and is often associated with a maternal history of intravenous drug abuse, malignancy, alcoholism, or human immunodeficiency virus infection (4). She was negative for all of these risk factors with the exception of coming from a high burden country. On ultrasonography, oligo-anhydroamnios of the fetus was detected. Due to poor prognosis in the pregnancy, we planned to terminate it because of high risk for the patient, subject to her decision and the recommendation of the chest consultant, at the 18th gestational week. Abortion was induced, after giving erythrocyte suspension of two units for maternal anemia. The patient has been well since and is continuing to receive antituberculous treatment.

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Parameters affecting outcomes of transumbilical and periumbilical median incisions in ovarian cancer patients

To the Editor,

With a great deal of interest, we read the article entitled: "Comparison of transumbilical and periumbilical median incisions in ovarian cancer surgery" by Yumru Çeliksoy et al. (1). The authors identified no differences in women in terms of infections, deep surgical site infections, evisceration and incisional hernias when comparing the two groups in their retrospective study.

Incisional hernia is a very common postoperative complication after midline incision. Recently, a retrospective study showed higher incisional hernia rates in patients with body mass index >25 kg/m² undergoing transumbilical incision (2). Moreover, in the same study no difference was shown comparing the use of PDS® (Ethicon) or Vicryl® (Ethicon) sutures for the abdominal closure.

We would like to ask the authors whether they identified any differences in infection or in hernia rates in obese, glycemic or diabetic patients. Furthermore, it would be of interest if the type of abdominal closure, such as PDS® or Vicryl® sutures, had an effect on the outcomes.

Last but not least, we would like to enquire about the cosmetic result after periumbilical median incisions as asymmetry might be identified due to slewing of the scalpel blade (3).

Once again, we would like to thank the authors for their excellent study.

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Author's Response**Dear Editor,**

We appreciate the authors of the letter for carefully reading and commenting on our paper.

We assessed the characteristics, including body mass index and diabetes mellitus, in both groups, and there was no significant difference. However, the absence of a subanalysis of patients who were overweight or obese may have been one of the study's limitations. Further prospective studies are required to answer the question of whether there is a difference between the incision types in obese patients.

To close the fascia, we used a continuous-suture technique with Polydioxanone (PDS) No. 1 slowly absorbable monofilament loop suture in all patients. We agree that a study comparing different types of suture materials for transumbilical incisions would be beneficial.

We observed no disparity in wound satisfaction scores between the two groups, according to the findings of a survey administered to surviving patients. However, when surveying the patients, we did not mention or show the alternative incision type; perhaps the findings would have been different had we done so.

Yours sincerely,

Harika Yumru Çeliksoy¹, Muhterem Melis Cantürk¹, Hamdullah Sözen², Engin Çelik³, Merve Baktoroğlu¹, Yavuz Salihoğlu¹, Samet Topuz¹

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Endometrioid endometrial cancer treated with open or laparoscopic approach: is there a dilemma?

To the Editor,

It was with a great deal of interest, we read the article entitled: "Surgical treatment of endometrioid endometrial carcinoma - laparotomy versus laparoscopy" by Baum et al. (1). The authors present the perioperative outcomes, pathologic findings and long-term oncological findings of their cohort of endometrioid endometrial cancer patients treated either by laparotomy or laparoscopy. The findings were, as expected, in favor of the laparoscopic group regarding the blood loss, hospital stay, intraoperative, and severe postoperative complications. No differences were identified in the nodal yield status, progression free and overall survival between the two groups (1). The study is in accordance with the findings of a recent metanalysis (2). ESGO/ESTRO/ESP guidelines for the management of patients with endometrial cancer recommend that a minimally invasive approach is preferred, even in high-risk endometrial cancer patients (3). Awareness is raised regarding the avoidance of any intra-peritoneal tumor spillage, including tumor rupture or morcellation (including in a bag), while if vaginal extraction risks uterine rupture, mini-laparotomy or use of an endobag is proposed (3). Moreover, the ESGO accreditation in endometrial cancer surgery is an award attributed to institutions that can offer optimal levels of surgical care, based on specific quality indicators among which the following: a minimum target of 60% of patients with early stage endometrial carcinoma need to be treated with minimally invasive surgery; >60% proportion of patients with body mass index >35 kg/m² need to be treated with minimally invasive surgery; less than 10% conversions from minimally invasive surgery to open surgery; and less than 1% proportion of early stage endometrial carcinoma cases with ruptured uterus (4).

Based on the above, ESGO considers a minimally invasive approach the standard of care for endometrioid endometrial cancer patients. Although, the findings of the LACC trial raised

concerns regarding the oncological safety of patients undergoing radical endoscopic surgery in cervical cancer patients (5), such concerns are not raised in endometrial cancer cohort studies. An older systematic review showed that the application of uterine manipulators had no clear correlation with endometrial cancer recurrence, although the included trials in the review were of low methodological quality (6). A recent meta-analysis showed that the use of a uterine manipulator for a minimally invasive approach in such patients does not increase the rate of recurrence and lymph-vascular space invasion (7). Furthermore, other studies propose user-friendly tips and tricks to optimize the application of minimal invasive approach (8). Last but not least, it should be highlighted that such patients should be treated by a gynecologic oncologist, or a trained surgeon specifically dedicated to gynaecological cancer in tertiary cancer centres, as supported by ESGO (4). Once again, we would like to thank the authors for their excellent retrospective study.

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Author's Response

Dear Colleagues,

We thank you for your general appreciation of our manuscript and kind comments. Our data highlight the superiority of the laparoscopic approach over open surgery for the treatment of endometrioid endometrial cancer in terms of overall morbidity, intraoperative complications, blood loss, post-surgical recovery, as well as the incidence and severity of postoperative complications in this population. Both approaches permitted a systematic pelvic and para-aortic lymphadenectomy with a sufficient amount of resected lymph nodes. The laparoscopic approach appears to be as safe as the conventional open technique, but provides a better surgical outcome and might therefore be more beneficial for the patient.

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CONGRESS CALENDER

INTERNATIONAL MEETINGS

(for detailed International Meeting please go website:

<http://www.medical.theconferencewebsite.com/conferences/obstetrics-and-gynaecology>)

June 06-09, 2023	The Society of Obstetricians and Gynecologists of Canada Annual Clinical Scientific Conference, Ottawa, Ontario, Canada
June 21-24, 2023	International Urogynecological Association (IUGA) 48 th Annual Meeting, The Hague, South Holland, Netherlands
June 25-28, 2023	European Society of Human Reproduction and Embryology (ESHRE) 39 th Annual Meeting, Copenhagen, Denmark
September 10-13, 2023	International Federation of Fertility Societies (IFFS) World Congress, Athens, Greece
October 01-04, 2023 Brussels, Belgium	European Society for Gynaecological Endoscopy (ESGE) 32 nd Annual Congress,
October 14-18, 2023	American Society for Reproductive Medicine (ASRM) 79 th Annual Meeting, New Orleans, LA, United States
October 16-19, 2023	33 rd ISUOG World Congress, Seoul, South Korea
October 18-22, 2023	19 th World Congress on Menopause, Melbourne, Australia
November 05-07, 2023	International Gynecologic Cancer Society (IGCS) 2023 Meeting, Seoul, South Korea
November 05-09, 2023	The 52 nd American Association of Gynecologic Laparoscopists (AAGL) Global Congress on Minimally Invasive Gynecologic Surgery (MIGS), Nashville, Tennessee, United States
November 23-25, 2023	The 31 st World Congress on Controversies in Obstetrics Gynecology & Infertility (COGI), Vienna, Austria

CONGRESS CALENDER

NATIONAL MEETINGS

(for detailed International Meeting please go website:
<http://www.kongre2022.com>)

June 02-03, 2023	TAJEV Fonksiyonel Tıp Kongresi, İstanbul, Türkiye
June 02-06, 2023	6. Karadeniz Jinekoloji ve Obstetrik Kongresi, Trabzon, Türkiye
June 09-11, 2023	1. Akdeniz Jinekoloji ve Obstetrik Kongresi, Adana, Türkiye
June 21-24, 2023	6. Minimal İnvaziv Jinekolojik Cerrahi Kongresi, İstanbul, Türkiye
October 05-08, 2023	5. Jinekoloji ve Obstetrikte Tartışmalı Konular Kongresi, Muğla, Türkiye
October 20-22, 2023	11. Ulusal Menopoz Osteoporoz ve Kadın Sağlığı Kongresi, İstanbul, Türkiye
October 25-28, 2023	Türkiye Maternal Fetal Tıp ve Perinatoloji Derneği Ultrasonografi Kongresi, İstanbul, Türkiye
November 01-05, 2023	10. Üreme Tıbbi ve Cerrahisi Derneği Kongresi, Antalya, Türkiye
November 11-12, 2023	Çukurova Kadın Doğum Günleri, Adana, Türkiye
November 16-19, 2023	11. Üreme Sağlığı ve İnfertilite Kongresi, Antalya, Türkiye