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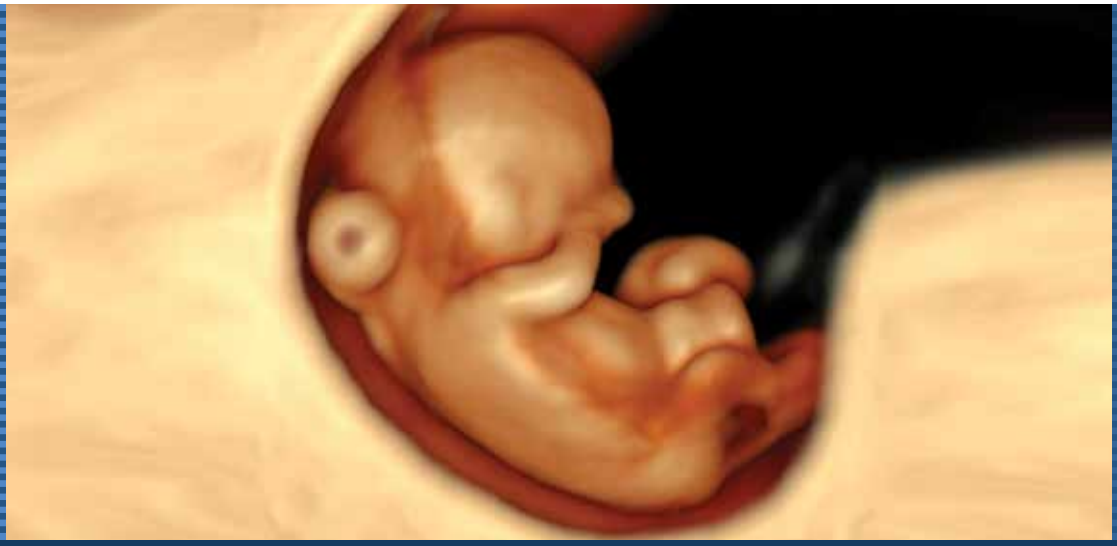

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Cover Picture: HDlive rendering mode of fetus at 10 weeks 2 days of gestation. *Tonni et al. (Page: 113)*

Original Investigations

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The target audience of Journal of the Turkish-German Gynecological Association includes gynaecologists and primary care physicians interested in gynecology practice. It publishes original work on all aspects of gynecology. The aim of Journal of the Turkish-German Gynecological Association is to publish high quality original research articles. In addition to research articles, reviews, editorials, letters to the editor and case presentations are also published.

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STARD checklist for the reporting of studies of diagnostic accuracy (Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, et al, for the STARD Group. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. Ann Intern Med 2003;138:40-4.) (http://www.stard-statement.org/),

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State concisely the purpose and rationale for the study and cite only the most pertinent references as background.

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Describe the plan, the patients, experimental animals, material and controls, the methods and procedures utilized, and the statistical method(s) employed. In addition to the normal peer review procedure, all randomized controlled trials (RCTs) submitted to the journal are sent to members of a team of professional medical statisticians for reviewing.

Address "Institutional Review Board" issues as stated above. State the generic names of the drugs with the name and country of the manufactures. Provide information on informed consent and ethics committee approval.

Results
Present the detailed findings supported with statistical methods. Figures and tables should supplement, not duplicate the text; presentation of data in either one or the other will suffice. Emphasize only your important observations; do not compare your observations with those of others. Such comparisons and comments are reserved for the discussion section.

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State the importance and significance of your findings but do not repeat the details given in the Results section. Limit your opinions to those strictly indicated by the facts in your report. Compare your finding with those of others. Provide information on the limitations of the study. No new data are to be presented in this section.

The main text of case reports should be structured with the following subheadings: Introduction, Case Presentation, Discussion.

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Number references in Arabic numerals consecutively in the order in which they are mentioned in the text starting with number "1". Use the form of the "Uniform Requirements for Manuscript Submitted to Biomedical Journals" (http://www.amaassn.org/public/peer/wame/uniform.htm). If number of authors exceeds seven, list first 6 authors followed by et al.

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Contents

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Editorial

It is my great pleasure to present you the second issue of Journal of the Turkish-German Gynecological Association *(J Turk Ger Gynecol Assoc)* in the publishing year of 2016.

In this latest issue, we have included many high quality manuscripts. One of them is a meta-analysis reviewing the recent literature and updating a previous systematic review and this study investigates the potential role of chorionic villus sampling in increasing the subsequent rate of occurrence of preeclampsia and gestational hypertension. You will also read an interesting paper determining the role of *BRCA1* and *BRCA2* gene mutations in the etiology of premature ovarian insufficiency in a Turkish population. The presence of mutations of *BRCA1* or *BRCA2* genes are associated with an increase in the risk of breast, ovarian, fallopian tube and peritoneal cancers. Herein is presented another highly scientific article reviewing cases with peritoneal cancer after prophylactic bilateral salpingo-oophorectomy and discussing the possible etiology of this rare entity as well as the possible changes in the management of such patients. As usual, we have included a quiz too, which we are sure that you will enjoy solving.

With each new issue of *J Turk Ger Gynecol Assoc*, we are trying to further increase the quality of our content. We are glad to see that this consistency in quality has translated well into our readership numbers. Number of hits we have received on PubMed has almost doubled over the past 18 months. Thank you for your interest.

Our submission traffic is also progressing in a healthy way. Due to *J Turk Ger Gynecol Assoc's* increasing international popularity and visibility, the number of international submissions we receive has almost tripled over the past 24 months and it is nice to see the quality of submissions is also rising.

Increasing submission numbers and the quality of these submissions makes our jobs as editors easier and harder at the same time. Thanks to the international support we receive from our reviewer community we can manage these submissions with ease. The average time from submission to first decision is 4 weeks for new submissions. We are planning to lower the average to 21 days within the next year. On behalf of the editorial board, I would like to thank our reviewers for their continuous support.

As you might know, we try to be as fast as possible to make the accepted manuscripts available to our readers. We publish the accepted papers within 4-6 weeks of the final decision. We will try to reduce this to 3 to 4 weeks within the next year as well.

Turkish-German Gynecological Education and Research Foundation's 11th congress (TAJEV 2016) was recently held in Antalya, Turkey and I am glad to report that it was a success. The scientific quality was very high and the organization received great interest from the international gynecology and obstetrics society. We have had more than 1600 participants and faculty members from 12 different contries.

During the congress we had keynote lectures with the world's most reputable speakers; Prof. Sara Brucker, Prof. Camran Nezhat, Prof. Serdar Bulun, Prof. Karl Oliver Kagan. In addition, there were two live surgeries, one from France the other from USA, with the moderation by Prof. Ceana Nezhat. I am sure that these lectures will positively contribute to the education and progress of your participants.

We have wasted no time and we have already started to work on TAJEV 2018. If you have any feedback regarding TAJEV 2016 that you would like to share with us, please do not hesitate to get in direct contact with us.

Sincerely,

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

The impact of a simulation-based training lab on outcomes of hysterectomy

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Abstract

Objective: To evaluate the impact of a simulation-based training lab on surgical outcomes of different hysterectomy approaches in a resident teaching tertiary care center.

Material and Methods: This retrospective cohort study was conducted at The University of Texas, Department of Obstetrics and Gynecology. In total, 1397 patients who had undergone total abdominal hysterectomy (TAH), vaginal hysterectomy (VH), total laparoscopy-assisted hysterectomy (TLH), or robot-assisted hysterectomy (RAH) for benign gynecologic conditions between 2009 and 2014 were included in the study. The comparison was made according to the year when the surgeries were performed: 2009 (before simulation training) and the combination of 2010-2014 (after simulation training) for each technique (TAH, VH, and LAH). Since a simulation lab for robotic surgery was introduced in 2010 at our institute, the comparison for robotic surgery was made between the combination of 2009-2010 as the control and the combination of 2010-2014 as the study group.

Results: The average estimated blood loss before and after simulation-based training was significantly different in TAH and RAH groups (317±170 mL versus 257±146 mL, p=0.003 and 154±107 mL versus 102±88 mL, p=0.004, respectively), but no difference was found for TLH and VH. The mean of length of hospital stay was significantly different before and after simulation-based training for each technique: 3.7±2.3 versus 2.9±2.2 days for TAH, 2.0±1.2 versus 1.3±0.9 days for VH, 2.4±1.3 versus 1.9±2.5 days for TLH, and 2.0±1.3 versus 1.4±1.7 days for RAH (p<0.01).

Conclusion: Based on our data, simulator-based training may play an integrative role in developing the residents' surgical skills and thus improving the surgical outcomes of hysterectomy. (J Turk Ger Gynecol Assoc 2016; 17: 60-4)

Keywords: Hysterectomy, simulation training, outcomes

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Introduction

Simulator-based training has recently evolved as an effective method in the training of surgeons (1). Developing the necessary eye–hand coordination before hands-on surgical practice may not only shorten the learning curve but also improve patient safety (2). By practicing in a simulation lab, residents can improve their psychomotor and cognitive skills in a risk-free setting (3-6). The benefit of simulator-based training has been increasingly evident since total laparoscopy-assisted hysterectomy (TLH) & robot-assisted hysterectomy (RAH) cases accounted for more than 30% of the total hysterectomies in the United States (7).

Hysterectomy is the most commonly performed gynecological surgical procedure after cesarean section; all residents should master these techniques during resident training (8). While the number of hysterectomies performed annually was 681,234 in 2002, it was calculated as 433,621 in 2010: the number in 2010 was lesser by 247,973 than that in 2002. Benign conditions still comprise the most common indications, with a rate of 90% (9, 10). Moreover, the use of new surgical techniques as well as more access to laparoscopy/robot-assisted approaches for hysterectomy has become more common than the use of abdominal hysterectomy (11-13). As a result, the overall real-case exposure time to perform each approach [total abdominal hysterectomy (TAH), vaginal hysterectomy (VH), TLH, and RAH has been probably compromised to achieve surgical competency. The importance of patient safety as well as limited resident work-hour necessitates new educational techniques for resident training.

Recent literature has shown that the skills gained in the simulation lab for laparoscopic/robotic techniques are transferable to real-case applications (14, 15). However, translation of this to actual clinical outcomes of patients is lacking. In addition, implementing simulation-based training for VH and TAH gains feasibility; however, no available data exist in the current

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literature. The aim of our study was to evaluate the impact of a simulation-based training lab on actual surgical outcomes of different hysterectomy approaches for benign cases in a resident teaching tertiary care center. We also investigated the impact of demographic data on patient selection when hysterectomy is needed.

Material and Methods

Simulator lab

The Mimic Technologies dV-Trainer platform (Mimic Technologies Inc.; Seattle, WA, USA) as a robotic surgery trainer, the 3-Dmed Trainer platform (3-DMEd; Franklin, OH, USA) as a laparoscopy trainer, and the Surgical Female Pelvic Trainer (SFPT) with Advanced Surgical Uterus (Limbs&Things; Bristol, UK) as an open surgery trainer have been used at our institution. All residents proceeded to a structured simulation-based training program implemented in 2009 for TAH, VH, and TLH and in 2010 for RAH. Residents should achieve at least 75% success in training exercises in order to be able to perform surgery on actual cases.

Study design

This retrospective cohort study was approved by the institution review board at The University of Texas Medical Branch. The study population consisted of the patients who had undergone hysterectomy for benign gynecologic conditions (such as leiomyoma, abnormal uterine bleeding, pelvic organ prolapse and/or urinary incontinence, endometriosis, adenomyosis, chronic pelvic pain, and endometrial hyperplasia without atypia) at John Sealy Hospital between 2009 and 2014. Patients with history of gynecological malignancy were excluded from the study. In total, 1397 patients were included in the study.

The patients' age, parity, number of previous surgeries, body mass index (BMI), estimated blood loss (EBL), intraoperative adverse events (IOAE), duration of postoperative hospital stay (HS), number of blood transfusions (BT), and operation room time (ORT) were obtained from the patient's medical records. EBL was calculated in millimeter (mL) and had been recorded during the surgery. IOAE were defined as urinary tract (at least the bladder or ureteral serosa), bowel (at least the bowel serosa), and/or vascular injuries. The length of HS was calculated by subtracting the day of surgery from the day of discharge. The number of BT was calculated in units, which were given to the patient during or following the surgery. ORT was calculated by subtracting the time when patients were taken to the operation room from the time when patients were physically removed after completion of surgery (wheels in and out). Further, the data for TAH, VH, and TLH were stratified according to the year when the surgeries were performed: 2009, which was used as a baseline before the simulation lab was introduced, and the combination of 2010-2014, which was used to assess the impact of the simulation. Since a simulation lab for robotic surgery was introduced in 2010 at our institute, the data for RAH was stratified according to the year when robotic simulation was introduced: 2009-2010 (before simulation) and the combination of 2010-2014 (after simulation). In addition, the postgraduate years (PGY) were later stratified into two groups: PGY2/3 and PGY4. We combined PGY2 and PGY3 because the number of patients on whom PGY2 residents performed surgery was very low to reach a better conclusion.

The outcomes of patients who underwent hysterectomy before simulation-based training were compared with the outcomes of patients who underwent hysterectomy after simulation-based training in terms of EBL, IOAE, length of HS, rate of BT, and ORT for each hysterectomy approach. In addition, the outcomes of hysterectomy performed by PGY2/3 residents were compared with the outcomes of hysterectomy performed by PGY4 residents.

Statistical analysis

SPSS 11.5 software (SPSS Inc.; Chicago, IL, USA) was used for statistical analysis. One-way analysis of variance (ANOVA), Student's t-tests, Mann–Whitney U test, and two-sample z-tests were performed where appropriate. One-way ANOVA was used to compare patient characteristics, and Mann–Whitney U test was used to compare the average EBL, the mean length of HS, the number of BT, and the mean ORT. Two-sample z-test was used to compare the rates of IOAE. A p value of 0.05 was considered as the level of statistical significance. Data are presented as mean±standard deviation (mean±SD) or percentage (%).

Of the 1397 patients, 41% (n=576) underwent TAH, 22% (n=305) underwent VH, 20% (n=272) underwent TLH, and 17% (n=244) underwent RAH. All patients' mean age, BMI, parity, and number of previous surgeries (±SD) were 45.4±9.7 years, 31.4±11.5 kg/m^2, 2.4±1.5, and 1.4±1.4, respectively. The patients who underwent VH were older than other patients (48.1±11.7 years, $p<0.05$). Among patients who underwent hysterectomy, BMI was the highest in patients who underwent RAH (32.9±8.1 kg/m^2, $p<0.05$). Parity, with a mean of 2.9±1.6, was the highest in patients who underwent VH, and a significant difference was found when compared with patients who underwent other types of hysterectomies. The number of previous surgeries was the lowest in the VH group and showed a significant difference in comparison with other groups. Data for the demographics are presented in Table 1.

Table 1. The comparison of demographic data of patients

	TAH	VH	TLH	RAH	p
Age (mean±SD years)	44.9±9.0	48.1±11.7	43.5±8.6	45.3±8.7	<0.05
BMI (mean±SD kg/m^2)	31.9±7.9	29.5±6.2	30.9±6.9	32.9±8.1	<0.05
Parity (mean±SD)	2.2±1.5	2.9±1.6	2.3±1.6	2.1±1.3	<0.05
The number of previous surgery (mean±SD)	1.4±1.4	1.1±1.1	1.6±1.4	1.3±1.2	<0.05

TAH: total abdominal hysterectomy; VH: vaginal hysterectomy; TLH: total laparoscopic hysterectomy; RAH: robot-assisted hysterectomy; BMI: body mass index

Table 2 presents the outcomes of hysterectomy obtained before and after simulation-based training for each hysterectomy technique. The average EBL was 317±170 versus 257±146 mL for TAH, 219±124 versus 180±101 mL for VH, 190±142 mL versus 149±104 mL for TLH, and 154±107 versus 102±88 mL for RAH. The average EBL was lower after simulation-based training for all approaches, but a significant difference was found for only TAH and RAH groups (p=0.003 and p=0.004, respectively). The mean lengths of HS was 3.7±2.3 versus 2.9±2.2 days for TAH, 2.0±1.2 versus 1.3±0.9 days for VH, 2.4±1.3 versus 1.9±2.5 days for TLH, and 2.0±1.3 versus 1.4±1.7 days for RAH. All groups showed a significant difference in terms of the mean lengths of HS obtained before and after simulation-based training (p<0.001 for all groups). Simulation-based training had a favorable impact on the length of HS in all approaches. With regard to the rates of IOAE, none of the groups showed a significant difference before and after simulation-based training. No significant difference was found between TAH and RAH groups in the rates of BT before and after simulation-based training. Because no blood transfusion was needed in VH and LAH groups before simulation-based training in our patient population, a comparison could not be performed for those groups. ORTs were not significantly different for all hysterectomy techniques when the introduction of simulation-based training was accepted as stratifying factor to reveal its impact.

Overall, the outcomes of all types of hysterectomies based on PGY were reported (Table 3). Although the average EBL, rate of IOAE, and rate of BL were less in PGY4 residents, no significant difference was found between PGY2/3 and PGY4 residents. The mean length of HS was shorter in PGY 4 residents than in PGY2/3 residents, and the difference was significant (p<0.001). The mean ORTs before and after simulation-based training were 210±84 versus 195±81 min (p=0.002).

Discussion

Information on evaluation of the impact of a simulator lab on actual hysterectomy outcomes in a tertiary center setting in gynecology is lacking in the literature. Training with simulators has been shown to improve residents' performance on the modules used in robotic and laparoscopic simulators in our previous studies (16, 17). Meanwhile, studies assessing the transferability of laparoscopic and endoscopic simulators to real-time performance have proved to be useful of simulator-based training (14, 15, 18, 19). A VH simulator has been shown to have beneficial effects in learning routine VH (20, 21). Although Greer et al. (21) have shown that structured multiple-component VH education is valid, the impact of VH simulation on surgical competence could be unproven. A pilot study concluded that residents' surgical skills and knowledge were better after simulator education for TAH; no clinical outcomes exist to reach a conclusion about the effectiveness of simulator education (22).

In our study, simulation-based education markedly shortened the outcomes of all hysterectomies in terms of HS. Although the average EBL was also positively influenced by simulation-based training in all techniques, only TAH and RAH showed a significant difference. With regard to the frequency of IOAE, simulation-based training did not seem to make a difference in any hysterectomy technique. Although a partial improvement in the results of ORT was observed in the RAH group, no improvement was observed in the other groups. On the other hand, ORT was better when hysterectomies were performed by PGY4 residents than when hysterectomies were performed by PGY2/3 residents, irrespective of simulation-based training.

In a database study by Igwe et al. (23), the authors found that

Table 2. Comparison of outcomes of each hysterectomy technique obtained before and after simulation-based training in terms of the average estimated blood loss (EBL), duration of postoperative hospital stay (HS), rate of intraoperative adverse events (IOAE), number of blood transfusions (BT), and mean operation room time (ORT)

	TAH (n=576)	VH (n=305)	TLH (n=272)	RAH (n=244)
EBL (mean±SD/mL)	2009 (n=78) 317±170 2010-2014 (n=498) 257±146 p 0.003	2009 (n=36) 219±124 2010-2014 (n=269) 180±101 p 0.054	2009 (n=40) 190±142 2010-2014 (n=232) 149±104 p 0.114	2009-2010 (n=34) 154±107 2011-2014 (n=210) 102±88 p 0.004
HS (mean±SD/day)	2009 (n=78) 3.7±2.3 2010-2014 (n=498) 2.9±2.2 p<0.001	2009 (n=36) 2.0±1.2 2010-2014 (n=269) 1.3±0.9 p<0.001	2009 (n=40) 2.4±1.3 2010-2014 (n=232) 1.9±2.5 p<0.001	2009-2010 (n=34) 2.0±1.3 2011-2014 (n=210) 1.4±1.7 p<0.001
IOAE (%)	2009 (n=78) 9% 2010-2014 (n=498) 10% p 0.345	2009 (n=36) 3% 2010-2014 (n=269) 4% p 0.351	2009 (n=40) 5% 2010-2014 (n=232) 7% p 0.538	2009-2010 (n=34) 5% 2011-2014 (n=210) 2% p 0.044
BT (mean±SD/unit)	2009 (n=78) 0.3±0.7 2010-2014 (n=498) 0.2±0.6 p 0.014	2009 (n=36) 0 2010-2014 (n=269) 0.007±0.1 p n/a	2009 (n=40) 0 2010-2014 (n=232) 0.03±0.2 p n/a	2009-2010 (n=34) 0.02±0.17 2011-2014 (n=210) 0.01±0.13 p>0.05
ORT (mean±SD/min)	2009 (n=78) 185±76 2010-2014 (n=498) 179±74 p 0.523	2009 (n=36) 178±68 2010-2014 (n=269) 168±66 p 0.318	2009 (n=40) 214±74 2010-2014 (n=232) 206±84 p 0.375	2009-2010 (n=34) 281±89 2011-2014 (n=210) 264±77 p 0.141

TAH: total abdominal hysterectomy; VH: vaginal hysterectomy; TLH: total laparoscopic hysterectomy; RAH: robot-assisted hysterectomy

Table 3. Comparison of postgraduate years (PGY) 2/3 and PGY4 in terms of average estimated blood loss (EBL), duration of postoperative hospital stay (HS), rate of intraoperative adverse events (IOAE), number of blood transfusions (BT), and mean operation room time (ORT)

	PGY-2/3 (n=547)	PGY-4 (n=850)	p
EBL (mean±SD/mL)	208±145	193±134	0.098
HS (mean±SD/day)	2.3±2.3	2.1±2.5	<0.001
IOAE (%)	6.4	6.9	0.341
BT (mean±SD/unit)	0.09±0.4	0.07±0.4	0.021
ORT (mean±SD/min)	210±84	195±81	0.002

the mean operative times were longer when a resident joined total laparoscopic hysterectomy (robotic versus conventional laparoscopy), but the duration was shorter when the attending surgeon operated alone. A significant difference was not found between the junior resident (PGY1/2) and senior resident groups (other PGYs) in the same study. In our study, we found that the performance of PGY4 residents with regard to ORT was significantly better than that of PGY2/3 residents for all techniques calculated. One explanation for this significant difference may be that our senior resident group was restricted to only PGY-4 residents in our study. The same study also examined the rates of complications in the attending-alone and the resident-involved groups (5.4% versus 6.8%, p=0.54). In addition, no significant difference existed between the junior resident and senior resident groups. Even when only the attending surgeon performed hysterectomy with a minimally invasive technique, the rates of complication stayed at a similar level in their study. The fact that all surgeries were performed under the direct supervision of a faculty in our study population may explain why we did not observe a significant difference before and after simulation-based education or between PGY2/3 and PGY4 residents in terms of the rate of IOAE. The hysterectomies included in this study were performed for residents' education only, and this may be another reason for the unchanged rate of IOAE before and after simulation-based education or between PGY2/3 and PGY4 residents.

According to our evaluation of patients' demographic data, while patients who underwent VH had the highest mean parity and age, the mean of the number of previous surgeries was the lowest in the VH group. Because age and parity are known to be risk factors for developing pelvic organ prolapse (24), patients with higher parity and older age may have had more advanced pelvic organ prolapse; therefore, this patient population more often underwent a VH procedure. Despite the ACOG opinion (25), the number of previous surgery seemed to be a factor affecting patient selection for the hysterectomy technique. Not surprisingly, it seems that suspected intraabdominal adhesions motivate surgeons to perform an abdominal approach rather than a vaginal approach. Our findings also showed that the patients who underwent RAH had higher BMI than those who underwent other hysterectomies. The robotic surgery technique is known to offer several advantages such as better instrument function without hand tremor and no need to cope with thick abdominal wall in obese patients. These advantages help the surgeon overcome the hardships encountered with traditional laparoscopy and perform surgery on obese patients more confidently. A study conducted by Geppert et al. (26) showed that RAH in obese women was associated with shorter hospitalization, fewer significant complications, and lesser EBL than TAH. Our results may be related to the fact that surgeons working at our institute tend to utilize the robotic technique in performing surgery on patients with higher BMI to reach the best clinical outcomes, which is correlated with the findings revealed by Geppert et al. (26).

One limitation of the study is that control group could not consist of longer consecutive years of data because of physical interruption by the hurricane Ike in 2008 in Galveston. Therefore, we included the data from 2009 and further consecutive years. Another limitation is that the constant shift in hysterectomies from open to minimally invasive hysterectomies throughout the study years may have potential effects on patient outcomes. However, this is a parallel change with nationwide institutions; therefore, it should not affect our overall results.

In conclusion, a simulator lab improves the outcomes of hysterectomy performed at a teaching institution and may play an adjunct role in developing the residents' surgical skills. The skills learned under simulation settings were at least partially transferable to actual surgical cases for all types of hysterectomies according to our results. Actual surgical outcomes of other large institutes using simulator lab will be helpful to reach a more precious conclusion about the impact of implementing simulator-based training on clinical outcomes.

Ethics Committee Approval: *Ethics committee approval was received for this study from the review board of the institution.*

Informed Consent: *N/A.*

Peer-review: *Externally peer-reviewed.*

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Effect of chorionic villus sampling on the occurrence of preeclampsia and gestational hypertension: An updated systematic review and meta-analysis

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Abstract

Objective: To perform a meta-analysis for an assessment of the risk of preeclampsia or gestational hypertension following chorionic villus sampling (CVS).

Data source: PubMed was systematically searched from its inception through January 2016.

Material and Methods: Nine reports were identified. A pre-specified scale was used to assess their quality.

Tabulation, integration, and results: We performed pooling into three subgroups with respect to the control group: A) Patients with no invasive prenatal diagnostic procedure served as a control group for comparison. The odds ratios for gestational hypertension (0.76, 95% CI 0.46–1.26), preeclampsia (0.83, 95% CI 0.42–1.67), and severe preeclampsia (0.49, 95% CI 0.04–5.78) or when hypertension categories were pooled (0.80, 95% CI 0.46–1.41) were not significantly different. B) Patients with midtrimester diagnostic amniocentesis and patients with no invasive prenatal diagnostic procedure were combined as a control group for comparison. The odds ratios for preeclampsia (1, 95% CI 0.46–2.18), severe preeclampsia (0.83, 95% CI 0.14–4.85), and pooled hypertension categories (1.07, 95% CI 0.63–1.84) were not significantly different. C) Patients with midtrimester diagnostic amniocentesis served as a control group. There was a significant difference in the odds ratio for preeclampsia between the CVS and amniocentesis groups (2.47, 95% CI 1.14–5.33). There was a marginal difference in the odds ratio for combined pregnancy-induced hypertension categories between the CVS and amniocentesis groups (1.61, 95% CI 1.02–2.53).

Conclusion: The available data do not indicate an increased risk of preeclampsia or gestational hypertension following first trimester CVS. The heterogeneity and retrospective design of existing studies are limiting factors for our analysis and findings.

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Keywords: Meta-analysis, chorionic villus sampling, preeclampsia, midtrimester diagnostic amniocentesis

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Introduction

Preeclampsia affects 5%–8% of all pregnancies. Together with the other hypertensive disorders of pregnancy, including gestational hypertension, it is a major contributor to maternal and perinatal morbidity and mortality (1, 2). Although significant advances have been made in elucidating the etiopathogenesis of preeclampsia, the identification of the specific cause(s) of this syndromic disorder remains elusive; therefore, it continues to be classified as a syndrome and not a single disease entity (3).

Current knowledge about the pathogenesis of preeclampsia suggests that altered placental development and disturbances in the transformation of the spiral arteries in early pregnancy play critical roles in the progression of events that initiate a spectrum of pregnancy-induced hypertensive disorders (4, 5). The first trimester diagnostic procedure of chorionic villus sampling (CVS) has been shown to cause some degree of bleeding and disruption at the feto-maternal placental interface, which is demonstrated by elevated maternal levels of alpha-fetoprotein and pregnancy-associated plasma protein A (6, 7). This disruption in the developing placental-decidual interface secondary to CVS during early pregnancy has been postulated to potentially cause subsequent abnormal placental development. A possible consequence of CVS could be an increased occurrence of hypertensive complications of pregnancy (8). Here, we will review the recent literature and update our previous systematic review and meta-analysis (9) to investigate the potential role of CVS in increasing the subsequent rate of occurrence of preeclampsia and gestational hypertension.

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Material and Methods

The current meta-analysis conforms to the guidelines outlined by the Meta-analysis of Observational Studies in Epidemiology (MOOSE) and was performed in accordance with the indicated statement (10).

Search strategy

We searched PubMed from its inception through January 2016 to identify studies that evaluated CVS and the subsequent occurrence of preeclampsia or gestational hypertension (Figure 1). The search terms and queries that were used in the PubMed search are provided in Table 1. We discovered additional studies by searching the bibliographies of the discovered studies, letters to editors, guidelines, and review articles and included these citations if they were found to be relevant to the subject matter being studies.

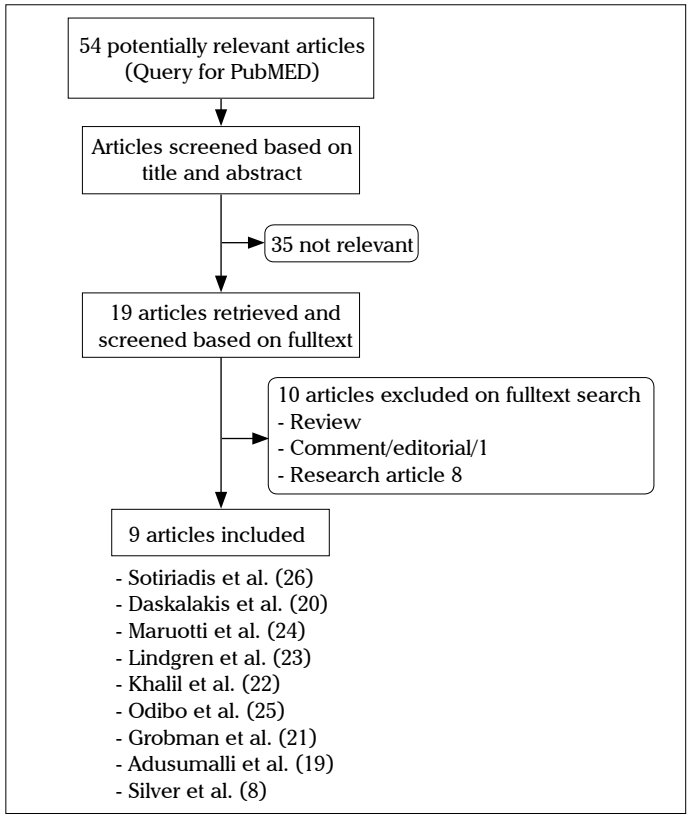

Figure 1. Search strategy

Study selection

The relevant studies were independently reviewed by two reviewers to grade them for quality and to determine their eligibility for inclusion in this systematic review and meta-analysis according to the following criteria:

a) Studies designed to compare CVS with a control group and/or amniocentesis and/or
b) Studies that report odds ratios (ORs) or data for the occurrence of preeclampsia and/or gestational hypertension as acute-onset, non-chronic hypertensive complications of pregnancy; and
c) Studies in the English language.

Assessment of reporting and study quality

The two reviewers independently and separately evaluated the reporting and study quality of the included studies. The two reviewers undertook this task using a pre-specified assessment scale, which was developed for the evaluation of the methodology used in retrospective clinical studies (11). Discussions about the interpretation and scoring of the checklist were undertaken before the initiation of the assessment and data abstraction procedure. Several items of the indicated quality assessment checklist were irrelevant and were accordingly removed. The maximum overall score that was possible using the customized quality assessment checklist was 22. To evaluate each individual study independently, associated spreadsheets were arranged for each reviewer for their assessment of the included studies.

Data abstraction

From the manuscripts that were determined to be appropriate for inclusion in the analysis, information regarding the year of the study, design of the study (retrospective versus prospective), sample size, nulliparity, maternal age, smoking status, body mass index, and hypertensive complications of pregnancy, which included gestational hypertension, preeclampsia, and severe preeclampsia, were extracted when available. Table 2 lists the characteristics of the studies and the extracted information. Two reviewers independently extracted the data, which were cross-checked by the authors (Table 3). We discussed any discrepancies between the two reviewers. Discrepancies were resolved by consensus among the authors.

Table 1. Search terms and query for PubMed search

Search terms	"Villus sampling," "chorionic villus sampling," "chorionic villi sampling," "chorionic villous sampling," "CVS," "invasive diagnostic procedure," "prenatal invasive," "hypertensive disorders," "preeclampsia," "pre-eclampsia," "pregnancy induced hypertension," "PIH," "eclampsia," "hypertension," "pregnancy," "proteinuria"
Query for PubMed	("Chorionic Villous Sampling"[TIAB] OR "Chorionic Villus Sampling"[TIAB] OR "CVS"[TIAB] OR "invasive diagnostic procedure"[TIAB] OR "Chorionic Villi Sampling"[MeSH Terms]) AND ("Hypertension, Pregnancy-Induced"[MeSH Terms] OR "Pre-eclampsia"[MeSH Terms] OR "preeclampsia"[TIAB] OR "Pregnancy induced hypertension"[TIAB] OR "PIH"[TIAB] OR "gestational hypertension"[TIAB] OR ("hypertensive disorder"[TIAB] AND "pregnancy"[TIAB]) OR ("hypertension"[TIAB] AND "pregnancy"[TIAB] AND "proteinuria"[TIAB]) OR ("hypertensive disorders"[TIAB] AND "pregnancy"[TIAB]) OR "eclampsia"[TIAB])

Table 2. Characteristics of the studies included in the meta-analysis

Study/year	Study design	Number of participants who underwent amniocentesis	Number of participants who underwent CVS	Number of participants with no invasive procedure	Maternal age control/ CVS groups	Nulliparity control/ CVS groups	Body mass index control/ CVS groups	Smoking status control/ CVS groups
Sotiriadis et al. (26)	Retrospective	-	437	2969	31.7/35.2	-	24.4/24.5	11%/9.8%
Daskalakis et al. (20)	Retrospective	6875	3243	-	30.0/35.5	41.1%/45.1%	28.4/28.3	6.5%/6%
Maruotti et al. (24)	Retrospective	-	219	553	-	-	-	-
Lindgren et al. (23)	Retrospective	21748	1984	47854	-	18.8%/15.3%	-	19.8%/15.4%
Khalil et al. (22)	Part of prospective ongoing trial	-	2278	28860	32.1/35.8	48.9%/35.2%	24.2/24.4	8.2%/8.8%
Odibo et al. (25)	Retrospective	-	5232	4136	31.6/37.8	33.1%/26.2%	-	8.7%/9.8%
Grobman et al. (21)	Retrospective	501	152	653	35.9/37.4	41.4%/38.8%	29.5/29.2	1.6%/1.3%
Adusumalli et al. (19)	Retrospective	-	1540	840	33.6/38.6	-	25.5/29.3	-
Silver et al. (8)	Secondary analysis of a randomized trial	1820	1878	-	-	-	-	-

BMI: body mass index; CVS: chorionic villus sampling

Table 3. Outcomes according to the type of hypertensive pregnancy disorder

	PRE			SPRE			GH			MAPHT		
Study/Year	CVS	AMNIO	Control	CVS	AMNIO	Control	CVS	AMNIO	Control	CVS	AMNIO	Control
Sotiriadis et al. (26)	14/437	-	37/2969	-	-	-	-	-	-	-	-	-
Daskalakis et al. (20)	78/3165	53/6822	-	-	-	-	44/3199	62/6813	-	122/3121	115/6760	-
Maruotti et al. (24)	5/219	-	48/553	-	-	-	-	-	-	-	-	-
Lindgren et al. (23)	-	-	-	-	-	-	-	-	-	98/1984	955/21748	2106/47854
Khalil et al. (22)	43/2278	-	654/28860	-	-	-	62/2278		795/28860	105/2278	-	1449/28860
Odibo et al. (25)	83/5096	-	203/4002	8/5096	-	45/4002	55/5096	-	80/4002	138/5096	-	283/4002
Grobman et al. (21)	5/152	12/501	8/653	-	-	-	-	-	-	-	-	-
Adusumalli et al. (19)	56/1540	-	33/840	28/1540	-	9/840	7/1540	-	4/840	76/1540	-	37/840
Silver et al. (8)	-	-	-	32/1878	13/1820	-	-	-	-	102/1878	64/1820	-

PRE: preeclampsia; SPRE: severe preeclampsia; GH: gestational hypertension; MAPHT: merged acute pregnancy-induced hypertensive disorders; CVS: chorionic villus sampling; AMNIO: amniocentesisT

Statistical analysis

Heterogeneity among the included studies was assessed using the I^2 statistic. Thresholds for the interpretation of the I^2 statistic were adapted from the Cochrane Handbook for Systematic Reviews of Interventions (12, 13). A guide to the interpretation of the I^2 statistic is provided in Table 4 (12). The DerSimonian–Laird random-effects model was applied in the statistical analyses that were performed in this meta-analysis. We used RevMan® version 5 (The Cochrane Collaboration; Copenhagen: The Nordic Cochrane Centre, Copenhagen, Denmark) for the calculation of effect sizes and the corresponding 95% confidence intervals (14). The results of scoring according to the customized checklist that was used to assess the methodological quality of the included studies are presented in Table 5. We used Cohen's kappa to determine the reliability of the checklist scores between the two reviewers. Values of Cohen's kappa were calculated using IBM SPSS® version 21 (IBM Corp.; Armonk, New York, USA) (15). We used the classification suggested by Landis and Koch for the interpretation of the values of Cohen's kappa (Table 6) (16). The prevalence rates of preeclampsia and merged acute pregnancy-induced hypertensive disorders among the pooling groups were compared using the Chi-square test with post hoc testing. For post hoc testing, we used adjusted standardized residuals and calculated p-values, which were evaluated for significance testing using the Bonferroni correction (17, 18).

Results

Our search of PubMed yielded nine studies that were eligible for consideration (8, 19–26). All of the identified studies were used in pooling and calculation of effect sizes, where appropriate, according to the pregnancy complication (Figure 1, Table 3). Midtrimester diagnostic amniocentesis was performed in four of the included studies, which were used as a control group for comparison where applicable (8, 20, 21, 23). We performed pooling into three subgroups according to the control group

used for comparison: A) Patients with no invasive prenatal diagnostic procedure whatsoever (no invasive) (first pooling group); B) Patients with no invasive prenatal diagnostic procedure (no invasive) combined with patients who underwent midtrimester diagnostic amniocentesis (second pooling group); and C) Only patients who underwent midtrimester diagnostic amniocentesis (third pooling group).

Table 4. Ranges for the interpretation of the I2 statistic according to the Cochrane Handbook for Systematic Reviews of Interventions

I2 statistic	Interpretation
0%–40%	might not be important
30%–60%	may represent moderate heterogeneity
50%–90%	may represent substantial heterogeneity
75%–100%	considerable heterogeneity

Table 5. Scores of the studies for methodological quality and respective values of Cohen's kappa

Study/year	Reviewer #1	Reviewer #2	Kappa value
Sotiriadis et al. (26)	19	17	0.752
Daskalakis et al. (20)	15	15	0.794
Maruotti et al. (24)	4	4	0.623
Lindgren et al. (23)	9	11	0.802
Khalil et al. (22)	15	15	1
Odibo et al. (25)	17	18	0.897
Grobman et al. (21)	16	15	0.893
Adusumalli et al. (19)	18	17	0.897
Silver et al. (8)	16	16	0.775

First pooling group

The prevalence rates of preeclampsia were 2.12% (206/9,722) in the CVS patient group and 2.59% (983/37,877) in the control group. The I^2 statistic was 92%. No significant difference was found in the OR for preeclampsia between the CVS and control groups (0.83, 95% CI 0.42–1.67) (Figure 2).

The prevalence rates of severe preeclampsia were 0.54% (36/6,636) in the CVS patient group and 1.12% (54/4,842) in the control group. The I^2 statistic was 95%. No significant difference was found in the OR for severe preeclampsia between the CVS and control groups (0.49, 95% CI 0.04–5.78) (Figure 3).

The prevalence rate of gestational hypertension was 1.39% (124/8,914) in the CVS patient group and 2.61% (879/33,702) in the control group. The I^2 statistic was 74%. No significant difference was found in the OR for gestational hypertension between the CVS and control groups (0.76, 95% CI 0.46–1.26) (Figure 4).

The combination of all patients with preeclampsia or gestational hypertension led to prevalence rates of 3.83% (417/10,898) in the CVS patient group and 4.75% (3,875/81,556) in the control group. The I^2 statistic was 96%. No significant difference was found in the OR for preeclampsia and gestational hypertension

Table 6. Classification of Landis and Koch for values of Cohen's kappa

Kappa value	Interpretation
<0.00	Poor
0.00–0.20	Slight
0.21–0.40	Fair
0.41–0.60	Moderate
0.61–0.80	Substantial
0.81–1.00	Almost perfect

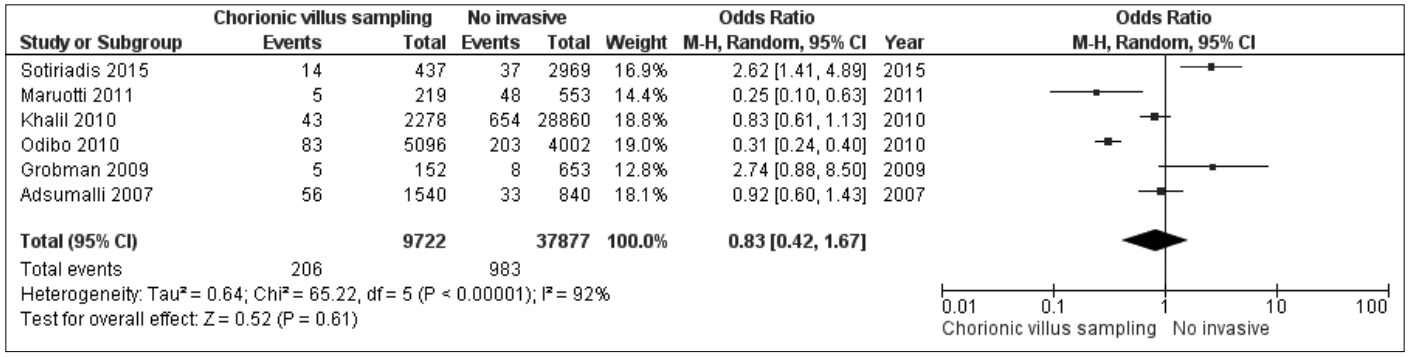

Figure 2. Forest plot of odds ratios for preeclampsia in the first pooling group

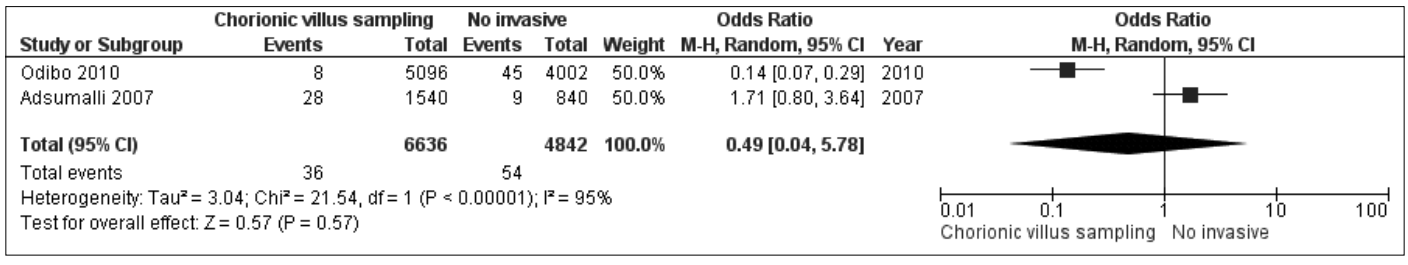

Figure 3. Forest plot of odds ratios for severe preeclampsia in the first pooling group

combined between the CVS and control groups (0.80, 95% CI 0.46–1.41) (Figure 5).

Second pooling group

The prevalence rates of preeclampsia were 2.2% (284/12,887) in the CVS patient group and 2.32% (1,048/45,200) in the pooled control group. The I^2 statistic was 96%. No significant difference was found in the OR for preeclampsia between the CVS and combined control groups (1.0, 95% CI 0.46–2.18) (Figure 6).

A diagnosis of severe preeclampsia occurred in 0.80% (68/8,514) of the CVS patient group and 1.01% (67/6,662) of the study subjects in the combined control groups. The I^2 statistic was 94%. No significant difference was found in the OR for severe preeclampsia between the CVS and combined control groups (0.83, 95% CI 0.14–4.85) (Figure 7).

Either preeclampsia or gestational hypertension was diagnosed in 4.06% (641/15,897) of the CVS patient group and 4.66% (5,009/111,884) of the combined control group. The I^2 statistic was 96%. No significant difference was found in the OR for combined acute pregnancy-induced hypertensive disorders between the CVS and combined control groups (1.07, 95% CI 0.63–1.84) (Figure 8).

Third pooling group

In two of the nine studies that were aggregated for this meta-analysis (20, 21), patients who underwent midtrimester diagnostic amniocentesis but not CVS were utilized as a control group to assess any potential relationship with preeclampsia. The prevalence rates of preeclampsia were 2.5% (83/3,317) in the CVS patient group and 0.89% (65/7,323) in the midtrimester diagnostic amniocentesis patient group. The I^2 statistic was 55%. A significant difference was observed in the OR for preeclampsia between the CVS patient group and the midtrimester diagnostic amniocentesis patient group (2.47, 95% CI 1.14–5.33) (Figure 9).

Moreover, in three of the included studies (8, 20, 23), data were available to evaluate merged acute pregnancy-induced hypertensive disorders in patients who only underwent midtrimester diagnostic amniocentesis. The prevalence rates of merged acute pregnancy-induced hypertensive disorders were 4.61% (322/6,983) in the CVS patient group and 3.74% (1,134/30,328) in the midtrimester diagnostic amniocentesis patient group. The I^2 statistic was 89%. A marginal difference was observed in the OR for merged acute pregnancy-induced hypertensive disorders between the CVS and midtrimester diagnostic amniocentesis patient groups (1.61, 95% CI 1.02–2.53) (Figure 10).

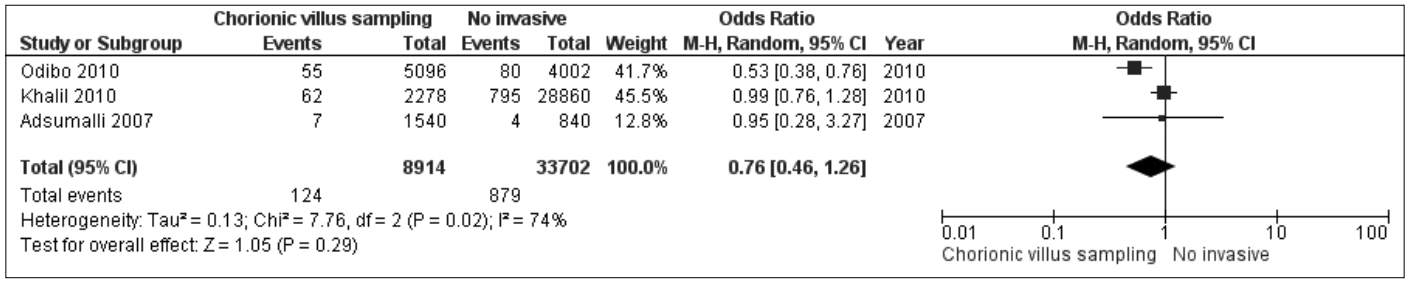

Figure 4. Forest plot of odds ratios for gestational hypertension in the first pooling group

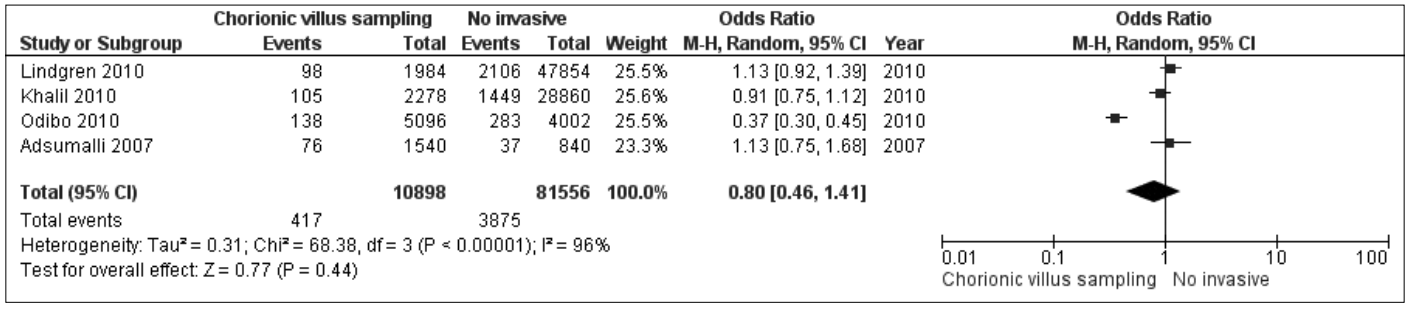

Figure 5. Forest plot of odds ratios for all pregnancy-induced hypertensive disorders in the first pooling group

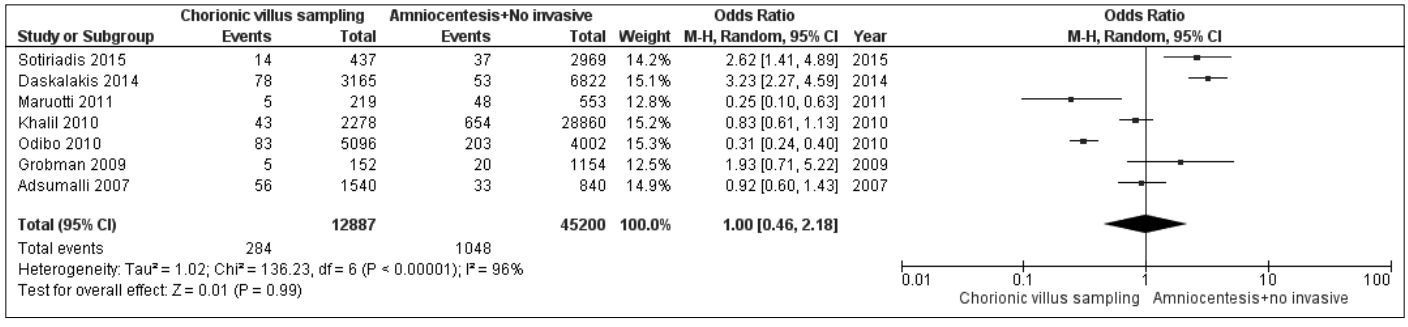

Figure 6. Forest plot of odds ratios for preeclampsia in the second pooling group

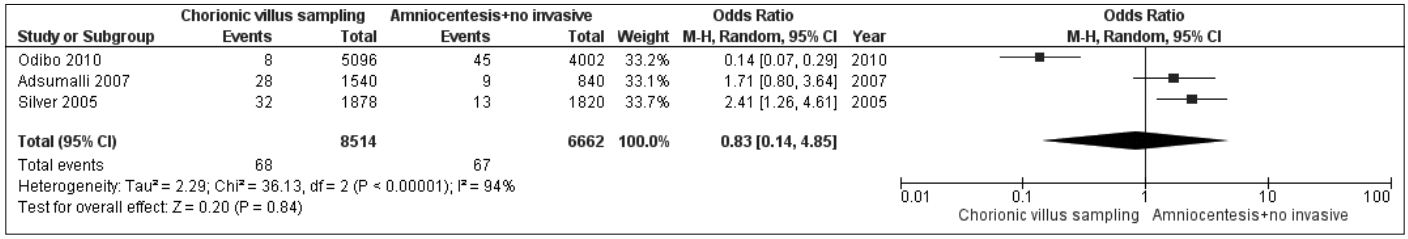

Figure 7. Forest plot of odds ratios for severe preeclampsia in the second pooling group

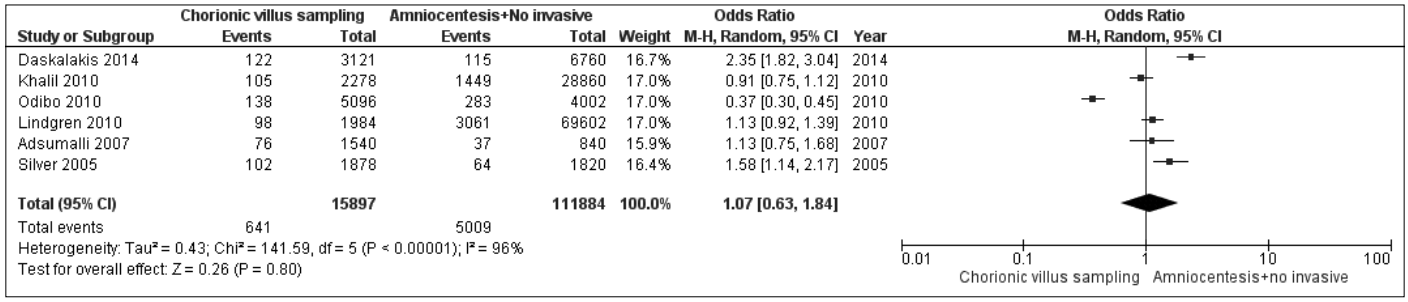

Figure 8. Forest plot of odds ratios for all pregnancy-induced hypertensive disorders in the second pooling group

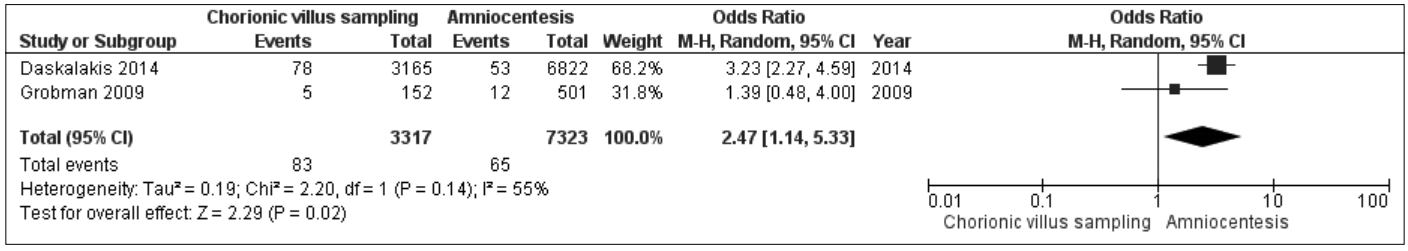

Figure 9. Forest plot of odds ratios for preeclampsia in the third pooling group

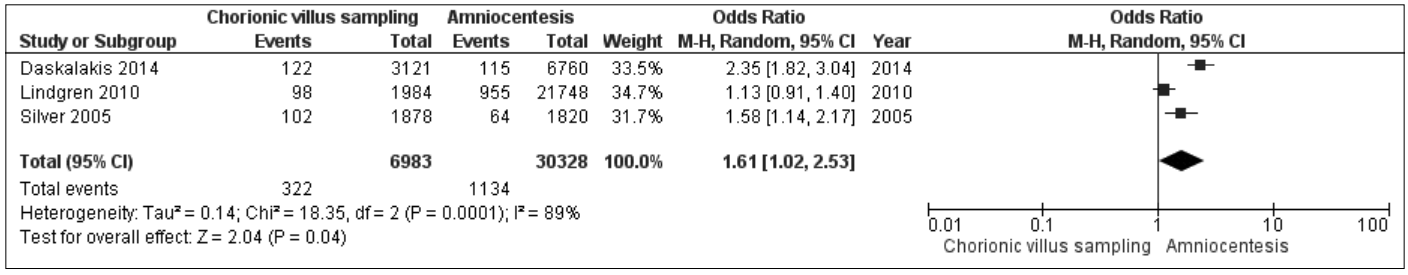

Figure 10. Forest plot of odds ratios for all pregnancy-induced hypertensive disorders in the third pooling group

Discussion

Among the studies that were included in the present meta-analysis, that by Silver et al. (8) constituted the stimulus for the subsequent research efforts. Two of the nine reports that met the criteria for this meta-analysis suggested that the risk for preeclampsia was reduced after a CVS procedure (24, 25). On the other hand, three reports suggested that the risk for preeclampsia was increased after a CVS procedure (8, 20, 26). No significant effect of CVS on the risk of subsequent preeclampsia in either way was reported in the other four reports that were identified for our analysis (19, 21–23). Considerable heterogeneity was noted during pooling of the studies. The observed values of the I^2 statistic during our analysis ranged between 55% and 96%.

For our analysis of the data from the collected nine studies (8, 19–26), we created three pools of patients, as indicated previously. In the first two pooling groups, we did not find evidence of an increased risk for preeclampsia, severe preeclampsia, or gestational hypertension following the performance of first trimester CVS. However, in the last pooling group, the risk of preeclampsia appeared to increase following CVS (Figures 9 and 10).

The prevalence rates of preeclampsia were between 1.6% and 3.6% and between 0.8% and 8.6% in the CVS and control groups, respectively, in the studies that were evaluated for this analysis. Most of the individually reported rates were considerably lower than the typically reported prevalence of preeclampsia of 5%–8% (2). Nevertheless, a recent prospective randomized study, which included only nulliparous women, reported an occurrence of

preeclampsia of approximately 7% (27). It is notable that the rates of nulliparity varied between 15.3% and 48.9% among the nine reports, with the largest study having a considerably lower rate of nulliparity in comparison with the other studies (Table 2). Therefore, the relatively low prevalence of preeclampsia among the pooled studies may in part be due to the low nulliparity rate and the retrospective structure of the included studies.

To investigate the sources of the differences between the three pooling groups, we constructed tables for the assessment of prevalence. In Tables 7 and 8, the rates of preeclampsia and merged acute pregnancy-induced hypertensive disorders according to the three pooling groups are provided. Firstly, the rates of preeclampsia were similar among the CVS arms of the three pooling groups (p=0.447) (Table 7). Secondly, Chi-square testing indicated a marginal difference for merged acute pregnancy-induced hypertensive disorders (p=0.042), which lost its significance after post hoc testing among the CVS arms of the three pooling groups (Table 8). However, the rates of preeclampsia and merged acute pregnancy-induced hypertensive disorders among the control arms of the three pooling groups were significantly different from each other (p<0.001) (Table 7, 8). Post hoc testing indicated that the rates were significantly lower in the control arm of the third pooling group (p<0.001). The significantly lower rates of preeclampsia and merged acute pregnancy-induced hypertensive disorders in the control arm of the third pooling group mostly originated from the study of Daskalakis et al. (20). In this study, it is notable that 83.1% of the study subjects underwent CVS for an indication of thalassemia, and the nulliparity rate was significantly higher in the CVS group (20). Daskalakis et al. (20) emphasized that anemia secondary to thalassemia is a protective factor against preeclampsia (28). Hence, they concluded that despite the purported protective effect of undergoing CVS in a thalassemic patient, CVS actually increased the risk for preeclampsia. In contrast, Hangrasertpong et al. (29) reported outcomes for thalassemia traits recently and debated the pros and cons of a purported protective effect of thalassemia against preeclampsia. Their data revealed a marginally higher risk of preeclampsia in patients that carried a thalassemia trait [OR 1.73 (95% CI 1.01–3.00)] (29). Another important weakness of the conclusions of Daskalakis et al. (20) was the performance of multiple regression analysis that excluded information regarding nulliparity and anemia. To strengthen their conclusions, nulliparity and the presence of anemia should have been included in the regression analysis. Similarly, Sotiriadis et al. (26) reported increased rates of preeclampsia after CVS [3.2% vs. 1.6%; OR 2.62 (95% CI 1.41–4.89)], but after controlling for confounding factors in their logistic regression analysis, the performance of CVS lost its effect. Thus, the results shown for the third pooling group are weakened and are likely due to a sampling error. Interpreted in this context, the data in Tables 7 and 8 suggest that midtrimester diagnostic amniocentesis has either no effect or perhaps an ironic protective effect in reducing the occurrence of preeclampsia. Figures 6 and 8 incorporate most of the available data and provide the best summary result, which indicates that CVS has no effect on the occurrence of preeclampsia.

Table 7. Prevalence of preeclampsia according to the three pooling groups

Pooling group	Chorionic villus sampling group	Control group
A	2.12%	2.59%
B	2.2%	2.32%
C	2.5%	0.89%

A: patients with no invasive procedures served as the control group (first pooling group); B: patients with no invasive procedures combined with patients who underwent midtrimester diagnostic amniocentesis served as the control group (second pooling group); C: patients who underwent midtrimester diagnostic amniocentesis but not chorionic villus sampling (CVS) served as the control group (third pooling group)

Table 8. Prevalence of merged acute pregnancy-induced hypertensive disorders according to three pooling groups

Pooling group	Chorionic villus sampling group	Control group
A	3.83%	4.75%
B	4.06%	4.66%
C	4.61%	3.74%

A: patients with no invasive procedures served as the control group (first pooling group); B: patients with no invasive procedures combined with patients who underwent midtrimester diagnostic amniocentesis served as the control group (second pooling group); C: patients who underwent midtrimester diagnostic amniocentesis but not chorionic villus sampling (CVS) served as the control group (third pooling group)

The grading scores of the investigations chosen for inclusion in this meta-analysis according to the checklist are shown in Table 5. The two investigations reported by Lindgren et al. (23) and Maruotti et al. (24) are conspicuous by the fact that their scores were low compared with the others. This finding was not expected, particularly for the Lindgren report given the large number of included subjects. The two reviewers indicated average scores for the other included studies. The scores that are indicated in Table 5 reflect the moderate methodology and reporting quality of the studies in this analysis.

It is important to note the limitations of the current meta-analysis. To begin with, none of the studies in this analysis were structured as a randomized prospective clinical trial designed to study the effect of CVS on the subsequent occurrence of preeclampsia. On the other hand, portions of two trials had some prospective components (8, 22). The report of Silver et al. (8) was a secondary analysis of a randomized trial, which was underpowered owing to difficulties in obtaining the necessary sample size (30). In addition, the report of Khalil et al. (22) indicated that their trial was part of an ongoing prospective study, but actually the outcomes were acquired retrospectively. Therefore, the trials of Khalil et al. and Silver et al. were analyzed with other trials. Amongst the studies included in this meta-analysis, only Adusumalli et al. (19) performed a power analysis. They calculated that 1471 patients were necessary in each arm to achieve sufficient statistical power to avoid the trial being underpowered. In the current meta-analysis, we observed considerable heterogeneity. As a result of the significant heterogeneity observed among the results, the power of this meta-analysis is limited (31).

In summary, the aggregated available information suggests that CVS does not have a significant adverse impact on future pregnancy outcomes with regard to the occurrence of acute hypertensive disease in the form of either gestational hypertension or preeclampsia. We acknowledge that our review is limited by considerable heterogeneity and the retrospective design of the available studies, which are prone to sampling error. Prospective randomized clinical trials are clearly required to determine with confidence if first trimester CVS performed via either the transvaginal or transabdominal route has a significant adverse impact upon the later development of pregnancy-specific hypertension.

Ethics Committee Approval: *N/A.*

Informed Consent: *N/A.*

Peer-review: *Externally peer-reviewed.*

Author Contributions*: Concept - A.B.; Design - A.B., M.B., B.T., J.N.M.Jr.; Supervision - A.B., M.B., B.T., J.N.M.Jr.; Materials - A.B., M.B., B.T., J.N.M.Jr.; Data Collection and/or Processing - A.B., M.B., B.T., J.N.M.Jr.; Analysis and/or Interpretation - A.B., M.B., B.T., J.N.M.Jr.; Literature Review - A.B., M.B., B.T., J.N.M.Jr.; Writer - A.B., M.B., B.T., J.N.M.Jr.; Critical Review - A.B., M.B., B.T., J.N.M.Jr.; Other - A.B., M.B., B.T., J.N.M.Jr.*

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Primary peritoneal cancer in *BRCA* carriers after prophylactic bilateral salpingo-oophorectomy

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Abstract

Objective: The presence of deleterious mutations in breast cancer *(BRCA)*-1 or *BRCA*-2 gene has a decisive influence on the development of various types of neoplasms, such as breast, ovarian, tubal, and peritoneal cancers. Primary peritoneal cancer is an aggressive malignancy which, due to the absence of a specific screening test, cannot be diagnosed in its early stages. As a risk-reducing option, prophylactic bilateral salpingo-oophorectomy and mastectomy are often proposed in *BRCA* gene carriers. The effectiveness of a preventive surgical treatment is, however, unclear in the development of peritoneal cancer.

Material and Methods: An extensive electronic search was performed in PubMed, Scopus, and Cochrane databases.

Results: The total number of patients who underwent prophylactic bilateral salpingo-oophorectomy was 1,830, of whom 28 presented with peritoneal cancer (1.53%). The age of the included patients ranged from 48 to 61 years. *BRCA*-1 was present in 9 out of 28 patients and *BRCA*-2 in 2 patients, while the type of *BRCA* was unclear in 17 patients. Salpingo-oophorectomy was performed in 23 out of 28 patients, while oophorectomy was carried out in 5 patients. The interval from initial risk-reducing surgical treatment to the presentation of peritoneal cancer ranged from 12 to 84 months.

Conclusion: Modification of the follow-up guidelines and increase in healthcare providers' awareness may reduce the risk of peritoneal cancer. (J Turk Ger Gynecol Assoc 2016; 17: 73-6)

Keywords: *BRCA* gene, peritoneal carcinoma, management, prophylaxis, etiology

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Introduction

Mutations in breast cancer *(BRCA)* genes are correlated with an increase in the risk of breast, ovarian, tubal, and peritoneal cancers. Premalignant dysplasias within the fallopian tubes have also been linked to *BRCA* gene mutations. The prevalence of *BRCA* mutations in the general population ranges between 0.07 and 0.24% for *BRCA-1* and 0.14 and 0.22% for *BRCA-2* (1). Genetic counseling and risk-reducing surgery, including prophylactic bilateral salpingo-oophorectomy and mastectomy, are often recommended in *BRCA* carriers. The median recommended age for prophylactic salpingo-oophorectomy in *BRCA* carriers is before the age of 40 years (in USA, before 35 years, while in Europe, before 40 years) or certainly after completion of family (2, 3).

Primary peritoneal cancer is an aggressive malignancy that cannot as yet be diagnosed early with screening tests. At present, the median age of peritoneal cancer diagnosis is 63 years, ranging from 44 to 74 years (4). The median survival rates vary from 11.3 to 17.8 months (5). Prophylactic salpingo-oophorectomy is used for cancer risk reduction in *BRCA* gene carriers and leads to decrease of ovarian/tubal/peritoneal cancer risk to 3.5-4.3% and breast cancer risk to 30-40% (1, 6). In a more optimistic study, prophylactic salpingo-oophorectomy was shown to be more effective in preventing ovarian, tubal, and breast cancers, with low surgical morbidity and complications including hematomas, cardiac arrhythmias, and injury of adjacent organs such as the bladder, ureters, and intestines (4). More specifically, in the above study, 3.4% of patients developed breast cancer and 0.8% developed peritoneal cancer. However, the follow-up period of that study was small (22 months) (4).

The aim of this report is to present a review of cases with peritoneal cancer after prophylactic bilateral salpingo-oophorectomy and to discuss the possible etiology of this rare entity as well as the possible changes in the management of such patients.

Material and Methods

We retrieved the included studies in our narrative review after performing an extensive electronic search in the PubMed (09/26/2015), Scopus (09/26/2015), and Cochrane (09/26/2015) databases. The search strategy adopted included the combination of the key words "*BRCA*" AND "peritoneal" AND ("carcinoma" OR "cancer" OR "neoplasm"). The reference list was also hand-searched for additional studies. Studies

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Table 1. Published cases of primary peritoneal cancer in *BRCA* carriers after prophylactic surgical treatment

First author, (ref) country	Publication type	No. of patients (%)	Age of patients (in years)	Type of *BRCA* genes	Type of prophylactic surgical treatment	Interval from initial prophylactic surgical treatment to detection of peritoneal cancer (in months)	Histological type
Bacha et al. (4) Brazil	Retrospective study	1/119 (0.8)	NR	*BRCA-1/2*	Salpingo-oophorectomy	NR	NR
Reitsma et al. (2) Netherlands	Prospective study	1/303 (0.3)	NR	*BRCA-1*	Salpingo-oophorectomy	50.4	NR
Rhiem et al. (8) Germany	Retrospective study	1/175 (0.6)	59	*BRCA-1*	Salpingo-oophorectomy	26	NR
Powell et al. (7) USA	Prospective study	2/111 (1.8)	NR	*BRCA-1/2*	Salpingo-oophorectomy	12 and 84	Ovarian origin
Rabban et al. (9) USA	Retrospective study	2/89 (2.2)	NR	*BRCA-1/2*	Salpingo-oophorectomy	22 and 62	Serous carcinoma
Carison et al. (11) USA	Prospective study	5/19 (26.3)	NR	*BRCA-1/2*	Salpingo-oophorectomy	NR	NR
Kauff et al. (12) USA	Prospective study	3/325 (0.9)	NR	*BRCA-1*	Salpingo-oophorectomy	Median: 16	NR
Maehle et al. (10) Norway	Prospective study	3/49 (6.1)	NR	*BRCA-1/2*	Salpingo-oophorectomy	NR	Serous carcinoma
Hill et al. (13) Canada	Case report	1	56	*BRCA-1*	Oophorectomy, hysterectomy	60	Serous papillary adenocarcinoma
Meeuwissen et al. (18) Netherlands	Retrospective study	1/152 (0.6)	61	*BRCA-1*	Salpingo-oophorectomy	14	Papillary serous carcinoma
Powell et al. (14) USA	Retrospective study	2/67 (2.9)	NR	*BRCA-2*	Salpingo-oophorectomy	60	NR
Kauff et al. (15) USA	Prospective study	1/98 (1)	NR	*BRCA-1/2*	Salpingo-oophorectomy	16.3	NR
Scheuer et al. (16) USA	Prospective study	1/233 (0.4)	48	*BRCA-1*	Salpingo-oophorectomy	NR	NR
Colgan et al. (17) Canada	Prospective study	1/60 (1.6)	NR	*BRCA-1*	Oophorectomy	NR	NR
Tobacman et al. (19) USA	Prospective study	3/29 (10.3)	NR	*BRCA-1/2*	Oophorectomy	NR	NR

Ref: reference; USA: United States of America; No.: number; NR: not referred; *BRCA*: breast cancer

written only in the English language were included. The reporting data on primary peritoneal cancer in *BRCA* carriers after prophylactic bilateral salpingo-oophorectomy of the retrieved studies were regarded as includable for this review. Abstracts, commentaries, review articles, conference papers, animal studies, and editorials were not included in this review.

Results

A total of 93 and 100 studies located in the PubMed and Scopus searches were retrieved, respectively, of which 13 studies met the inclusion criteria of our review (2, 4, 7-17). No further studies were retrieved from the Cochrane database. Two additional studies were found through hand-searching of references (18, 19). The search strategy followed is depicted in Figure 1 (flow diagram).

The major characteristics of the studies included in our review (demographics, publication type, histological type, type of *BRCA* gene, type of prophylactic surgical treatment, interval from initial prophylactic surgical treatment, and interval from initial prophylactic surgical treatment to detection of peritoneal cancer [in months]) are presented in Table 1.

Nine prospective studies, five retrospective studies, and one case report were included in the study. The total number of patients who underwent prophylactic bilateral salpingo-oophorectomy was 1830, of whom 28 had peritoneal cancer (1.53%). The age of the included patients ranged from 48 to 61 years. *BRCA-1* was present in 9 out of 28 patients and *BRCA-2* in 2 patients, while the type of *BRCA* was unclear in 17 patients. Salpingo-oophorectomy was performed in 23 out of 28 patients, while oophorectomy was carried out in 5 patients. The interval from initial risk-reducing surgical treatment to the presentation of peritoneal cancer ranged from 12 to 84 months. Regarding the histological type, this was mentioned in only 5 studies, but serous type was mentioned for all patients.

Discussion

For many years, it was believed that the ovarian and peritoneal epithelia share a common embryonal (mesonephric) origin. However, ovarian and primary peritoneal cancers are histologically similar to the Mullerian epithelium. For this reason, another peritoneal cancer etiology theory suggested that primary ovarian epithelial, tubal, and primary peritoneal cancers

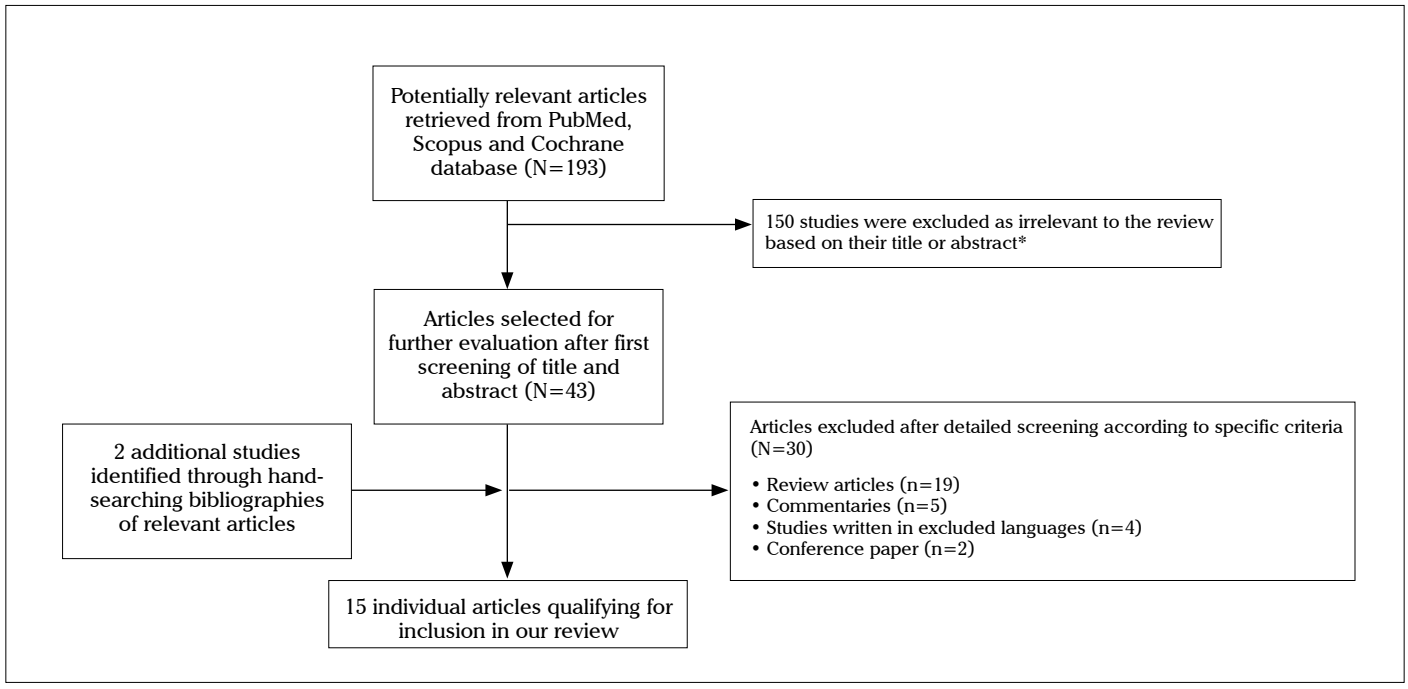

Figure 1. The greater part of studies was retrieved in both databases

are all Mullerian in origin, and they are now considered to be a single disease entity. The majority of these cancers originate from the fimbriated end of the fallopian tube, but some of them derive from components of the secondary Mullerian system (20). It is thus believed that the etiology of such a rare cancer could be found in the fallopian tube remnants after prophylactic salpingo-oophorectomy. Compared to ovarian cancer, primary peritoneal cancer is characterized by loss of heterozygosity at chromosomal loci as well as overexpression of the human epidermal growth factor receptor 2 (HER2) oncogene on immunohistochemical analysis (21).

Primary peritoneal cancer is a rare malignancy mimicking ovarian cancer. In *BRCA* carriers, the lifetime risk of presenting with primary peritoneal cancer has been estimated to be 1.3%, and this risk should be explained when counseling patients for prophylactic salpingo-oophorectomy (22). This was also found in the present review, which demonstrated that the risk is around 1.53%. It has been shown that *BRCA* carriers are diagnosed at a younger mean age than patients without a mutation (60 versus 70 years), but the overall median survival is in favor of the *BRCA* carriers (148 versus 41 months) (22). Primary peritoneal cancer can be found many years after prophylactic salpingo-oophorectomy, and it usually presents at advanced stages (6). In the present review, the interval from initial surgery to presentation of peritoneal cancer ranged from 12 to 84 months. For this reason, long-term follow-up is required in such patients; and although it is very unlikely to be diagnosed early, our hospitals guidelines recommend that gynecologic examination, transvaginal ultrasound, and serum cancer antigen 125 (CA-125) be carried out on an annual basis. However, as was recently pointed out, there is a lack of follow-up protocols in the current literature (23). More specifically, although dual-energy X-ray absorptiometry testing, annual serum CA-125, and pelvic examination have been suggested, a recent cohort questionnaire-based study reported that some women do not have access to follow-up or have no support for their menopausal symptoms by health providers who either did not recommend follow-up testing or advised against hormone replacement therapy (HRT) use in younger patients with no other contraindication to the use of HRT (23, 24). Regarding possible future changes in the management of such patients, a recent review proposed that the source of primary peritoneal cancer in *BRCA* carriers after prophylactic salpingo-oophorectomy could be the appendix (25). It is therefore suggested by a research group that prophylactic appendectomy should also be considered in *BRCA* carriers to eliminate the risk of primary peritoneal cancer (25).

The possible mechanisms that could explain the origin of peritoneal cancer after prophylactic salpingo-oophorectomy include the presence of the precancerous lesion serous tubal intraepithelial carcinomas (STIC) during salpingectomy and the possibility that STIC would have metastasized to the peritoneum before the prophylactic procedure (26). Moreover, the second hypothesis could suggest that the salpingo-oophorectomy was incomplete, meaning that there might be an ovarian or tubal remnant postoperatively. Furthermore, the third hypothesis could include the primary origin of the peritoneal cancer. Although, the appendix hypothesis is also presented in the current literature by one research group, we do not believe that this hypothesis could explain the pathogenetic mechanism (25).

This current report has several limitations. First, the number of studies and of patients included is small, which underlines the rarity of cancer represented after risk-reducing surgical treatment in *BRCA* gene carriers. Concerning our selected search strategy, it could be considered limited due to the exclusion of abstracts, commentaries, review articles, conference papers, animal studies, as well as editorials. Restriction to only English language literature could also have limited the range of our search.

In conclusion, the role of prophylactic salpingo-oophorectomy in *BRCA* carriers is crucial for the prevention of breast/ovarian cancer. Peritoneal cytology at the time of prophylactic adnexectomy, is also advised in order to assist in the diagnosis of peritoneal carcinomatosis. However, there is still a risk of peritoneal cancer for such patients that they should be aware of. Changes and better organization in the follow-up guidelines and increase in healthcare providers' awareness should be considered to reduce the risk of peritoneal cancer.

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BRCA1 and *BRCA2* sequence variations detected with next-generation sequencing in patients with premature ovarian insufficiency

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Abstract

Objective: Although the association between *BRCA1* and *BRCA2* gene mutations and breast and ovarian cancer is known, there is insufficient data about premature ovarian insufficiency (POI). However, several studies have reported that there might be a relationship between POI and *BRCA1* and *BRCA2* gene mutation. Therefore, in the present study, we aimed to investigate the role of *BRCA1* and *BRCA2* gene mutations in the etiology of POI in a Turkish population.

Material and Methods: The cohort was classified into two groups: a study group, consisting of 56 individuals diagnosed with premature ovarian insufficiency (and who were younger than 40 years of age, had an antral follicle count <3-5, and FSH levels >12 IU/I), and a control group, consisting of 45 fertile individuals. A total of 101 individuals were analyzed by next-generation sequencing to detect *BRCA1* and *BRCA2* gene mutations.

Results: We detected four new variations (p.T1246N and p.R1835Q in BRCA1 and p.I3312V and IVS-7T>A in BRCA2) that had not been reported before.

Conclusion: We did not find an association between the BRCA1 and BRCA2 gene mutations and premature ovarian insufficiency. However, larger, functional studies are needed to clarify the association. (J Turk Ger Gynecol Assoc 2016; 17: 77-82)

Keywords: Premature ovarian insufficiency, *BRCA1*, *BRCA2*, next generation sequencing, *in vitro* fertilization

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Introduction

Premature ovarian insufficiency (POI), characterized by a loss of function of the ovaries before the age of 40 years (1), is a health problem in women that affects 1% of the population (2). The spontaneous pregnancy rate is low in POI, and although approximately 5–10% of women with POI are still able to conceive, most women with POI have a permanent loss of fertility. The association between POI and rare genetic disorders, some autoimmune and viral diseases, chemotherapy, and radiotherapy are well known. However, the underlying mechanism is still unknown. It has been speculated that there might be a relationship between POI and *BRCA1* and *BRCA2* gene mutations (3–5). There are a limited number of studies in the literature on single cases or related to small patient groups investigating *BRCA1* and *BRCA2* mutations in patients with premature ovarian insufficiency.

BRCA1 and *BRCA2* genes are responsible for DNA repair. Mutations in these genes result in unrepaired DNA damage, leading the cell to apoptosis (6). Loss of function in *BRCA* genes in oocytes also leads them to the apoptotic pathway and may cause early depletion of ovarian reserves (4). Although the association between *BRCA1* and *BRCA2* gene mutations and breast, ovarian, and prostate cancer is clearer, there is insufficient data concerning POI. Recently, it has been speculated that there might be a relationship between POI and *BRCA1* and *BRCA2* gene mutations (5). Although *BRCA1* and *BRCA2* genes have been analyzed in several studies in Turkish population, these studies only analyzed the relationships of *BRCA1* and *BRCA2* gene variations and breast cancer (7-9).

Next-generation sequencing is a valuable tool that analyzes up to gigabases of DNA reads at a high speed and with a low cost per base. This method has also been used in worldwide collaborative projects, such as the International Genome Consortium (ICGC) (10) and The Cancer Genome Atlas (TTGA) (http://cancergenome.nih.gov). Because of the large size of *BRCA1* and *BRCA2* genes (5592 bp and 10257 bp, respectively) and lack of mutation hot spots, these genes need useful prescreening strategies, such as next-generation

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sequencing; therefore, we used the MiSeq Illumina sequencer (MiSeq, Illumina Inc.; San Diego, CA, USA) to detect the variants of *BRCA1* and *BRCA2* genes.

There are only a limited number of studies about the importance of *BRCA1* and *BRCA2* gene mutations in the etiology of POI in the literature. Therefore, the association of POI and *BRCA1* and *BRCA2* gene mutations is unclear. In the case of detecting variations related to breast-ovarian cancer, these patients might be referred for screening and follow-up programs for breast/ovarian cancer before the age of 40 years. Hence, we aimed to investigate the role of *BRCA1* and *BRCA2* gene mutations in the etiology of POI. According to our knowledge, this study is the first study in Turkish patients to assess the genetic predisposition to premature ovarian failure. Also, it is the first study to analyze the whole *BRCA1* and *BRCA2* genes by next-generation sequencing in Turkish patients.

Material and Methods

To determine the mutations and variants in the target exon sequences of *BRCA1* and *BRCA2*, we sequenced and analyzed these genes using next-generation sequencing technology in Turkish patients with premature ovarian failure and in control subjects.

Patients

We enrolled 101 individuals referred to the Zekai Tahir Burak Women's Health Training and Research Hospital who fulfilled the exclusion and inclusion criteria and who accepted to participate in our study. The study group consisted of 56 individuals who had been referred to the IVF unit due to infertility problems and who were younger than 40 years of age with an antral follicle count <3-5 and FSH levels >12 IU/I. The control group consisted of 45 individuals who had been referred to the Family Planning Unit due to contraception and who had spontaneous pregnancies before. None of the individuals had a history of internal systemic disease, pelvic-ovarian surgery, or familial breast/ovarian cancer. Informed consent was obtained from all the patients and controls. This study was approved by Baskent University Institutional Review Board and Ethics Committee (Project No: KA13/297) and was supported by Baskent University Research Fund and Turkish German Gynecology Education and Research Foundation.

Next-generation sequencing analyses

Genomic DNA was obtained from 200 μL peripheral blood samples from each individual using the QIAamp DNA Blood Mini Kit (Qiagen Inc.; Hilden, Germany) according to the manufacturer's instructions.

Primer design was performed for the coding regions of *BRCA1* and *BRCA2* genes. These primers were used to construct a library containing the essential nucleotide sequences. Thirty-eight primers for *BRCA1* and 40 for *BRCA2* were used to amplify 19 and 20 amplicons, respectively. The sizes of the amplicons varied between 299 and 5504 bps. PCRs were performed on isolated DNA samples, using the designed primers, and the reactions were checked by 2% agarose gel electrophoresis. PCRs belonging to each individual were mixed to obtain PCR pools, which had all the amplicons of each individual in one tube. While mixing, the amplification efficiency and the length of the amplicons were taken into consideration; the volume for each PCR was directly proportional to the length of the amplicon and inversely proportional to the efficiency of the reaction, which was estimated by gel electrophoresis. The PCR pools for each individual were purified using the NucleoFast® 96 PCR kit (MACHEREY-NAGEL GmbH; Düren, Germany). The purified pools were quantified using a ND1000 (Thermo Fisher Scientific Inc.; Wilmington, DE, USA) micro volume spectrophotometer and standardized to 0.2 ng/μL, which was needed for the sample preparation step. The samples were prepared for next-gene sequencing using the NexteraXT sample preparation kit (Illumina Inc.; San Diego, CA, USA). Sequencing was performed using the Next Generation Sequencing MiSeq Illumina sequencer (Illumina Inc.; San Diego, CA, USA). Obtained sequences were aligned to the reference genome (GRCh37/hg19) using MiSeq Reporter software (Illumina Inc.; San Diego, CA, USA).

Analysis of the variants

The data were analyzed on IGV 2.3 software (Broad Institute; Cambridge, MA, USA). The clinical outcomes of the variations found on the samples were estimated using the following databases: Ensembl (http://www.ensembl.org/index.html) and dbSNP (http://www.ncbi.nlm.nih.gov/SNP/) for minor allele frequencies; SIFT (http://sift.jcvi.org/), Mutation Taster (http://www.mutationtaster.org/), and Polyphen II (http://genetics.bwh.harvard.edu/pph2/) for the effects of amino acid changes on the protein; HSF (http://www.umd.be/HSF3/) for the mutations that affect the splicing pattern.

The established variants were cross-checked with Align GVGD (http://agvgd.iarc.fr/) and the breast cancer databases UMD-*BRCA1*/*BRCA2* (http://www.umd.be/*BRCA1*/, and http://www.umd.be/*BRCA2*/).

Statistical analysis

To examine the association between *BRCA* variations and POI, Fisher's-Exact Test and Student's t-test were used. The outcome was considered statistically significant when the p value was below 0.05.

Results

Of the 101 women included in our study, 56 were in the study group and 45 in the control group. The mean ages were 33.4 years (±4.5) and 29.4 years (±6.1), respectively. The difference in ages between the control and study groups was statistically significant. The mean E2 level in the study group was 52.3 pg/mL (±82.1), and the FSH level was 23.1 IU/mL (±10.1).

Next-generation analysis results

We identified a total of 11 *BRCA1* and 13 *BRCA2* sequence variants in the study group. Two of the variants detected in the study group have not been reported in the BIC and UMD-*BRCA1*/*BRCA2* databases previously. Of these novel variants, c.3737C>A was in *BRCA1* and c.9934A>G were in *BRCA2*

Table 1. Variants in the *BRCA1* and *BRCA2* genes in the study and control groups

Exon	cDNA	Protein	SIFT (0-1)	Variation class (UMD)	Polyphen–HumDiv	Polyphen–HumVar	GVGD	Domain	Control group % (n:45)/ Study group % (n:56)
BRCA1									
7	c.536A>G	p.Y179C	0	1	-	0.85	Class45	-	2 (1)/0 (0)
10	c.1067A>G	p.Q356R	-	1	-	-	Class0	*BRCTassoc*	16 (7)/14 (8)
10	c.2077G>A	p.D693N	-	1	-	-	Class0	*EIN3*	20 (9)/9 (5)
10	c.2612C>T	p.P871L	-	1	-	-	Class0	*EIN3*	67 (30)/55 (31)
10	c.3113A>G	p.E1038G	-	1	-	-	Class0	-	60 (27)/54 (30)
10	c.3119G>A	p.S1040N	-	1	0.01	-	Class0	-	2 (1)/2 (1)
10	c.3541G>A	p.V1181I	0.26	1	-	0.01	Class0	-	0 (0)/2 (1)
10	c.3548A>G	p.K1183R	-	1	-	-	Class0	-	62 (27)/54 (30)
10	c.3737C>A	p.T1246N	0	Not reported	-	-	Class0	-	0 (0)/2 (1)
10	c.1456T>C	p.F486L	0.22	-	-	-		*BRCTassoc*	2 (1)/0 (0)
10	c.1648A>C	p.N550H	0.01	-	0.99	0.88		-	2 (1)/0 (0)
12	c.4342A>G	p.S1448G	0.01	3	0.33	-	Class0	-	0 (0)/2 (1)
14	c.4535G>T	p.S1512I	0.01	1	-	0.13	Class0	-	2 (1)/0 (0)
15	c.4837A<G	p.S1613G	-	1	-	-	Class0	-	64 (29)/54 (30)
15	c.4883T>C	p.M1628T	-	1	-	-	Class0	-	2 (1)/0 (0)
15	c.4956G>A	p.M1652I	-	1	-	-	Class0	*BRCT*	4 (2)/2 (1)
23	c.5504G>A	p.R1835Q	0.02	Not reported	*BRCT* domain	-	Class0	*BRCT*	2 (1)/0 (0)
BRCA2									
3	IVS2-7T>A	-	-	Not reported	-	-	-	-	2 (1)/0 (0)
10	c.865A>C	p.N289H	-	1	-	-	Class0	-	9 (4)/13 (7)
10	c.1114A>C	p.N372H	-	1	-	-	-	-	47 (19)/45 (26)
10	c.1368G>C	p.E456D	0.04	-	0.85	0.32	Class0	-	2 (1)/0 (0)
11	c.2971A>G	p.N991D	-	1	-	-	Class0	-	9 (4)/11 (6)
11	c.5744C>T	p.T1915M	-	1	-	-	Class0	-	4 (2)/4 (2)
11	c.4258G>T	p.D1420Y	0	1	0.03	0.01	-	-	4 (2)/2 (1)
11	c.6853A>G	p.I2285V	0.12	2	0.61	0.14	Class25	-	0 (0)/2 (1)
11	c.6100C>T	p.R2034C	-	1	-	-	Class0	-	0 (0)/2 (1)
11	c.3318C>G	p.S1106R	0	-	1	1	-	-	2 (1)/0 (0)
11	c.2919G>A	p.S973S	-	-	-	-	-	-	2 (1)/0 (0)
18	c.8187G>T	p.K2729N	0.07	3	1	0.93	Class35	*BRCA2DBD_OB1*	2 (1)/2 (1)
22	c.8851G>A	p.A2951T	-	1	-	-	Class55	*BRCA2DBD_OB2*	2 (1)/4 (2)
26	c.9581C>A	p.C3194Q	0.12	3	1	0.95	-	-	0 (0)/2 (1)
27	c.9934A>G	p.I3312V	0.88	Not reported	0	0	Class0	-	0 (0)/2 (1)
27	c.10234A>G	p.I3412V	-	1	-	-	Class0	-	0 (0)/2 (1)
27	c.9976A>T	p.K3326X	-	1	-	-	-	-	4 (2)/2 (1)

Classification UMD database: 1 - Neutral, 2 - likely neutral or contradictory neutral/UV, 3 - UV, 4 - likely causal or contradictory deleterious/UV, 5 - Causal. Neutral variant: non-causal variant in terms of disease risk, present in less than 1% of the general population, designated as "less likely" for Align-GVGD, "benign" for PolyPhen, and "not clinically important" for BIC. Polymorphism: neutral variant present in more than 1% of the general population, Predicted neutral: considerable evidence for neutrality but no final GGC decision. UV: unclassified variant, designated as "unknown" for BIC. Predicted causal: considerable evidence for pathogenicity but no final GGC decision, Causal mutation: causal or pathogenic mutation in terms of disease risk, designated as "most likely" for Align-GVGD, "damaging" for PolyPhen, "pathogenic" for UMD-Predictor, and "clinically important" for BIC PolyPhen results for each variant were classified as benign (score ≤0.5), possibly damaging (0.5< score <2), probably damaging (score >2), and unknown.
C/P: P: Patient group; C: Control group, patient numbers with * indicates homozygous variant, patient number without * indicates heterozygote variant.
SIFT score: Ranges from 0 to 1. The amino acid substitution is predicted damaging is the score is <=0.05, and tolerated if the score is >0.05.
GVGD: Align GVGD scores amino acid substitutions on a 7-scale scoring system, from C0 to C65. C0: Neutral, C15-25 intermediate, as changes to protein structure or function are uncertain, and C35 scores or higher are considered as likely deleterious.
UMD: Universal Mutation Database; SIFT: Sorting Tolerant From Intolerant; GVGD: Grantham Variation Grantham Deviation; BIC: Breast Cancer Information Core; *BRCT*: *BRCA* C-terminus

genes. In contrast, in the control group, 14 *BRCA1* and 12 *BRCA2* sequence variants were detected. Two of them were novel: c.5504G>A in *BRCA1* and IVS2-7T>A in *BRCA2* genes. All the detected variants are shown in Table 1.

Each *BRCA1* variant was seen in different numbers of individuals (Table 1). For example, c.4342A>G was seen only in one individual in the study group. However, c.3113A>G was seen in 27 individuals in the control group and in 30 individuals in the study group. *BRCA2* variants were also seen in different numbers of individuals in each group. As an example, c.1368C>G was seen in only one individual in the control group; however, c.1114A>C was detected in 19 individuals in the control group and in 20 individuals in the study group.

Discussion

According to the best of our knowledge, this study was the first to perform *BRCA1* and *BRCA2* gene sequencing using next-generation sequencing methods in Turkish patients with premature ovarian insufficiency. Different variants were detected in *BRCA1* and *BRCA2* genes.

There are only a few relevant studies in the literature investigating the relationship between *BRCA1* and *BRCA2* mutations and premature ovarian failure and/or ovarian reserve or ovarian stimulation (3–5,11). For the first time, Oktay et al. (4) showed the relationship between ovarian stimulation and BRCA1 mutations and concluded that there might be a possible link between gene repair and infertility and breast/ovarian cancer risks. Then, Titus et al. (11) showed the association between *BRCA1*-related DNA double-strand break repair and ovarian aging in mice and humans. Finch et al. (3) found that women carrying a *BRCA* mutation experience menopause earlier, on average, than women who have no mutations, although the difference is small and does not affect fertility. Santoro (12) commented on Finch et al.'s (3) study that BRCA mutations appear to have normal fertility in a study group. A recent study concluded that *BRCA1* germ-line mutations may be associated with reserved ovarian reserve (5). Another study investigated the effects of *BRCA1* and *BRCA2* mutations on female infertility (13). Finally, a recent study (14) reported that patients with *BRCA* gene mutations showed a normal ovarian response in IVF compared to patients with no *BRCA* mutations. A survey reported that knowledge of *BRCA* mutations affects the marriage and childbearing decisions of the patients (15). However, most of the studies used different study groups from our study, with other studies mostly including patients who had IVF treatment with *BRCA1* and *BRCA2* mutations (14), whereas our study group consisted of women diagnosed with POI.

BRCA1 encodes an 1863 amino acid protein. It has three major domains: first, the N-terminal RING finger (amino acids 18–136); second, consisting of three nuclear localization signals in the central region; and third, the tandem of two *BRCA1* C-terminus (*BRCT*) domains (i.e., *BRCT1*: amino acids 1642–1736; *BRCT2*, amino acids 1756–1835) at the C-terminus (16). Many inherited cancer-associated *BRCA1* mutations have been found within the RING and *BRCT* domains, indicating that both domains are involved in suppressing breast and ovarian cancer (17).

We detected two variations in *BRCA1* and two in *BRCA2* that have not been reported before. The first one in *BRCA1* was p.T1246N and was detected only in one patient with POI but was not detected in the control group. Although it does not correspond to any domain, the SIFT score was 0, which means the amino acid substitution can be predicted to be damaging. The GVGD score shows that amino acid substitution is not deleterious. However, our results are not sufficient to conclude whether the variation involves a polymorphism.

The other variation detected in *BRCA1* was p.R1835Q, which corresponds to the *BRCT* domain and was seen in only one individual in the control group but none in the study group. According to the SIFT score of 0.02, the amino acid substitution can be predicted to be damaging. The *BRCT* (*BRCA1* carboxyl terminal domain) domain is an evolutionary conserved module that exists in a large number of proteins, from prokaryotes to eukaryotes. Most of the proteins that contain the *BRCT* domain participate in DNA damage checkpoint or DNA repair pathways. However, the function of the domain is still controversial. It is known that germ-line mutations in the *BRCT* domain lead to 50% of familial breast cancers (18). Most *BRCT* domain mutations cause a truncated *BRCA1* protein. It has been shown that loss of the *BRCT* domain leads to tumor formation in mice (19). Therefore, the *BRCT* domain has an important role in the cellular process of DNA damage. Because *BRCT* repeats are found in different proteins associated with the regulation of the DNA damage response, such as *BARD1*, *53BP1*, and *MDC1*, this individual in the control group did not show any clinical signs although she had the mutation in the *BRCT* domain. Other proteins that have a *BRCT* domain might function properly to protect tumor formation in this individual. The risk of developing breast cancer by the age of 70 years for *BRCA1* mutation carriers is between 57% and 65% and between 45% and 57% for *BRCA2*. The risk of developing ovarian cancer by the age of 70 years for *BRCA1* mutation carriers is between 39% and 59% and between 11% and 18% for *BRCA2*. However, the overall risk for younger age (<40) is reported to be lower for ovarian cancer in *BRCA1* and *BRCA2* mutation carriers (20-22). It is also known that *BRCA* mutations in oocytes may lead to early depletion of the ovarian reserve (4). There are also other factors that affect breast-ovarian cancer, such as age, gender, family history of breast-ovarian cancer, and mutations other than *BRCA1* and/or *BRCA2* genes (*ATM, TP53, CHEK2, PTEN, CDH1, STK11, PALB2*) (23). Because we did not have other clinical data, such as family history or mutations in other genes of this individual, it was not possible to predict the clinical outcome.

We detected two new variations in *BRCA2*. The first one was in the intergenic sequence IVS-7T>A, which was detected only in one patient in the control group but none in the study group. Because the intergenic sequence is a non-coding region, there might not be an effect on a gene or a protein, thus resulting in no clinical signs in the patient. The second new variation p.I3312V was detected in only one case in the study group (1/45). The SIFT score was 0.88, which means that the amino acid substitution is tolerated. Although the new variation was detected in a patient with POI, this might not be related with the disease because the amino acid substitution is tolerated

and does not correspond to any domain. Therefore, the variation might be only a rare polymorphism seen in the Turkish population.

We detected 17 different variations in *BRCA1* and 17 in *BRCA2*. Six of 17 *BRCA1* variations corresponded to a domain, whereas only 2 of 17 variations corresponded to a domain in *BRCA2*. Oktay et al. (4) found nine variations in *BRCA1* and *BRCA2* that might be associated with premature ovarian failure and/or ovarian reserve. Of those, only one of them corresponded to the *BRCT* domain; however, another five variations did not correspond to any domain. Wang et al. (5) detected 13 different variations in *BRCA1* and 10 in *BRCA2*. Five of them correspond to *BRCA* domains. Of them, only one corresponded to the *BRCT* domain.

Our study has a limited number of individuals in both the study and control groups. In addition, our study lacks the confirmation of the detected variations by Sanger sequencing. However, the depth of coverage of our study was >100x in 98% of the patients. We repeated the results when the depth of coverage was <20x. Current next-generation sequencing guidelines for inherited disorders do not define quality parameters to provide concrete guidance for confirmatory analysis. In a recent next-generation sequencing laboratory standards paper, the College of American Pathologists justifies that "Sufficient depth of coverage and quality parameters should not expect false positives in their filtered data" (24). Implementing NGS-based tests according to diagnostic standards is a challenge for individual laboratories. To facilitate the implementation of NGS into routine laboratory practice several studies done such as the Dutch Society for Clinical Genetic Laboratory Diagnostics (VKGL) working group. And also, in a recent paper researchers have been emphasized that the necessity of Sanger confirmation of next-generation sequencing variants lower than 30x depth of coverage might need to be explored (25).

As a conclusion, we did not detect an association between POI and the *BRCA1* and *BRCA2* gene variations. However, functional studies are needed to clarify the variations of *BRCA1* and *BRCA2* genes because there are conflicting results about the association of *BRCA1* and *BRCA2* variations. In case of detecting variations that are related to breast-ovarian cancer, these patients might be referred for screening and follow-up programs for breast-ovarian cancer before they reach the age of 40 years. We also detected new variations in *BRCA1* and *BRCA2* genes both in the study and control group, which have not been reported before. Therefore, next-generation sequencing is a valuable tool to detect gene variations of large genes in a fast and cost-effective way.

Ethics Committee Approval: *Ethics committee approval was received for this study from the ethics committee of Başkent University (Project No: KA13/297).*

Informed Consent: *Written informed consent was obtained from patients who participated in this study.*

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Author Contributions: *Concept - N.K.Y., Y.K.T., F.İ.Ş.; Design - N.K.Y., Y.K.T., F.İ.Ş.; Supervision - N.K.Y., F.İ.Ş.; Funding - N.K.Y., S.E., F.İ.Ş.; Materials - N.K.Y., İ.K., S.Y., S.E.; Data Collection and/or Processing - P.H.K., Y.K.T., F.İ.Ş.; Analysis and/or Interpretation - N.K.Y., P.H.K., Y.K.T., F.İ.Ş.; Literature Review - N.K.Y., P.H.K., Y.K.T., İ.K., S.Y., S.E., F.İ.Ş.; Writer - N.K.Y., P.H.K., Y.K.T., F.İ.Ş.; Critical Review - N.K.Y., F.İ.Ş.*

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Effect of body mass index and age on *in vitro* fertilization in polycystic ovary syndrome

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Abstract

Objective: The aim of this study was to investigate age-related variations in the effect of body mass index (BMI) on *in vitro* fertilization (IVF) outcomes.

Material and Methods: This was a cohort study conducted by retrospectively investigating the IVF cycles of 653 polycystic ovary syndrome (PCOS) patients under the age of 40 years who were diagnosed based on the Rotterdam criteria in a private IVF clinic between 2005 and 2015. The study included data from 653 IVF cycles of PCOS patients. The patients were classified into three groups based on their BMI, i.e., normal weight (n=299), overweight (n=208), and obese (n=146). The patients were also grouped by age: 562 patients were under the age of 35 years and 91 patients were above the age of 35 years. Then, BMI- and age-related variations in the IVF cycle parameters and clinical pregnancy rates of patients with PCOS were investigated. The Mantel–Haenszel Chi-square statistical assessment method was used to determine whether the effect of BMI on IVF outcomes varies with age.

Results: Variations in cycle variables with BMI and age showed that IVF cycles were negatively affected by increases in obesity and age. Clinical pregnancy rates were found to be lower in the obese group than in the other groups, particularly in the age group above 35 years; however, this difference could not be proven statistically.

Conclusion: The present study evaluated obesity and clinical pregnancy rates in IVF cycles in PCOS patients according to age groups, and particularly in the obese group, the clinical pregnancy rates were observed to be lower in the age group ≥35 years than in the other BMI groups; however, this difference was found to be statistically insignificant. (J Turk Ger Gynecol Assoc 2016; 17: 83-90)

Keywords: PCOS, body mass index, age, IVF outcome

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Introduction

There are numerous potential causes of female infertility, and a systematic approach is necessary to effectively identify them in patients. Polycystic ovary syndrome (PCOS) is the most frequently observed cause of treatable infertility. It is commonly encountered among young women and accounts for nearly 70% of cases involving anovulatory infertility (1). *In vitro* fertilization (IVF) is an option that is recommended for infertility with no response to medical treatment in PCOS (2). Nearly half of all women with PCOS are either obese or overweight, and most exhibit the abdominal phenotype (3). Recently, Lindsay et al. (4) showed that weight loss has a corrective effect on reproductive outcomes in all obese infertile patients. In 2015, Carmina et al. (5) demonstrated that weight loss increased spontaneous fertility rates in anovulatory PCOS patients.

The literature contains different opinions about the role of body mass index (BMI) in IVF cycles. A study by Loveland et al. (6) demonstrated that implantation rates and pregnancy rates decrease with increasing BMI. In 2006, Dechaud et al. (7) showed that obesity does not have any adverse impact on IVF, and Metwally et al. (8) showed that obesity does not affect oocyte quality or clinical pregnancy rates in IVF/intracytoplasmic sperm injection (ICSI) cycles in 2007.

Because weight loss in PCOS has positive effects on hormonal, metabolic, and clinical parameters, recent studies have focused on the effect of BMI during IVF cycles in PCOS patients. Studies of IVF in PCOS also suggest different opinions about BMI: Fedorcsák et al. (9) and Mulders et al. (10) demonstrated that obese women with PCOS show gonadotropin resistance in IVF cycles; therefore, the need for follicle-stimulating hormone (FSH) and cycle cancellations increases. A study by McCormick et al. (11) demonstrated that patients with PCOS who, according to their BMI, were leaner rather than obese exhibited more positive responses to assisted reproductive technology cycles; however, these patients also exhibited no remarkable differences with respect to clinical

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outcomes. In 2014, Bailey et al. (12) investigated the effect of BMI on the characteristics and outcomes of IVF cycles in PCOS and found lower clinical pregnancy rates in an obese PCOS group than in a lean group and a lower rate of ovarian hyperstimulation syndrome (OHSS) in the obese group.

A woman's age is also an important factor in IVF success (13, 14). The IVF success rate reduces with increasing age in PCOS, as in all infertile patient groups (15, 16).

The literature contains a very limited number of studies that investigate age and BMI together. Heijnen et al. (17) reported that although BMI has a significant and negative effect on female fertility, this effect gradually decreases as women approach their mid-thirties and also in younger women receiving IVF. Above the age of 36 years, on the other hand, the effect of BMI on fertility becomes minimal. PCOS patients were not included in this study. The study by Metwally et al. (8) that investigated 426 IVF/ICSI cycles did not exclude PCOS patients and reported that although obesity does not affect oocyte quality, there is a reduction in embryo quality in patients under the age of 35 years.

The hypothesis of the present study assumed that the effect of BMI on the IVF cycles of PCOS patients may vary with age. The present study aimed to investigate age-related variations in the effect of BMI on IVF outcomes.

Material and Methods

This was a cohort study conducted by retrospectively investigating the IVF cycles of 653 PCOS patients under the age of 40 years, who were diagnosed based on the Rotterdam criteria in Gürgan IVF clinic between 2005 and 2015. The study protocol was approved by the local ethics committee, and all patients included in the study provided informed consent.

The patients were diagnosed with PCOS in accordance with the diagnostic criteria of the European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine Rotterdam Consensus Meeting 2003 (18). Based on this set of criteria, two of the following three criteria are necessary for confirming a diagnosis:

- Anovulation or Oligoovulation
- Biochemical or Clinical Hyperandrogenism
- Polycystic ovaries identified by ultrasound examination

Weight and height information was obtained from the patient database, and the following formula was used to calculate the BMI value: BMI=weight/height^2 (kg/m^2). Patient classification was performed based on BMI, with the patients being divided into three groups according to the World Health Organization's (WHO's) system for obesity (WHO, 2000). As such, normal weight was defined as a BMI value between 18.5 and 24.9 kg/m^2; overweight was defined as a BMI value between 25 and 29.9 kg/m^2; obese was defined as a BMI value of ≥30 kg/m^2 (19).

Patients were also classified into two groups based on age, i.e., under and above the age of 35 years (Table 1).

Female patients above 40 years of age, with basal FSH levels of >12 IU/L, and with frozen embryo transfer cycles were excluded from the study. Couples with systemic diseases such as diabetes and hypo- or hyperthyroidism, psychiatric diseases, and drug use in either the woman or the man were excluded. Although it was planned to have patients with a BMI of <18.5 to form a low-weight group, the number of patients in this group was very low; therefore, patients with a BMI of <18.5 were excluded.

In all patients, controlled ovarian hyperstimulation (COH) and (ICSI were performed using a classic ovulation induction protocol. For COH, long agonist and antagonist protocols were applied; gonadotropins were administered using step-up, step-down, and constant regimens. Ovulation induction was performed using urinary FSH (Metrodin; Serono, Geneva, Switzerland), recombinant FSH (Gonal F; Serono, Geneva, Switzerland or Puregon; Organon, Oss, The Netherlands), or urinary and recombinant FSH, and ovulation stimulation was performed using human chorionic gonadotropin (hCG) (Pregnyl; Organon, Brussels, Belgium).

The cycle parameters that were evaluated for this study included the protocols used in ovulation induction, total drug dose, ovulation induction time, cycle day of hCG, number of follicles and estradiol levels on the hCG day, endometrial thickness on the hCG day, number of oocytes retrieved, number of mature oocytes, number of embryos developed, number of grade 1 embryos, and number of embryos transferred. The IVF outcome parameters were the presence/absence of clinical pregnancy and multiple pregnancy rates. Clinical pregnancy was diagnosed by the embryo reaching the end of week 6, along with a proper image of the gestational sac, and the establishment of regular rhythmic heartbeats.

In the statistical assessment, firstly the effects of BMI and age on the cycle parameters and the rates of clinical pregnancy achieved were evaluated separately. Bivariate analyses such as the Mann–Whitney U test, Chi-square test, and/or Fisher's exact test were used to evaluate group comparisons and results were summarized as median (minimum–maximum) and frequencies, where appropriate. In addition, the Mantel–Haenszel Chi-square statistical assessment method was used to determine whether the effect of BMI on IVF outcomes varies with age. Values of p less than 0.05 were considered to be significant. SPSS for Windows 11.5 (SPSS Inc.; Chicago, IL, USA) was used for statistical analysis.

Results

The study included data from 653 IVF cycles of PCOS patients. The patients were classified into three groups based on their BMI, i.e., normal weight (n=299), overweight (n=208), and

Table 1. Number of patients by BMI and age

BMI (kg/m^2)	n	Age (years)	n
Normal weight	299		
Overweight	208	<35	562
Obese	146	≥35	91
Total	653	Total	653

BMI: body mass index

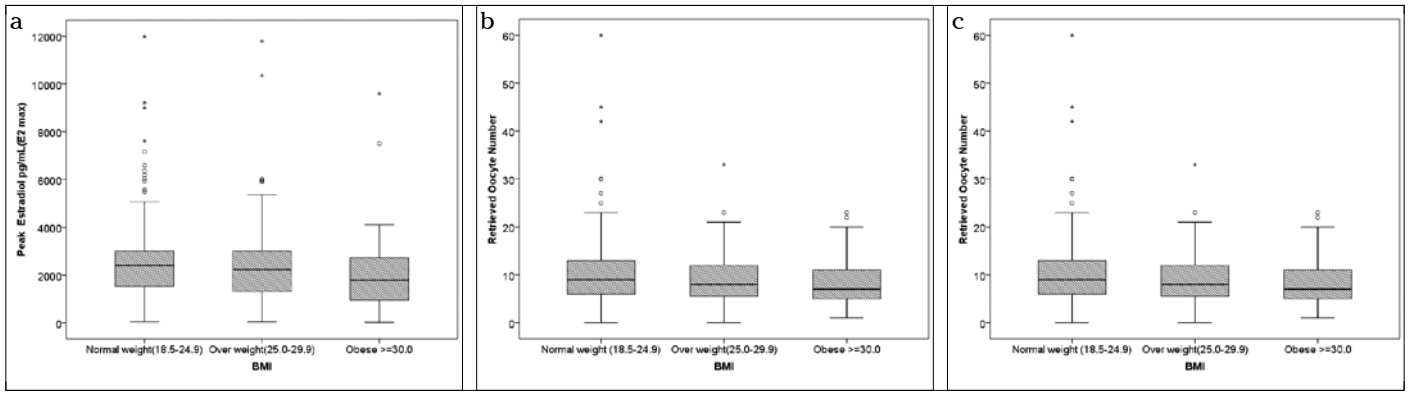

Figure 1. a-c. Distribution of maximum E2 levels (a), number of oocytes retrieved (b), and number of mature oocytes (c), according to BMI group

Table 2. Demographic characteristics of the couples included in the study

Parameter	Mean±SD	Median (min.-max.)
Female		
Age	29.30±4.81	29.00 (16.00-39.00)
BMI (kg/m^2)	26.45±5.12	25.59 (11.75-46.65)
FSH (IU/L)	3.57±1.83	3.35 (0.10-19.00)
LH (IU/L)	1.44±0.99	1.69 (0.10-2.30)
E2 (pg/mL)	47.98±190.01	23.00 (8.00-2899.00)
Male		
Age (years)	33.58±5.52	33 (13-65)
Sperm count	39761455±45713180	36000000 (12-910000000)
Motility (%)	55.91±15.29	58.00 (2.00-93.00)
Progression (%)	22.31±9.51	23.00 (1.00-50.00)

BMI: body mass index; FSH: follicle-stimulating hormone; LH: luteinizing hormone; E2: estradiol

Table 3. Results of sperm analysis

Sperm analysis	Frequency	Percentage
Normospermia	193	29.6
Teratozoospermia	178	27.3
Oligoasthenoteratospermia	137	21.0
Asthenoteratozoospermia	73	11.2
Azoospermia	68	10.4
Immotile sperm	4	0.6
TOTAL	653	100.0

obese (n=146). When the patients were grouped by age, 562 patients were under the age of 35 years and 91 patients were above the age of 35 years (Table 1).

The demographic characteristics of the couples included in the study are summarized in Table 2.

Details obtained from an examination of the male factor in the couple are presented in Table 3.

The data obtained from comparing the cycle parameters according to the BMI groups are summarized in Table 4. No

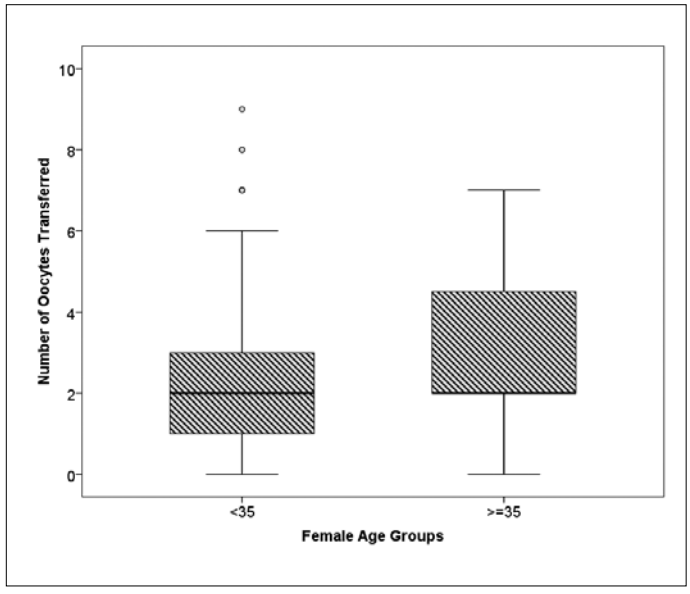

Figure 2. Distribution of number of embryos transferred (etemb) according to age group

difference was found between the groups in the distribution of induction protocols, ovulation induction times, and total drug doses (p=0.320, 0.180, and 0.298, respectively). There was no statistically significant difference in the total number of follicles on the hCG day, endometrial thickness, embryo transfer days, number of embryos developed, number of grade 1 embryos, and number of embryos transferred between all three groups (p=0.846, 0.529, 0.886, 0.176, 0.833, and 0.639, respectively). Of the cycle parameters, the maximum estradiol (E_2) levels, number of oocytes retrieved, and number of mature oocytes were found to be significantly lower in the obese group than in the normal weight group (p=0.007, 0.001, and 0.047, respectively) (Figure 1). The rates of OHSS, clinical pregnancy, and multiple pregnancy were not different between the groups (p=0.933, 0.129, and 0.121, respectively).

When the same parameters were compared between the group under the age of 35 years and the group aged 35 years and above, there was a difference in favor of the group aged 35 years and above only in the number of embryos transferred

Table 4. Comparison of IVF cycle parameters in PCOS patients according to BMI group

Parameter	BMI			
	Normal weight BMI (18.5-24.9)	Overweight BMI (24.9-29.9)	Obese BMI ≥30	p
GnRH treatment (long agonist protocol)	149 (58.9%)	100 (59.5%)	59 (51.8%)	0.367
Duration of GnRH treatment (days)	24.0 (20.0-44.0)	24.0 (21.0-32.0)	24.0 (20.0-36.0)	0.123
Protocols of OI				
Step-down	178 (66.9%)	111 (60%)	74 (57.8%)	0.320
Step-up	36 (13.5%)	34 (18.4%)	21 (16.4%)	
Constant	52 (19.5%)	40 (21.6%)	33 (25.8%)	
E2 max (pg/mL)	2411 (43-11985)[a]	2229 (38-11800)	1796 (19-9600)[b]	0.007*
Number of retrieved oocytes	9 (0-60)[a]	8 (0-33)[ab]	7 (1-23)[b]	0.001*
Number of mature oocytes	7 (0-55)[a]	7 (0-30)[ab]	6 (0-21)[c]	0.047*
Duration of OI (days)	8 (0-28)	9 (0-27)	9 (0-24)	0.180
Total dose of FSH (IU)	2550 (0-3150)	2887 (0-3075)	3375 (0-5625)	0.298
hCG day	12 (7-32)	13 (6-30)	12 (7-24)	0.007*
≥17 mm follicle on hCG day	1 (0-9)	1 (0-8)	1 (0-7)	0.846
15-17 mm follicle on hCG day	4 (0-14)	4 (0-14)	3 (0-12)	0.342
10-14 mm follicle on hCG day	9 (0-35)	9 (0-41)	8 (0-28)	0.587
Endometrial thickness on hCG day (mm)	9.9 (0-14.5)	9.9 (5-13.8)	9.5 (0-14.3)	0.529
Day of transfer				0.886
D2	33 (45.8%)	22 (30.6%)	17 (23.6%)	
D3	202 (44.3%)	152 (33.3%)	102 (22.4%)	
D4	19 (52.8%)	12 (33.3%)	5 (13.9%)	
D5	30 (44.1%)	19 (27.9%)	19 (27.9%)	
D6	3 (60%)	1 (20%)	1 (20%)	
Number of embryos	6 (0-25)	5 (0-24)	5 (0-21)	0.176
Number of grade 1 embryos	2 (0-8)	2 (0-9)	2 (0-8)	0.639
Number of grade 1 embryos (Frag0+Frag<10%)	2 (0-13)	2 (0-13)	2 (0-19)	0.833
OHSS	5 (1.7%)	4 (1.9%)	3 (2.1%)	0.933
Clinical pregnancy	101 (33.7%)	78 (37.5%)	40 (27.3%)	0.129
Multiple pregnancy	48 (53.3%)	37 (50%)	12 (33.3%)	0.121

*A value of $p<0.05$ is statistically significant.

IVF: *in vitro* fertilization; PCOS: polycystic ovary syndrome; BMI: body mass index; GnRH: gonadotropin-releasing hormone; OI: ovulation induction; D: day of embryo; E2 max: peak estradiol level; FSH: follicle-stimulating hormone; hCG: human chorionic gonadotropin; OHSS: ovarian hyperstimulation syndrome

a: 0 cells (0%) expected count less than 5. The minimum expected count is 0.

b: Computed only for a 2x2 table.

c: The standardized statistic is 1.330.

($p<0.001$) (Figure 2). The number of embryos developed, number of grade 1 embryos, clinical pregnancy rates, and OHSS rates were higher in the group aged under 35 years; however, this was not statistically significant (Table 5).

The obesity and clinical pregnancy rates in PCOS patients were evaluated according to age groups, and particularly in the obese group, the clinical pregnancy rates were observed to be lower in the group aged ≥35 years than in the other BMI groups; however, this difference was analyzed using Mantel–Haenszel Chi-square statistics and found to be statistically insignificant (Mantel–Haenszel Chi-square statistic=1.34, $p=0.247$) (Table 6, Figure 3).

Discussion

The present study investigated the BMI- and age-related variations in the IVF cycle parameters and clinical pregnancy rates of

Table 5. Comparison of IVF cycle parameters in PCOS patients according to age group

	Female Age		
Parameter	**<35**	**≥35**	**p**
GnRH treatment (long agonist protocol)	262 (57.2%)	46 (59.7%)	0.677
Duration of GnRH treatment (days)	24 (20–37)	24 (21–44)	0.782
Protocols of OI			
Step-down	395 (61.7%)	58 (68.2%)	0.527
Step-up	89 (16.2%)	11 (12.9%)	
Constant	109 (22.1%)	18 (18.8%)	
Peak estradiol (pg/mL) (E2 max)	2221 (38–11985)	2316 (19–5900)	0.620
Number of retrieved oocytes	9 (0–60)	8 (0–21)	0.189
Number of mature oocytes	7 (0–55)	6 (0–19)	0.390
Duration of OI (days)	8 (0–27)	9 (0–28)	0.156
Total dose of FSH (IU)	2925 (0–3150)	3525 (0–5625)	0.134
hCG day	12 (7–30)	12 (6–32)	0.481
≥17 mm follicle on hCG day	1 (0–9)	1 (0–6)	0.809
15–17 mm follicle on hCG day	4 (0–14)	4 (0–12)	0.836
10–14 mm follicle on hCG day	9 (0–41)	7.5 (0–30)	0.291
Endometrial thickness on hCG day (mm)	9.9 (0–14.5)	9.6 (4.6–14)	0.350
Day of transfer			0.735
D2	60 (10.9%)	12 (13.5%)	
D3	392 (71.5%)	64 (71.9%)	
D4	33 (6%)	3 (3.4%)	
D5	59 (10.8%)	9 (10.1%)	
D6	4 (0.7%)	1 (1.1%)	
Number of embryos	5 (0–25)	5 (0–15)	0.530
Number of transferred embryos	2 (0–9)	2 (0–7)	<0.001*
Number of grade 1 embryos (Frag0+Frag<10%)	2 (0–19)	2 (0–11)	0.111
OHSS	10 (1.8%)	2 (2.2%)	0.678
Clinical pregnancy	193 (34.3%)	26 (28.5%)	0.140
Multiple pregnancy	83 (47.2%)	14 (58.3%)	0.304

*A value of $p<0.05$ is statistically significant.
IVF: in vitro fertilization; PCOS: polycystic ovary syndrome; GnRH: gonadotropin-releasing hormone; OI: ovulation induction; D: day of embryo; E2 max: peak estradiol level; FSH: follicle-stimulating hormone; hCG: human chorionic gonadotropin; OHSS: ovarian hyperstimulation syndrome

patients with PCOS. The variations in cycle variables with BMI and age showed that the IVF cycles were negatively affected by increases in obesity and age. The clinical pregnancy rates were found to be lower in the obese group than in the other groups, particularly in the age group above 35 years; however, this difference could not be proven statistically.

In the first part of the present study, in which the relationship of the cycle parameters with BMI in IVF cycles in PCOS patients was investigated, there were reductions in the number of oocytes retrieved, maximum E_2 levels, and number of mature oocytes in the obese group compared with the normal weight group ($p=0.001$, 0.007, and 0.047, respectively), but no significant difference was found in other cycle parameters between BMI groups.

The literature contains several studies that report that oocyte maturation is impaired in PCOS (20), oocyte quality and fertilization capacities are reduced (21), and, in addition, obesity contributes to such impaired maturation in PCOS (22–24). Fedorcsák et al. (9) reported that the number of oocytes retrieved was low in PCOS patients. A study by Robker et al. (25) with PCOS subgroups reported that the number of oocytes retrieved and the fertilization rates were lower in an obese group. The results of the present study seemed to be consistent with these literature data.

Regarding the age-related variations in the cycle characteristics, when the same parameters were compared between the group under the age of 35 years and the group aged 35 years

Table 6. Evaluation of obesity and clinical pregnancy rates according to age group in PCOS patients

BMI			Female Age n (%)	
			<35	≥35
Normal weight (18.5–24.9)	Clinical pregnancy	Yes	92 (42%)	9 (34.6%)
Overweight (25.0–29.9)	Clinical pregnancy	Yes	68 (43.6%)	10 (41.7%)
Obese ≥30.0	Clinical pregnancy	Yes	33 (35.5%)	7 (22.6%)

PCOS: polycystic ovary syndrome; BMI: body mass index

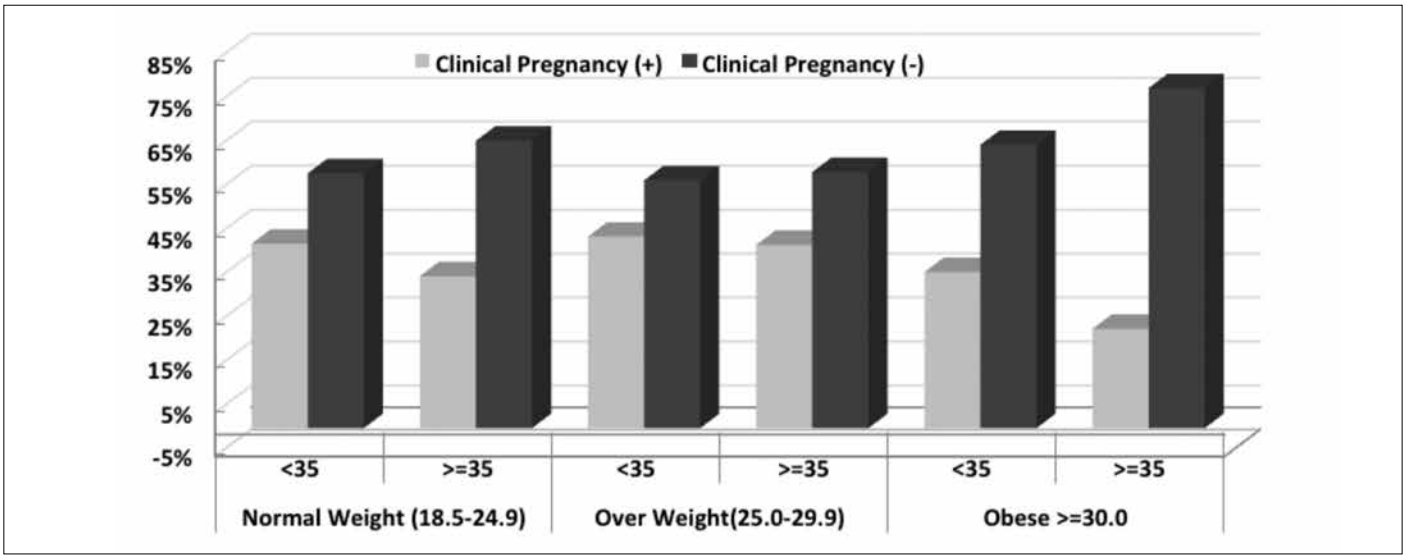

Figure 3. Evaluation of obesity and clinical pregnancy rates according to age group in PCOS patients

and above, there was a difference in favor of the group aged 35 years and above only in the number of embryos transferred ($p<0.001$) (Figure 2). The number of embryos developed, number of grade 1 embryos, clinical pregnancy rates, and OHSS rates were higher in the group aged under 35 years; however, this was not statistically significant. The higher number of embryos transferred in the group aged 35 years and above is a natural outcome of transferring more embryos at advanced ages. Whether in PCOS patients, it is a generally accepted fact in the literature that advanced maternal age has a negative effect on all parameters in IVF cycles (14, 26–29). In the present study, although a statistically significant difference could not be demonstrated, it was seen that all parameters were negatively affected in the group aged 35 years and above.

The present study evaluated obesity and clinical pregnancy rates in IVF cycles in PCOS patients according to age group, and particularly in the obese group, the clinical pregnancy rates were observed to be lower in the age group aged ≥35 years than in the other BMI groups; however, this difference was found to be statistically insignificant.

Considering studies regarding BMI in IVF cycles in PCOS patients, a study by Bailey et al. (12), which specifically compared BMI and clinical pregnancy rates in PCOS patients, and in a similar manner to the data of the present study, showed a reduction in the clinical pregnancy rates in obese PCOS patients compared with thin PCOS patients.

A retrospective study by Gorelick et al. (31), which investigated 5208 IVF cycles with different diagnoses, included 439 cycles in PCOS patients and demonstrated that obesity did not have any effect on IVF outcomes in other groups, whereas among PCOS patients, obesity led to a twofold increase in the risk of failure in IVF treatment. In women with PCOS, various negative effects were identified, not only in the implantation rate but also in pregnancy and live birth outcomes (30).

McCormick et al. (11) previously conducted a study comparing obese and lean women with PCOS with obese and lean women without PCOS. The results of this study showed a higher number of retrievable oocytes among lean women with PCOS than among obese women with PCOS. However, the study also reported similar rates of clinical pregnancy and live birth between all groups. It is important to note that this study had a very small and limited sample size, with only six lean patients with PCOS included.

The data from the present study are in line with those from the three major studies mentioned above. No study could be identified in the literature that investigated the variation in the adverse effects of obesity on the IVF cycle data in PCOS patients with age. A study of IVF published in 2011 by Luke et al. (32) evaluated the age-related effect of obesity on IVF. This study analyzed the data of 45,000 embryo transfers and concluded that higher obesity levels (i.e., BMI values) resulted in a considerable increase in the inability to achieve clinical pregnancy via the use of autologous oocytes but resulted in no differences in the use of donor oocytes. Besides, it reported that the adverse effects of obesity were more evident in the group aged under 35 years (31). The present study observed the negative effects

of obesity more specifically in the group aged above 35 years. This discrepancy is because the present study included only PCOS patients in the study group. In addition, the comparison in the abovementioned study was made between autologous and donor oocytes, which made it difficult to compare with the present study.

We believe that this study is the first to investigate age-related variations in the effects of BMI on IVF cycles in PCOS patients. The most important limitation of the present study is its retrospective nature. The retrospective examination of data from the last 10 years provided a higher number of cycles but prevented a statistically significant result from being achieved as it increased the heterogeneity of the data.

In conclusion, the present study evaluated the obesity and clinical pregnancy rates in IVF cycles in PCOS patients according to age groups, and particularly in the obese group, the clinical pregnancy rates were observed to be lower in the age group aged ≥35 years than in the other BMI groups; however, this difference was found to be statistically insignificant. Therefore, well-conceived, randomized controlled studies are required, which would investigate the effect of BMI and age on each other in PCOS patients, create a homogeneous and a higher number of subgroups, and include a larger patient population.

Ethics Committee Approval: *Ethics committee approval was received for this study from the ethics committee of Turgut Özal University School of Medicine.*

Informed Consent: *Written informed consent was obtained from patients who participated in this study.*

Peer-review: *Externally peer-reviewed.*

Author Contributions*: M.N.K., Z.K.; Design - M.N.K., Z.K.; Supervision - T.G.; Materials - M.N.K., Z.K., T.G.; Data Collection and/or Processing - Z.K., T.S.; Analysis and/or Interpretation - M.N.K., C.A.; Literature Review - M.N.K., Z.K.; Writer - M.N.K., Z.K.; Critical Review - M.N.K., T.G.*

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PGD management scheme for older females with balanced translocations: Do older females have less chance of balanced embryo transfer?

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Abstract

Objective: Carriers of reciprocal and Robertsonian translocations have a higher risk of experiencing infertility and repeated miscarriages. It is well established that with advancing maternal age, the risk of aneuploidies in embryos increases. In this study, the chance of developing balanced embryos in translocation carriers with advanced maternal age was analyzed to establish a management scheme for couples seeking fertility treatment and preimplantation genetic diagnosis (PGD).

Material and Methods: Biopsy was performed on cleavage-stage embryos. Multicolor fluorescence in situ hybridization was used for PGD. The translocation carriers underwent a total of 55 cycles of PGD. Genetics diagnosis and cycle outcomes of PGD cases were examined.

Results: This study showed that the chance of obtaining a balanced embryo from the Robertsonian translocation carriers was significantly less when the maternal age is advanced. Similar rates for balanced embryos were obtained from the reciprocal translocation carriers.

Conclusion: The results of this study show that maternal age plays an important role and that genetic counselling and planning for a PGD cycle in translocation carriers, particularly for Robertsonian carriers, must be accordingly adapted. (J Turk Ger Gynecol Assoc 2016; 17: 91-5)

Keywords: PGD, maternal age, human embryo, translocation, PGD counselling

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Introduction

Couples with known chromosomal abnormalities and structural changes opt for preimplantation genetic diagnosis (PGD) to avoid prenatal diagnosis or miscarriages. PGD is a method of selecting unaffected embryos from patients with translocations or single gene disorders. In humans, the incidence of structural changes, including translocations, is frequent (1, 2). Translocations are grouped in two categories: reciprocal, the most common form, and Robertsonian. Reciprocal translocations occur due to an exchange of two ends of non-homologous chromosomes, whereas the rearrangement of two acrocentric chromosomes arises in Robertsonian translocation carriers occurring in 1 in 900 live births (3). The phenotype of balanced reciprocal and Robertsonian translocation carriers is normal; however, they usually have a lower chance of successful natural reproduction (1, 4). Therefore, such couples opt for PGD to select a balanced embryo and increase their implantation and pregnancy rates. PGD is a highly invasive technique that necessitates *in vitro* fertilization followed by polar body or embryo (cleavage or blastocyst stage) biopsy.

It is well established that there is a higher risk of aneuploidy in embryos with advancing maternal age (5). Additionally, advanced maternal age has been associated with higher levels of meiotic errors (6). Therefore, for these patients, genetic counselling holds great importance. These patients must be thoroughly informed about the risks of reproductive outcomes, and treatment strategies should be explained to these couples. In this study, we aimed to investigate if there was any effect of maternal age on the chromosomal status of embryos as well as preimplantation embryo development and to develop a management scheme for these patients.

Material and Methods

This retrospective study analyzed PGD results, the number of balanced embryos, and embryo transfer, followed by the analysis of pregnancy rates in carriers undergoing assisted reproductive technology treatments in Bahçeci ART Centre.

Patient information

Translocation carriers were subdivided according to maternal age (<35 years and advanced maternal age of ≥35 years) and translocation type, i.e., reciprocal and Robertsonian. A total of 20 couples with maternal ages of <35 years with 37 PGD cycles were analyzed within the reciprocal translocation carrier group. The advanced maternal age group of recipro-

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cal translocation carriers included 15 couples. Twelve couples with 17 PGD cycles were involved within the Robertsonian carrier group of maternal age of <35 years and three couples with eight cycles of PGD were in the advanced maternal age group. The couples undergoing PGD were counselled by an infertility specialist and medical doctors. PGD and limitations, including the risk of misdiagnosis due to mosaicism and technical errors, were explained to the couples. Prior to PGD cycles, the patients were informed about the procedure, and the potential risks were explained to and informed consent was obtained from each patient; ethical approval was granted. Chromosome banding techniques were used to obtain the karyotypes of all patients. The translocations of carriers were confirmed using fluorescence *in situ* hybridization (FISH). For FISH analysis, a total of two sub-telomeric probes and one centromeric probe were used.

Controlled ovarian stimulation and embryology

Stimulation treatment was applied for each patient as previously described (7). Briefly, to induce ovulation, human chorionic gonadotrophin (hCG) (5,000 IU; Ovidrelle; Merck Serono, UK) was injected, followed by the aspiration of follicles transvaginally after 35–36 h of the hCG injection. Hyaluronidase treatment was applied to oocytes following 2 h of culturing. Sperms were washed and prepared for the Intra-cytoplasmic sperm injection (ICSI) procedure by discontinuous colloidal silica gel gradient (PureSperm; Nidacon, Sweden). Only meiosis II (MII) stage oocytes were microinjected, and the oocytes were cultured for 16–18 h following ICSI in a 5% CO_2 and 5% O_2 incubator (INB-203C; IKS International, Netherlands). Embryo scoring was assessed in terms of the number of cells and presence of even and uneven cells.

Embryo biopsy and PGD

Embryos were biopsied on day 3 of embryonic development (Octax™, MTG; Munster, Germany). Embryos with less than six cells and more than 20% fragmentation were not biopsied. Three hundred eighty-nine embryos, of which 227 were from the reciprocal translocation carriers with maternal age of <35 years and 162 were from those with advanced maternal age, were biopsied. One hundred eighteen embryos, of which 131 obtained from the Robertsonian translocation carriers with maternal age of <35 years and 49 from those with advanced maternal age, were biopsied. Poly-L-lysine coated slides were used for FISH analysis, and one cell was analyzed for each embryo (Thermo Scientific; Darmstadt, Germany). Details of the translocations for each couple and the list of probes used are listed in Table 1. The poly-L-lysine coated slides were washed to remove excess probe hybridization as described by the manufacturer, and each nucleus was analyzed by counterstaining with 0.15 ng/mL of 4, 6-diamidino-2-phenylindole (DAPI, Medimiks; İstanbul, Turkey). Two expert scientists analysed the outcome of the PGD for translocations.

Statistical analysis

GraphPad prism v6 (GraphPad Software, Inc.; La Jolla, California, USA) was used for statistical analysis. The significance of obtaining balanced embryos in the translocation carriers with respect to maternal age was evaluated by the Chi-square test.

Results

Overall, 79% (1154/1457) of the oocytes collected matured to the MII stage, and 72% (832) of these were normally fertilized with two pronuclei. Seventy four percent (619) of the normally developing embryos were biopsied for PGD (Table 2). Sixty age-matched patients with no chromosomal rearrangements were analyzed for the developmental rate as a control group within the same time period. Ninety three percent (551/594) of the oocytes obtained from the control group developed to the MII stage, and 79% (438) of these fertilized. The maturation and fertilization rates were similar between the two groups (Table 2).
Overall, 63 of 389 (16%) embryos from the reciprocal translocation carriers were chromosomally balanced, and eight of these were transferred, resulting in two pregnancies. The chances of obtaining balanced embryos from the reciprocal translocation carriers grouped in the maternal age<35 years (16%, 38/227) and advanced maternal age (15%, 25/162) groups were similar (Table 3). The number of balanced embryos was further analyzed according to the sex of the carrier. From 19 female carriers, 175 embryos were obtained. Of these, 14% (24) were balanced. The chances of obtaining balanced embryos from male carriers were also at similar levels (18%, 39/214).
In the Robertsonian translocations, a total of 187 embryos were biopsied, and 42% (78) of these embryos were shown to be balanced (Table 3). When the number of balanced embryos was further investigated according to the maternal age groups, considerably fewer balanced embryos (30%, 17/56, $p<0.05$) were obtained from the advanced maternal age group than from the younger patients who were <35 years (45%, 59/131). Similar to the reciprocal translocation carriers, the number of balanced embryos obtained from the female (38%, 35/92) and male (45%, 43/95) carriers was similar (Table 3).

Discussion

In this study, the role of maternal age in obtaining balanced embryos from translocation carriers was investigated. Overall, among the reciprocal translocation carriers, there was no difference in the number of balanced embryos between the advanced maternal age group and younger females. However, in the Robertsonian translocation carriers, it was observed that the number of balanced embryos was significantly decreased in the advanced maternal age group. Therefore, for the Robertsonian carriers, there may be an adverse effect of maternal age on PGD outcome. Advanced maternal age is well known to increase the risk of aneuploid pregnancy (8). This may be more noticeable in Robertsonian translocation carriers because the most commonly found aneuploidies in embryos include the same chromosomes that are also involved in Robertsonian translocations, such as chromosomes 13, 14, 15, 21, and 22 (9-12). In the present study, the majority of embryos in Robertsonian translocation carriers involved chromosomes 13, 14, and 21. Therefore, it is possible that the effect of maternal age on PGD outcome is due to the fact that these chromo-

Table 1. Patient information list. Patient ID with maternal age at the time of oocyte retrieval, karyotype, and list of probes used in PGD analyses are listed. All probes are from Cytocell (UK) and Abbott Molecular Inc (USA)

Patient ID	Maternal age	Karyotype	FISH probes used
1	39	46,XY, t(1;2)(p?36;p?14-16)	LPT 1p (Green), CEP 1 (Spectrum Orange), LPT 2p (Red)
2	41	46,XY, t(1;2)(q42;q14.2)	LPT 1p (Green), LPT 1q (Red), TelVysion 2q (Spectrum Orange)
3	30	46,XX, t(1;5)(q22;q23)	LPT 1q (Green), CEP 1 (Spectrum Orange), LPT 5q (Red)
4	30	46,XY, t(1;6)(q23.1;q21)	LPT 1q (Green), CEP 1 (Spectrum Orange), LPT 6q (Red)
5	40	46,XY, t(1;9)(p32;q22)	LPT 1p (Green), LPT 1q (Red), TelVysion 9q (Spectrum Orange)
6	34	46,XX, t(1;10)(p?34;p11.2)	LPT 1p (Green), CEP 10 (Spectrum Aqua), LPT 10p (Red)
7	40	46,XX, t(1;10)(p32;q21.3)	LPT 1p (Red), CEP 10 (Spectrum Aqua), LPT 10q (Green)
8	38	46,XY, t(1;10)(p22;q22)	LPT 1p (Red), CEP 10 (Spectrum Aqua), LPT 10q (Green)
9	41	46,XY, t(1;16)(q21;q12)	LPT 1q (Red), CEP 1 (Spectrum Orange), LPT 16q (Green)
10	39	46,XY, t(1;22)(q12;q11.2)	LPT 1q (Red), CEP 1 (Spectrum Orange), LPT 22q (Green)
11*	36	46,XY, t(2;5)(p11.2;q33)	LPT 2p (Red), LPT 2q (Green), LPT 5q (Red)**
12	31	46,XY, t(2;10)(q?23;p?13)	LPT 2q (Green), CEP 10 (Spectrum Aqua), LPT 10p (Red)
13	29	46,XX, t(2;10)(q36;q22)	TelVysion 2q (Spectrum Orange), CEP 10 (Spectrum Aqua), LPT 10q (Green)
14	38	46,XX, t(2;18)(p15;p11.2)	LPT 2p (Red), TelVysion 2q (Spectrum Orange), LPT 18p (Green)
15*	30	46,XY, t(3;5)(q12;p12)	LPT 3p (Green), LPT 3q (Red), LPT 5p (Red)**
16	33	46,XY, t(3;10)(p21.3;p15)	TelVysion 3p (Spectrum Green), CEP 10 (Spectrum Aqua), LPT 10p (Red)
17	32	46,XX, t(4;10)(q31.2;q21.2)	TelVysion 4q (Spectrum Orange), CEP 10 (Spectrum Aqua), LPT 10q (Green)
18	33	46,XY, t(5;10)(q13;q24)	LPT 5q (Red), CEP 10 (Spectrum Aqua), LPT 10q (Green)
19	24	46,XY, t(5;11)(q33;p15)	LPT 5q (Red), CEP 11 (Spectrum Aqua), TelVysion 11p (Spectrum Green)
20	37	46,XX, t(5;20)(q31;q13.3)	LPT 5q (Red), TelVysion 20p (Spectrum Green), TelVysion 20q (Spectrum Orange)
21*	33	46,XY, t(5;22)(q22;qter)	LPT 5p (Red), LPT 5q (Red), LPT 22q (Green), LSI 22 (Spectrum Green)**
22	25	46,XX, t(7;10)(p13;p11.2)	LPT 7p (Green), CEP 10 (Spectrum Aqua), LPT 10p (Red)
23	28	46,XY, t(8;22)(q23.2;qter)	LPT 8q (Red), CEP 8 (Spectrum Aqua), LPT 22q (Green), LSI 22 (Spectrum Green)**
24	34	46,XY, t(9;10)(p13;q11.2)	LPT 9p (Red), CEP 9 (Spectrum Aqua), LPT 10q (Green)
25	36	46,XY, t(9;12)(q22;q24.3)	TelVysion 9q (Spectrum Orange), CEP 9 (Spectrum Aqua), LPT 12q (Green)
26	29	46,XX, t(9;12)(q22.3;q13.3)	TelVysion 9q (Spectrum Orange), CEP 9 (Spectrum Aqua), LPT 12q (Green)
27*	30	46,XX, t(9;20)(q34;q13)	TelVysion 9q (Spectrum Orange), CEP 9 (Spectrum Aqua), TelVysion 20q (Spectrum Orange)**
28	28	46,XX, t(10;12)(q26;q24)	LPT 10q (Green), CEP 10 (Spectrum Aqua), TelVysion 12q (Spectrum Orange)
29	27	46,XX, t(10;14)(q22.3;q13)	LPT 10q (Green), CEP 10 (Spectrum Aqua), LPT 14q (Red)
30	32	46,XX, t(11;22)(q11.2;q13.3)	TelVysion 11q (Spectrum Orange), CEP 11 (Spectrum Aqua), LPT 22q (Green), LSI 22 (Spectrum Green)**
31	35	46,XX, t(13;17)(q?14;q21)	LPT 13q (Green), CEP 17 (Spectrum Aqua), TelVysion 17q (Spectrum Orange)
32	26	46,XY, t(13;22)(p11.2;q13.1)	LPT 13q (Red), LPT 22q (Green), LSI 13 (Spectrum Orange), LSI 22 (Spectrum Green)**
33*	36	46,XY, t(16;22)(q23.1;q13)	LPT 16q (Green), CEP 16 (Spectrum Aqua), LPT 22q (Green)**
34	31	46,XX, t(17;19)(q11.2;p13.3)	TelVysion 17q (Spectrum Orange), CEP 17 (Spectrum Aqua), LPT 19p (Green)
35	38	46,XY, t(19;22)(p13.3;q11.2)	LPT 19p (Red), TelVysion 19q (Spectrum Orange), LPT 22q (Green)
36	35	45,XY, t(13;14)(q10;q10)	LPT 13q (Green), LPT 14q (Red), LSI 13 (Spectrum Orange)
37	39	45,XX, t(13;14)(q10;q10)	LPT 13q (Green), LPT 14q (Red), LSI 13 (Spectrum Orange)

38	31	45,XY, t(13;14)(q10;q10)	LPT 13q (Green), LPT 14q (Red), LSI 13 (Spectrum Orange)
39	40	45,XX, t(13;14)(q10;q10)	LPT 13q (Green), LPT 14q (Red), LSI 13 (Spectrum Orange)
40	29	45,XY, t(13;14)(q10;q10)	LPT 13q (Green), LPT 14q (Red), LSI 13 (Spectrum Orange)
41	28	45,XY, t(13;14)(q10;q10)	LPT 13q (Green), LPT 14q (Red), LSI 13 (Spectrum Orange)
42	29	45,XX, t(13;14)(q10;q10)	LPT 13q (Green), LPT 14q (Red), LSI 13 (Spectrum Orange)
43	33	45,XX, t(13;14)(q10;q10)	LPT 13q (Green), LPT 14q (Red), LSI 13 (Spectrum Orange)
44	31	45,XY, t(13;14)(q10;q10)	LPT 13q (Green), LPT 14q (Red), LSI 13 (Spectrum Orange)
45	31	45,XY, t(13;14)(q10;q10)	LPT 13q (Green), LPT 14q (Red), LSI 13 (Spectrum Orange)
46	28	45,XX, t(13;15)(q10;q10)	LPT 13q (Green), LPT 15q (Red), LSI 13 (Spectrum Orange)
47	35	45,XY, t(13;21)(q10;q10)	LPT 13q (Green), TelVysion 21q (Spectrum Orange), LSI 13 (Spectrum Green), LSI 21 (Spectrum Orange)**
48	21	45,XX, t(14;21)(q10;q10)	LPT 14q (Red), TelVysion 21q (Spectrum Orange), LSI 21 (Spectrum Orange)**
49	30	45,XY, t(14;21)(q10;q10)	LPT 14q (Red), TelVysion 21q (Spectrum Orange), LSI 21 (Spectrum Orange)**
50	31	45,XX, t(14;22)(q10;q10)	LPT 14q (Red), LPT 22q (Green)

ID: identification data; CEP: chromosome enumeration probe
*two cycles of IVF and PGD, **two rounds of FISH analysis

somes are the most commonly observed chromosomal abnormalities in embryos. Therefore, for the Robertsonian carriers, we cannot distinguish the segregation pattern of these chromosomes from aneuploidy that may be observed in non-carriers as well. The likelihood of obtaining a balanced embryo did not seem to be affected by the sex of the carrier. Similar findings have also been previously reported for reciprocal translocation carriers (8). These findings are particularly important to counsel patients prior to and during a PGD cycle. Understanding all these factors and the possible risks and outcomes of PGD are essential for effective counselling. Furthermore, evaluating couples' motives and considerations while opting for PGD would provide valuable information for improving genetic counselling and clinical care for these couples. Therefore, the results of this study provide significant information to establish a management scheme for translocation carriers undergoing PGD.

One of the drawbacks of this study was that we only analyzed the chromosomes involved in translocation. However, mitotic recombination of these chromosomes has been suggested to interrupt segregation of the normal chromosomes, leading to aneuploidies (13-18). Therefore, some aneuploid chromosomes that were not tested by FISH may have been missed. With the use of array-based comparative genomic hybridization (aCGH), translocation as well as aneuploid embryos would have been detected. However, FISH is still widely used to detect translocations (19, 20). This is mostly due to the size of breakpoints involved in translocation because in some cases, even the high-resolution aCGH cannot detect the translocated segment (21). Additionally, neither FISH nor aCGH could distinguish a balanced embryo from a normal one.

To conclude, advancing maternal age may adversely affect the chromosome complement in embryos. The chances of attaining a balanced embryo were significantly reduced in Robertsonian translocation carriers compared to those in younger females. Therefore, it is important to counsel carriers, particularly those with advanced maternal age, undergoing PGD.

Table 2. Table showing the number of patients, oocytes retrieved with the number of meiosis II (MII) stage oocytes, and biopsied embryos

	Translocation Carriers	Control Group
Number of patients	50	60
Number of retrieved oocytes	1457	594
Number of injected oocytes (MII)	1154	551
Number of biopsied embryos	619	438

Table 3. Table showing the number of reciprocal and Robertsonian translocation carriers, number of biopsied embryos, and number of balanced embryos

	Reciprocal cases			Robertsonian cases		
	Total	Maternal age <35	Maternal age ≥35	Total	Maternal age <36	Maternal age ≥36
Number of patients	35	20	15	15	12	3
Number of biopsied embryos	389	227	162	187	131	56
Number of balanced embryos	63 (16%)	38 (16%)	25 (15%)	78 (42%)	59 (45%)	17 (30%)

Ethics Committee Approval: *Ethics committee approval was received for this study from the ethics committee (Approval number: BSG0020).*

Informed Consent: *Written informed consent was obtained from patients who participated in this study.*

Peer-review: *Externally peer-reviewed.*

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Mucinous borderline ovarian tumors: Analysis of 75 patients from a single center

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Abstract

Objective: To analyze the clinicopathologic features, recurrence and survival rates, reproductive history, and treatment of patients with mucinous borderline ovarian tumors (mBOTs).

Material and Methods: Patients with a diagnosis of mBOT were evaluated retrospectively. Patients with borderline ovarian tumors other than mucinous type and concomitant invasive cancer were excluded.

Results: A total of 75 patients were identified. Median age was 38 years. The most common symptom was pain (42.7%). Median CA-125 level was 23.5 IU/mL (range, 1–809 IU/mL). Median tumor size was 200 mm (range, 40-400 mm), and 6.7% of mBOTs were bilateral. Thirty-six (48%) patients underwent staging surgery. Two patients (5.9%) had nodal involvement. One patient received platinum-based adjuvant chemotherapy. One (1.3%) patient had recurrence. None of the patients died because of the ovarian tumor. A total of 43 patients had conservative surgery.

Conclusion: Prognosis of mBOTs is excellent, and fertility-sparing surgery should be considered in the reproductive age group. Furthermore, the necessity of staging surgery is controversial. (J Turk Ger Gynecol Assoc 2016; 17: 96-100)

Keywords: Borderline ovarian tumor, mucinous, surgical approaches

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Introduction

Borderline ovarian tumors (BOTs) comprise 10-20% of ovarian malignancies (1). Although they are similar to malignant epithelial ovarian tumors in some of the histologic characteristics, these types of tumors do not have destructive stromal invasion (2). Their prognosis is much better than that of carcinoma (3). BOTs can be divided into serous, mucinous, endometrioid, clear cell, and Brenner types (4). Median age at diagnosis is 10-20 years younger than that of invasive ovarian cancer, and BOTs are usually diagnosed at an early stage (4, 5).

Up to 30-50% of BOTs are mucinous (4). Mucinous BOTs (mBOTs) were classified into "intestinal" or "Mullerian" (endocervical) types. However, according to a new classification, endocervical mBOT is a part of seromucinous tumors and intestinal mBOT is accepted as mBOT (6). mBOTs are rarely associated with pseudomyxoma peritonei. However, 75% of tumors associated with pseudomyxoma peritonei have an appendiceal origin (7). In the past, guidelines often recommended removal of the appendix in patients with mBOT. However, appendectomy is controversial today, and some authors suggest appendectomy only if the appendix appears macroscopically abnormal (4). More than 90% of patients with mBOT have stage I disease, and fewer than 10% are bilateral (7).

The purpose of this study was to analyze the clinicopathologic features, recurrence and survival rates, reproductive history, and treatment of patients with mBOT.

Material and Methods

The patients who were diagnosed in our institution between January 1990 and April 2014 with a final diagnosis of mBOT were evaluated retrospectively. Patients with BOT other than mucinous type and patients with concomitant invasive cancer were not included. Information about the patients' pathologic reports, medical records, and operation notes were extracted from the computerized database of the gynecologic oncology department. The clinical, surgical, and pathologic details (age, menopausal status, history of infertility, complaint at admission, tumor size, bilaterality, type of operation, nodal involvement, CA-125 levels, histologic subtype, and follow-up) of patients were obtained from the archives. Ethics committee approval was received from the local ethics committee of the hospital where this research was conducted. Written informed consent was not received from patients due to the nature of the study.

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The treatment of patients with these tumors was altered during the period of research. The staging surgery was decided according to the time of the diagnosis of the tumor (intraoperatively vs postoperatively) and the opinion of the surgeon. For the premenopausal women who desire pregnancy, fertility-sparing surgery (conservative surgery) was preferred. Radical surgery (total abdominal hysterectomy and bilateral salpingo-oophorectomy) was done for women who are not willing to be pregnant again. Preservation of the uterus and minimum one ovary was performed for the fertility-sparing surgery. This description contains unilateral adnexectomy (UA), unilateral cystectomy (UC), UA with contralateral cystectomy (UA+CC), and bilateral cystectomy (BC), with or without staging surgery. All of the patients had laparotomy for surgery.

Samples gained from surgery were assessed by pathologists who are skilled in gynecologic pathology. Seventy-five patients with mBOT were included in the study; 14 of these 75 patients were sent to our institution from other hospitals because of having mBOT. After the pathologic specimens were confirmed as BOT by our pathologists, we finalized the diagnosis of patients as BOT. For identification of BOT, World Health Organization (WHO) diagnostic norms were utilized. The criterion for being BOT is showing uncharacteristic epithelial proliferation lacking stromal invasion. Staging surgery was performed by using 1988 International Federation of Gynecology and Obstetrics (FIGO) staging system for ovarian carcinoma.

Patients were taken to abdominal ultrasonography, performed pelvic examination, CA-125 levels, blood biochemistry, and complete blood count every 3 months for 2 years, every 6 months till the fifth year, and once a year subsequently. If required, thoracic, abdominal, or pelvic computed tomography was performed. The time between the surgical treatment and the patients' last visit was assessed as follow-up time. Time to recurrence was defined as the period between surgery and relapse.

Statistics

Data assessment was made by using the SPSS 11.5 for windows (SPSS Inc.; IL, USA). P values <0.05 were accepted as statistically significant.

Results

Clinicopathologic factors

A total of 250 patients having a final diagnosis of BOT between January 1990 and April 2014 in our institution were identified, of whom 175 with cell types other than mucinous were excluded. The remaining 75 patients had mBOT. Median age at diagnosis was 38 years (range, 16-77 years). Sixty percent of the patients were premenopausal, and 4% had a history of infertility. The most common symptom before diagnosis was pain (42.7%), and 25.3% of patients had sensation of bloating. The characteristics of the patients are shown in Table 1.

Preoperative CA-125 levels of 46 patients were known. Median CA-125 level was 23.5 IU/mL (range, 1-809 IU/mL). Eighteen patients had a CA-125 level above 35 IU/mL. Median tumor size was 200 mm (range, 40-400 mm). Only 6.7% of mBOTs were bilateral.

Table 1. The clinical characteristics of the patients, n=75

Characteristics	Median (range), n (%)
Age	38 (16-77)
Menopausal status	
Not known	14 (18.7)
Premenopausal	45 (60)
Postmenopausal	16 (21.3)
History of infertility	
Not known	33 (44)
Yes	3 (4)
No	39 (52)
Complaint at admission	
Absent	14 (18.7)
Abdominal pain	32 (42.7)
Sensation of bloating	19 (25.3)
Abnormal vaginal bleeding	3 (4)
Urinary incontinence	1 (1.3)
Not known	6 (8)

Stage I disease was observed in 45% and stage III only in 3% of patients. Among 75 patients, 43 (57%) had conservative surgery. Among these 43 patients, 34 had UA, 5 had UA+UC, 3 had UC, and 1 had BC. Staging surgery was not performed in 39 (52%) patients. Thirty-six (48%) patients underwent staging surgery. Eleven of these 36 patients had staging surgery at the second operation (re-staging). Among these 36 patients, 34 had lymphadenectomy. Median number of harvested lymph nodes was 52 (range, 21-105). These numbers were 36 (range, 21-67) and 19 (range, 0-38) for pelvic and para-aortic regions, respectively. Among the 34 patients, two (5.9%) had nodal involvement. One patient had pelvic and one had para-aortic involvement. Omentectomy was performed in 53% of patients, and omental metastasis was not detected in any of these patients. Appendectomy was performed in 44 (58.7%) patients, and none of the patients had involvement of the appendix. No other pathologic finding was detected in the appendectomy speciments. Abdominal cytology was reported in 47 patients of which five (6.7%) had positive cytology, reported as atypical cells. No patient was reported to have microinvasion or micropapillary growth pattern. Only one patient received platinum-based adjuvant chemotherapy (Carboplatin flacon, 450 mg/45 mL; Eczacıbaşı, Turkey). Reason of adjuvant therapy was nodal involvement in this patient. None of the patients had adjuvant radiotherapy. Median follow-up time was 51 months (range, 1-222 months). The clinical features and the type of the operations are shown in Table 2.

Recurrence and survival

Median follow-up time was 51 months (range, 1-222 months). Only one (1.3%) patient had recurrence. None of the patients died because of the ovarian tumor. The patient with recurrent

Table 2. The pathologic and surgical characteristics of patients, n=75

Characteristics	Median (range), n (%)
Mean tumor size (mm)	200 (40-400)
Bilaterality	5 (6.7)
Type of operation	
UA	34 (45.3)
UA+CC	5 (6.7)
UC	3 (4.0)
BC	1 (1.3)
TAH+BSO	30 (40.0)
BA	2 (2.7)
Staging surgery	36 (48.0)
Restaging	11 (14.7)
Abdominal cytology	47 (62.6)
Omentectomy	40 (53.3)
Peritoneal biopsy	3 (4.0)
Appendectomy	44 (58.7)
Lymphadenectomy	34 (45.3)
Nodal involvement (n=34)	2 (5.9)

UA: unilateral adnexectomy; UA+CC: unilateral annexectomy+contralateral cystectomy; UC: unilateral cystectomy; BC: bilateral cystectomy; TAH+BSO: total abdominal hysterectomy+bilateral salpingoophorectomy; BA: bilateral adnexectomy

mBOT had recurrence twice: her first recurrence was after 61 months following the first surgery and the second recurrence was 91 months after first surgery. She had right unilateral salpingooferectomy (USO) in the first operation conducted in another institution. Preoperative CA-125 level was unknown. Resurgery for staging had not been performed. She had left ovarian cystectomy after both the first and second recurrences. Both pathology results revealed mBOT. She has been free of disease for 116 months after the second recurrence, and she had a term pregnancy after her second recurrence.

Reproductive history

A total of 43 patients had conservative surgery in the first operation. Median age of these patients was 29 years (range, 16-40 years). Among these patients, desire for pregnancy was reported in the files of 14 patients. In all, 12 patients got 14 pregnancies. Eleven pregnancies went to term, 1 pregnancy terminated with abortion, and the pregnancy outcomes of 2 patients were not known.

Discussion

BOTs constitute 10-20% of ovarian malignancies (1), and 30-50% of BOTs are mucinous (4). In the literature, there is limited data evaluating only mBOTs. We retrospectively analyzed patients with mBOT.

It is known that BOTs are usually seen in young women. We found that median age at diagnosis was 38 years and that 60% of patients were premenopausal. There are similar findings in the literature regarding median age at diagnosis. A retrospective study from Italy assessed 43 patients with BOT and reported the median age as 49 years (8). Ayhan et al. (9) evaluated recurrence and prognostic factors in 100 patients with BOT and found that the mean age at diagnosis was 41.7 years. Similarly, Uysal et al. (10) reported the mean age of patients with BOT as 37.7 years, and Desfeux et al. (11) identified that the mean age at diagnosis was 45 years. All these studies evaluated not only patients with mBOT but also the other patients with other types of BOT.

In our series, the most common symptoms were pain (42.7%) and sensation of bloating (25.3%). In the literature, there were studies evaluating patient complaints. Although these studies include patients with all types of BOT, they don't specifically focus on only the patients with mBOT . Like our study, in a French retrospective multicenter study, Fauvet et al. (12) evaluated 360 women treated for BOT and pointed out that the most common symptom at diagnosis was pelvic pain (27%). Ayhan et al. (9) reported that the most common complaints at admission were abdominal mass (37%) and abdominal pain (29%). In the study by Messalli et al. (8), 49% of patients with BOT were asymptomatic and in premenopausal patients the most common symptom was menstrual disorders (44%).

CA-125 level is elevated in patients with epithelial ovarian tumors and used in diagnosis and follow up (13). In our study the mean preoperative CA-125 level was 23.5 IU/mL (range, 1-809 IU/mL) and 18 patients had a CA125 level above 35 IU/mL. An article that aimed to understand the outcomes of women with mBOT identified that carcinoembryonic antigen (CEA) could be used in the diagnosis and follow-up (14). Engelen et al. (13) analyzed preoperative and postoperative serum levels of tumor markers. They reported that CA 19-9 level was elevated more than CA-125 and CEA levels in mBOT. Gotlieb et al. (15) pointed out that 70% of patients with serous BOT had elevated CA-125 levels, while 30% of patients with mBOT did. CA-125 level does not seem to be a good marker in the diagnosis and follow-up of patients with mBOT.

mBOTs are characterized by their large size. Messalli et al. (8) analyzed patients with BOT retrospectively and found that serous BOTs (20-230 mm) were smaller than mBOTs (40-354 mm). Brown and Frumovitz (14) examined mucinous tumors of the ovary-not only mucinous tumors of low malignant potential but also benign mucinous cystadenoma and invasive mucinous ovarian carcinoma-and reported that mucinous tumors are large cystic mass. The mean size was 18 cm, and they were usually unilateral (14). In a population-based study from California that compared characteristics of different types of BOTs pointed out that the mean tumor size of serous BOTs was 9.8 cm, while it is 16.4 cm in mBOTs. In that study, 28.7% of serous BOTs were bilateral, while 4.7% of mBOTs were (16). Our findings were similar with the literature. We found that median tumor size was 200 mm and that only 6.7% of mBOTs were bilateral.

Staging surgery is controversial in patients with BOT. Many authors reported that patients with mBOT that were limited to

the ovary did not have lymph node metastases. A review from Italy, which was about early stage BOTs, included 15 studies—a total of 948 cases—and showed that 69 (6%) patients had stage I disease, 10.3% had stage II, 19.2% had stage III, and 0.6% had stage IV (17). However, this study included patients with all types of BOT, not only mBOTs. These ratios were for both serous BOTs and mBOTs. Romagnolo et al. (18) compared the laparoscopic and laparotomic approach in patients with BOT. They found that in 35 patients with mBOT, 34 patients had stage I disease. Brown and Frumovitz (14) reported that in three series that included 146 patients with mBOT, none had lymphatic metastases. Kleppe et al. (4) investigated the incidence of mucinous neoplasm in the appendix in patients with mBOT and found that appendices of 13 patients with mBOT were removed and all of them were microscopically normal. Our findings were similar to the literature. Our results showed that staging surgery was performed in 48% of patients and that only 3% of patients had stage III disease. Among 34 patients who had lymphadenectomy, only 2 had nodal involvement. Omental metastases, peritoneal implants, involvement of the appendix, microinvasion, or micropapillary growth pattern were not seen in any of these patients.

One patient received platinum-based adjuvant chemotherapy (Carboplatin flacon, 450 mg/45 mL; Eczacıbaşı, Turkey). The reason of adjuvant therapy was nodal involvement in this patient. None of the patients had adjuvant radiotherapy. Only one (1.3%) patient had recurrence. None of the patients died due to the ovarian tumor. Our results showed that the prognosis of patients with mBOT was excellent. We did not compare the survival and recurrence rate between staged and unstaged patients, as no patients died because of mBOT and only one patient had recurrence. Winter et al. (19) compared the survival and recurrence rate between staged and unstaged patients with all types of BOT. They showed that there was no difference between staged and unstaged patients. In a retrospective study that aimed to show the prognostic importance of each step of the surgical staging in patients with serous BOT reported that by each skipping step of the surgical staging the recurrence risk of the patient increased (20). Trillsch et al. (21) analyzed the age-dependent differences in patients with all types of BOT and point out that younger patients had higher disease recurrence risk.

It is known that BOTs are usually seen in young women, and these patients may have desire for pregnancy. In the present study, median age of patients who had conservative surgery was 29 years. In many other studies, it was said that conservative surgery in patients with BOT could be acceptable (22-24). In our study, only one patient who had USO in the initial surgery (conservative group) had recurrence. She had recurrence twice. In both recurrences, she had cystectomy, and she has been free of disease since then. In our series, desire for pregnancy was reported in the files of 14 patients. We did not calculate the pregnancy rate, since we did not know whether the remaining 61 patients had desire for pregnancy.

Our study is a retrospective study. Furthermore, it does not compare survival and recurrence rates, and not calculate the pregnancy rates. One should consider these limitations in assessing our findings.

In conclusion, as prognosis of patients with mBOT is excellent, fertility-sparing surgery should be considered in the reproductive age group. Furthermore, the necessity of staging surgery is controversial, since mBOT is a clinically benign tumor.

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Evaluation of vaginal agenesis treated with the modified McIndoe technique: A retrospective study

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Abstract

Objective: Retrospective analysis of cases that have undergone neovagina operation because of congenital vaginal agenesis was objected.

Material and Methods: Seven cases applying with the complaints of primary amenorrhea or inability to have sexual intercourse were in the study. The cases were diagnosed with congenital vaginal agenesis and operated at Mustafa Kemal University Training and Research Hospital between 2011 and 2014. Vaginoplasty by the modified McIndoe method was performed in all cases. The main complaint, chromosomal analysis, duration of operation, preoperative and postoperative vaginal length, complications, postoperative treatment, and satisfaction from the sexual intercourse were all evaluated.

Results: Average age of our patients was 28.14±8.61 (19-39) years. One patient was 46XX-45X0 mosaic Turner syndrome), 1 patient was 46XY (testicular feminization), and other 5 patients were 46XX. The average duration of operation was 2.7±0.56 (2–3.5 h). Postoperative infection was observed in 1 patient. In this infected patient, graft failure occurred and debridement was performed in reoperation. No early complications were seen in the others. Preoperative and postoperative average vaginal lengths were 1.85±0.62 (1–3 cm) and 8.71±1.11 (7–10 cm), respectively. Dyspareunia occurred in 2 cases that were not able to use dilatator regularly: 1 because of cancelation of marriage and the other because of postoperative infection; regular sexual life was achieved in remaining 5 (71%) cases.

Conclusion: Although there is no consensus about the ideal method of making a functioning vagina among different specialties. The modified McIndoe technique is the most applied method by gynecologists and simple, minimally invasive and with low morbidity.

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Keywords: Vaginal agenesis, modified McIndoe technique, vaginoplasty

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Introduction

Vaginal agenesis is a rare malformation of the Mullerian duct. Since lower vagina is usually normal and middle and upper 2/3 of vagina are absent, it can also be thought as aplasia or dysplasia of the Mullerian duct. Some researchers defended that absence of vagina is not a real syndrome but is a part of symptom complex. When absence of the uterus accompanies with vaginal agenesis, this is called as Mayer-Rokitansky-Küster-Hauser Syndrome (MRKH) (1). Also, vaginal agenesis may be encountered as a part of androgen insensitivity syndrome (testicular feminization), Turner syndrome, Morris syndrome, or combined congenital defects (2).

Incidence of vaginal agenesis changes between 1/4000 and 1/10000. Diagnosis is often made at adolescence due to amenorrhea or coital problems (3, 4). Abnormalities of sexual organs during this period may cause personality problems and poor body image. Also, inability to get pregnant during adulthood may cause low self-confidence (5, 6).

There are several nonsurgical and surgical methods for treatment of vaginal agenesis. The purpose of the treatment is to create an adequate passageway for penetration and to facilitate satisfactory sexual intercourse. The McIndoe (Abbe-McIndoe-Reed) technique is the most frequently mentioned procedure in literature (7-9).

In this study, we aimed to present the retrospective results of the modified McIndoe technique that we used in 7 patients who admitted with complaint of primary amenorrhea or inability to have sexual intercourse.

Material and Methods

The diagnosis of congenital vaginal agenesis was made in 10 cases who were admitted with the complaints of primary amenorrhea and/or inability to have sexual intercourse to Mustafa Kemal University Training and Research Hospital between 2011 and 2014. Vaginal reconstruction was performed to all. Seven cases whom data was reached of were enrolled in the study. The main complaint, chromosomal analysis, duration of operation, preoperative and postop-

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erative vaginal length, complications, postoperative treatment, and satisfaction from the sexual intercourse were all evaluated. Cases were followed up 1-3.5 years postoperatively. All cases underwent clinical examination, pelvic ultrasonography, vaginometry, karyotyping, and MR during preoperative evaluation. Written informed consent was taken from all participants before the operation. Operation was performed in lithotomy position and under general anesthesia. The modified McIndoe technique was applied to all cases. Between bladder and rectum, , transverse incision was made on the blind vagina and a cavity up to peritoneal level (Douglas pouch) was formed by dissecting about 8-10 cm in depth (Figure 1).

At this stage, plastic surgery team was invited to operation. Full thickness skin grafts were obtained bilaterally. Then the skin graft was placed on a mould (Silimed, ERA Medical; Rio de Janeiro, Brazil) and sutured to vaginal apex in such a way that dermal side touches to the mould. Subsequently, the mould (covered with skin graft) was placed and sutured into vaginal cavity (Figure 2).

The patients were administered to the absolute bed rest, and special diet was given for 7 days. After prosthesis was taken out, povidone iodine and saline dressing was applied. Patients were discharged by warning for never removing the moulds (dilatator) given to them during 3 months except the need of toilet and bath in order to prevent contraction of vagina. Patients were allowed to have sexual intercourse only after 3 months. All patients were controlled at every week during the first month and consequently on every month. Patients were informed that they should always replace the mould into the vagina after washing with soap and applying estrogenous cream when they remove it. Afterwards, they were recommended to use the dilatator just at nights for 3 months. Six months after the surgery, if the patient was married, regular sexual intercourse was recommended. If she was unmarried, dilatator use for 1 hour 3 times a week was advised.

The data was analyzed using SPSS version 21.0 (SPSS Inc.; Chicago, IL, USA). Continuous variables were expressed as mean±standard deviation. Local Ethics Committee approved the study.

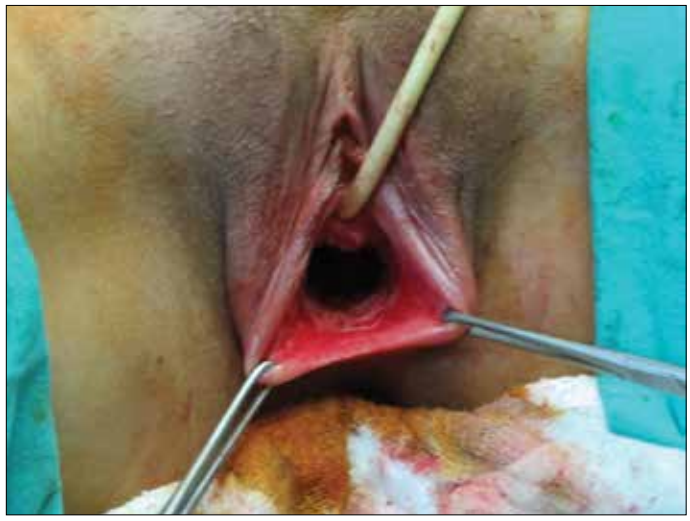

Figure 1. Transverse incision between the rectum and bladder and a cavity up to peritoneal level

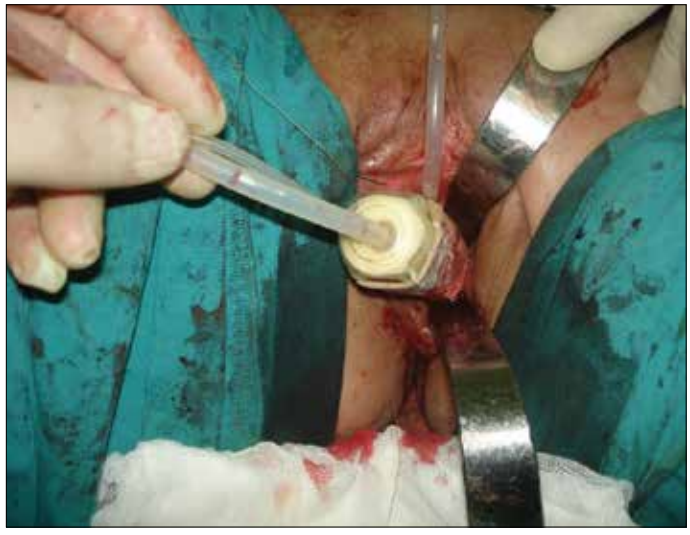

Figure 2. The mould (covered with the skin graft) is being placed into the vagina

Results

Mean age of our cases was 28.14±8.61 (19-39) years. One of cases was engaged, and the others were married. Of the cases, 5 were admitted with complaint of primary amenorrhea, 1 was admitted with inability to have sexual intercourse and 1 with desire of child. On examination, external genitalia were normal in appearance in all of the cases and blind vagina was determined in all of them. In 2 cases, uterus and ovaries were not found. Mosaic Turner Syndrome and testicular feminization were diagnosed in them. Of the remaining cases, uterus and ovaries were seen in 3, and ovaries were seen but uterus in 2. Magnetic resonance images were normal in all cases except in 1 who was diagnosed as mosaic Turner syndrome. In this case, an ectopic kidney was found in the right pelvis. According to chromosomal analysis, 1 patient was 46XX-45X0 (mosaic Turner Syndrome), 1 patient was 46XY (testicular feminization), and the other 5 patients were 46XX. Patients were operated by using a full-thickness skin graft by the modified McIndoe technique. Mean time of operation was 2.7±0.56 h (2-3.5 h). Postoperative infection developed in 1 case, and graft failure also occurred in this patient. Therefore, she could not use dilatator. No early complications were seen in other cases, and all of them used dilatator regularly. Preoperative and postoperative findings of cases were summarized in Table 1.

Case 2 who was infected was reoperated and debridement was done. Length of vagina was measured as 6 cm in later controls. She described rare and painful sexual intercourse and she was advised for continuing to use dilatator. The patients were followed postoperatively for an average of 28 months (10-41 month). Vaginal lengths were 1.85±0.62 (1-3 cm) preoperatively and 8.71±1.11 (7-10 cm) postoperatively. Skin grafts were obtained with success in all patients and pseudomembranous metaplasia developed in all cases except that who was infected. Again except that infected case, regular dilatator use was

Table 1. Preoperative and postoperative findings of cases

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Age	36	19	19	39	21	28	35
Complaint	Primary amenorrhea Desire of child	Primary ameno-rrhea	Primary ameno-rrhea	Primary ameno-rrhea	Primary ameno-rrhea	Primary ameno-rrhea	Unability to have sexual intercourse
Chromosomal analysis	46XX	46XX	46XX	46XX-45XO	46XX	46XY	46XX
Gynecological examination	Blind vagina Normal external genitalia	Blind vagina Normal external genitalia	Blind vagina Normal external genitalia	Blind vagina Normal external genitalia	Blind vagina Normal external genitalia	Blind vagina Normal external genitalia	Blind vagina Normal external genitalia
Ultrasound	No uterus, ovaries were viewed	2 cm uterus, ovaries were viewed	No uterus, ovaries were viewed	Uterus and ovaries were not viewed	Hematometra in uterus, ovaries were viewed	Uterus and ovaries were not viewed	Rudimentary uterus, ovaries were viewed
Breast developement	Tanner stage 4	Tanner stage 4-5	Tanner stage 3	Tanner stage 3	Tanner stage 3	Tanner stage 4-5	Tanner stage 4
Duration of operation (hr)	3	2	3	3	3.5	2	2.5
Preop vaginal length (cm)	2	1	1.5	3	1.5	2	2
Length of Neovagina (cm)	8	10	9	7	8	9	10
Complication	None	Infection, tissue rejection	None	None	None	None	None
Dilatator usage	Yes	No, using currently	Yes	Yes	Yes	Yes	Yes
Satisfaction from sexual intercourse	Yes	Rare, shrinkage, disparaneua	Yes	Yes	Bachelor	Yes	Yes
Postop time past (month)	36	13	10	35	37	41	24

achieved by all patients. One case was engaded and she left the fiance in postoperative period. She was lost at the end of 1-year-time follow-up and we do not have any information about her current sexual life. The infected case is going on using dilatator and she describes disparaneua. Apart from these 2 cases, remaining 5 cases (71%) have satisfactory sexual life currently.

Discussion

Patients having vaginal agenesis are usually admitted with the complaint of primary amenorrhea at 14-16 years of age. Medications to induce menstruation may have already been given to those cases. Despite in the past making a new vagina have been postponed until just before marriage,, today, performing the operation at 17-20 years of age when patient reaches emotional maturity and intellectual reliability is the accepted approach (1).

Surgical and non-surgical methods have been used to correct vaginal agenesis. The aims of treatment are to provide sufficient vaginal depth for penetration and to facilitate satisfactory sexual intercourse. There are surgical methods, such as the Vecchietti techique, Davidoff technique, McIndoe technique (Abbe-McIndoe-Reed), and intestinal vaginoplasty, and non-surgical methods that are based on continuous dilatation of vagina with a dilatator (7-9).

Despite several available methods to treat vaginal agenesis, the most suitable surgical technique depends on surgeon's experience, advantages and disadvantages of the technique, and also patient preference. Although there is no consensus on the best approach to vaginal agenesis, McIndoe vaginoplasty is the most frequently applied surgical technique (10). Advantages of the original McIndoe technique are feasibility, high success rates, and low morbidity. Possibility of partial or total obliteration is its disadvantage (7-9). We also preferred this technique because of its simplicity, low complication rate, and lack of requirement for abdominal approach.

This procedure consists of creation of a vaginal canal by dissecting the potential neovaginal space that is subsequently covered by a full-thickness skin graft. Several modifications of this technique, especially regarding the material adopted for the canal lining, have been proposed. These include the use of the peritoneum, amnion, allogenic epidermal sheets, Interceed absorbable adhesion barrier, and autologous buccal mucosa (11, 12).

Moreover, in vitro autologous vaginal cell cultures obtained from biopsies from the vaginal vestibule were used for the epithelization of the neovaginal walls by Panici for the first time in 2007. Autologous tissue does not carry the risk of infection or allogenic tissue rejection. Since there is no need to "mesh" the material, epithelization is rapid and no visible scars remain. Additionally, this physiological vaginal tissue plays an important role in the achievement of a normal sexual life. Disadvantages include that the procedure can only be performed in centers that have dedicated tissue culture laboratories (12).

The etiology of MRKHS is still poorly understood and is considered as multifactorial disorder. Several studies have investi-

gated mutations in developmental genes, the analysis of gene expression profiling on cultured cells harvested from vaginal vestibulum biopsy became a feasible approach, focused some genes (13). In this study, we only used chromosomal analysis.

In the Vecchietti technique, traction rather than dilatation is needed to create a new vagina. Classical Vecchietti operation was an abdominal surgery that was done abdominally through a Pfannenstiel incision (14); however, this procedure was modified laparoscopically since it has high complication rates (15). This traction process may be too painful, and patients may not tolerate it easily (14, 16, 17). The Vecchietti technique was used by Fedele et al. (18) and Brucker et al. (19) in 110 and 101 patents, respectively. The achieved vaginal length was well within limits, and nearly 60% of patients engaged in sexual intercourse without dyspareunia.

Another surgical approach is vaginoplasty by using intestinal grafts. Advantages of this procedure over others are; not requiring mould and dilatation for long time, mucus serving as a natural lubricant, sufficient vaginal length, possibility of early coitus and well long-term results (20). On the other hand, necessity for laparotomy, serious risk of infection, intestinal stenosis, and fistula formation are the disadvantages. Additionally, vagina made from intestinal graft is less sensitive and prone to produce more mucous; patients may have to carry peds continuously (21).

In the study by Bastu et al. (2), no dyspareunia was seen in patients who had vaginal length 8 cm or more postoperatively. However, success of surgery does not correlate only with postoperative vaginal length. For a successful operation, a functional and sensitive, as well as sufficiently long, vagina should be created. For this reason, adequate metaplasia development in graft is important for elasticity and sensitivity in newly formed vagina.

Sexual satisfaction when the McIndoe technique was used has been reported as 80-90% in the literature (22, 23). In our study, considering dyspareunia in 2 patients who were not able to use dilatator regularly, 1 because of cancelation of marriage and the other because of postoperative infection, sexual satisfaction rate was 71% (5 in 7). Our study is limited because of its' low number of cases; according to our findings, regular dilatator use in patients who does not have a regular sexual relationship is important in order to reach painless and satisfactory sexual intercourse for long period.

Limitations of our study are low number of cases and short duration of post-operative follow-up since some cases were lost to follow-up. Further studies with higher number of subjects and longer period of postoperative follow-up would provide more reliable results about long-term effects of the surgery.

In conclusion; currently there is no consensus on ideal technique among different specialties to create a functional vagina. The McIndoe technique used most frequently by gynecologists has advantages of simplicity, minimal invasiveness, and low morbidity. However, regular usage of the dilatator is the cornerstone of this procedure. Cases should be managed individually, and treatment should be planned and applied by a multidisciplinary team.

Ethics Committee Approval: *: Ethics committee approval was received for this study from the Local Ethics Committee.*

Informed Consent: *Written informed consent was obtained from patients who participated in this study.*

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Application of da Vinci® Robot in simple or radical hysterectomy: Tips and tricks

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Abstract

The first robotic simple hysterectomy was performed more than 10 years ago. These days, robotic-assisted hysterectomy is accepted as an alternative surgical approach and is applied both in benign and malignant surgical entities. The two important points that should be taken into account to optimize postoperative outcomes in the early period of a surgeon's training are how to achieve optimal oncological and functional results. Overcoming any technical challenge, as with any innovative surgical method, leads to an improved surgical operation timewise as well as for patients' safety. The standardization of the technique and recognition of critical anatomical landmarks are essential for optimal oncological and clinical outcomes on both simple and radical robotic-assisted hysterectomy. Based on our experience, our intention is to present user-friendly tips and tricks to optimize the application of a da Vinci® robot in simple or radical hysterectomies.

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Keywords: da Vinci®, robot-assisted hysterectomy, lymphadenectomy, robotics

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Introduction

Robotic simple hysterectomy was first performed by Diaz-Arrastia et al. (1) in 2002, while robotic radical hysterectomy was first performed by Sert et al. (2) in 2006. These days, robotic-assisted hysterectomy is widely accepted as an alternative surgical approach and is applied in both benign and malignant gynecological surgical entities (3, 4). Post-operative outcomes can be optimized by taking into consideration the main aims of a disease-free outcome combined with functional preservation. Overcoming any technical challenge leads to an improved surgical operation including patients' safety, surgeons' fatigue, cost, and operative time. Based on our experience, our aim is to present user-friendly tips and tricks to optimize the application of a da Vinci® (Intuitive Surgical Inc.; CA, USA) robot in simple or radical hysterectomies.

Preoperative management

The day prior to surgery, a clear liquid diet is offered, while mechanical bowel preparation is based on surgeons' preferences. Some surgeons offer their patients a bowel preparation such as magnesium citrate the afternoon before the procedure. Based on our experience, we believe that there is no need for any mechanical bowel preparation as this approach has been proved to be safe and effective to both robotic and laparoscopic procedures. All anti-inflammatory drugs and blood thinners, such as aspirin, clopidogrel, and warfarin, are withheld for at least 7 days before surgery, and patients with medical indications can instead have low molecular weight heparin. Preoperative assessment by an experienced anesthetist in robotic surgery is essential for challenging patients e.g., obese or elderly with comorbidities. General anesthesia is induced, and the patient is placed in the lithotomy position. We tend to position the patient prior to anesthesia induction to achieve the best possible uterine manipulation. Intraoperative antibiotics (e.g., co-amoxiclav or cephalosporin and metronidazole) are administered as a single dose based on the Centers for Disease Control and Prevention antibiotic guidelines.

Set-up

A patient was positioned in the modified lithotomy position with her legs on Allen stirrups (5). A Foley urinary catheter was inserted, and we tended to use bilateral ureteric stents, in our radical hysterectomies in particular, that are immediately removed postoperatively. We applied methylene blue on hours 12, 3, 6, and 9 in the vaginal fornices. This can help to intraoperatively recognize the edge of the cervix to the vagina; sometimes, using this, we avoid the use of any uterine manipulators and complete the entire operation with a swab on a stick in the vagina. Otherwise, two Vicryl® (Ethicon Inc.; USA) sutures are applied on the cervix (hours 12 and 6) to make the traction of the uterus at the end of the operation through the vagina easier. Different types of uterine manipulators can be used such as ZUMI® (Cooper Surgicals; CT, USA), BARD® (BARD Inc.; Billerica, MA, USA), HUMI® (UNIMAR; Wilton, CT,

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USA), Clermont-Ferrand® (Karl Storz; Germany), HOHL® (Karl Storz; Germany), Endopath® (Ethicon Endo-Surgery Inc.; OH, USA), Hourcabie (by Jacques Alain Hourcabie) (5, 6). We prefer the use of either the Sparkman followed by a McCartney tube at the time of colpotomy or V-Care® (ConMed EndoSurgery; Utica, NY, USA) uterine manipulator with the use of an internally inflated balloon to preserve the pneumoperitoneum. As the Trendelenburg position is also intraoperatively fundamental, to have a clear view in the surgical field, we used a gel pad placed under the patient with the aim of preventing her from sliding in the Trendelenburg position. After trocar insertions, we checked the Trendelenburg position with an inclinometer, which can be found as a smartphone application to achieve an angle of 25–30°. However, enough Trendelenburg is achieved when the bowels can remain pushed cephalad of the promontory to have access to the field as well as to avoid bowel injury. However, this is sometimes difficult in obese patients with central adiposity or in patients with other comorbidities that do not allow a deep Trendelenburg position.

Pneumoperitoneum and trocar placement

Pneumoperitoneum (up to 15–20 mmHg) is achieved either with a Veress needle in the middle of the umbilicus through Palmer's point or with the open Hasson technique. All ports were placed under direct vision. We placed the 12-mm primary trocar for the camera using Visiport™ (Covidien; USA) in the umbilicus or above it in a way that the distance of the uterine fundus from the umbilical port would be at least 10 cm. The correct placement of ports is crucial for avoiding robotic arm collision. Surgeons should avoid tunneling in the abdominal fat during trocar insertion. The operative trocars should be bilaterally positioned in an "M" or arch shape. More specifically, the robotic arms on the right were placed at least 8–10 cm apart, with the lower port slightly above the anterior superior iliac spine and the upper port triangulated between the umbilical port and lower port. The left-sided port was then placed parallel to the lower right-sided port. A 12-mm assistant port was then placed on the cephalad and to the left of the umbilical trocar. We prefer side docking, and we used bipolar diathermy at 40 in the left main port, scissors in the right main port with monopolar diathermy at 40 cut and coagulation, and ProGrasp™ (Intuitive Surgical Inc; CA, USA), Cadiere™ (Intuitive Surgical Inc; CA, USA) or Maryland™ (Intuitive Surgical Inc; CA, USA) forceps at the third arm. Additionally, 12 mmHg intra-abdominal pressure was intraoperatively used because of the deep Trendelenburg position, which could be decreased down to 8 mmHg if requested by the anesthetist for the patient's safety.

Technique of simple hysterectomy

Round ligaments are coagulated with bipolar diathermy and incised with monopolar scissors, and broad ligaments are opened. When we planned to perform pelvic lymph node dissection, we laterally incised the round ligaments. Ureters were bilaterally identified, and infundibulopelvic (IP) pedicles were taken with bipolar and monopolar. Laparoscopic clips could be used for extra hemostasis. We prefer to use hemalocks on IP pedicles. The bladder peritoneum was reflected. Uterine vessels were skeletonized and were then taken with bipolar and monopolar. Methylene blue spots were identified on hours 12, 3, 6, and 9. At the moment we used a Sparkman manipulator for uterine manipulation, we changed it to a McCartney tube™ (LiNA Medical ApS; United Kingdom). The vagina was entered either anteriorly or posteriorly between uterosacral ligaments but was always on top of the manipulator's (V-Care®) cervical cap or on top of the McCartney tube, and the dissection was circumferentially continued using monopolar scissors and bipolar diathermy. A uterine specimen was extracted through the vagina by pulling cervical sutures. A glove with a 9×9-cm swab was inserted in the vagina to maintain the pneumoperitoneum to minimize the cost in V-Care manipulator use; otherwise, the McCartney tube can be used for the same reason, but the cost would be higher. The vaginal vault was closed with a continuous v-loc barbed suture from the right to the left (7). No knot was necessary at the end, and it should be mentioned that it locked after the second stich. V-loc® (Covidien, USA) suture facilitates the easy suturing of the vault.

Technique of radical hysterectomy

The uterus is preferred to be instrumented with the V-Care® manipulator or a swab on a stick to avoid tumor contamination. Two Vicryl® sutures were used on the cervix (hours 12 and 6) to retract the specimen at the end of the operation. Round ligaments were coagulated with bipolar diathermy and incised with monopolar scissors, and broad ligaments opened. Paravesical and pararectal spaces were developed to identify cardinals, parametrial web, and lateral parametrium. The ureters were bilaterally identified and followed to the crossing with the uterine arteries. Bilateral ureteric stenting can optimize this step. We started with pelvic lymph node dissection bilaterally (the technique is going to be described in the following paragraph). Pelvic lymph nodes are sent for frozen section, and if proven positive, then para-aortic lymph node dissection is suggested higher to the level of the inferior mesenteric artery or up to renal vessels to clarify the extent of radiation field. The completion of radical hysterectomy is following if the lymph nodes are negative. More specifically, parametrial division was performed at the origin of the uterine vessels from the internal iliac artery and vein. The rectovaginal space was caudally developed caudally to the upper vaginal third; the ureters were separated from the peritoneum, and uterosacral ligaments were dissected depending on the type of radical hysterectomy. Bladder reflection followed to the upper vaginal third, and the ureters were followed to the entrance in the parametrial tunnel. A space was created above the ureter with monopolar, and the ventral part of the vesicouterine ligament was transected, and the ureter was unroofed. We tend to keep infundibulopelvic ligaments until the completion of radical hysterectomy to achieve better traction. At the end of the operation, they are taken with bipolar and monopolar, and hemalocks were used. If the adnexae were preserved, tubo-ovarian pedicles were divided along the lateral wall of the uterus, including the broad ligament. The vagina was entered anteriorly or posteriorly between the utero-

sacral ligaments on top of the manipulator, and dissection was circumferentially continued with monopolar and bipolar. The next steps are similar to simple hysterectomy.

Technique of nerve-sparing radical hysterectomy

Nerve-sparing radical hysterectomy can improve the quality of life by reducing urinary, rectal, and sexual dysfunction. The retroperitoneal space was opened to identify the ureter. The ureter was laterally removed, and the hypogastric nerve was identified posteriorly and medially to the ureter. Keeping the ureter and hypogastric nerve always under vision, the peritoneum was cut toward the pouch of Douglas. The rectum was dissected from the posterior part of the vaginal wall till the elevator muscle of the anus was reached. More specifically, the pararectal space was opened up to the level of the uterine veins with a Prograsp™ or Maryland™ forceps, preserving the branches of the pelvic splachnic nerves. The cardinal and the uterosacral ligaments were identified and dissected posteriorly to the paravesical area, and the hypogastric nerves that lie laterally to the uterosacral ligaments were spared. The branches of pelvic splachnic nerves can be found below the dissected uterine veins, which direct in the direction of the inferior hypogastric plexus. It is important to preserve the intact bladder branch of the inferior hypogastric plexus. With a Maryland™ forceps, the ureteric tunnel was dissected. The ureteric tunnel was unroofed, and the veins were identified, ligated and cut, while the ureter was laterally pushed. With this surgical step, the ureteric and bladder branches of the inferior hypogastric plexus were laterally moved. The bladder branch passing under the inferior vesical vein can be visible and preserved. The remaining operation is continued as described above.

Technique of pelvic lymph node dissection

The pelvic side wall was entered overlying the external iliac vessels. We suggest good traction of the round ligament remnant using the third arm. A thorough dissection of lymph nodes from the common iliac vessels down to the external iliac vessels follows. The dissection is suggested to start from the external iliac artery down to the deep iliac circumflex vein and to return toward the internal iliac artery with a U turn. The dissection was performed after recognizing the genitofemoral nerve laterally, the ureter medially, the deep iliac circumflex vein inferiorly, and the inferior mesenteric artery superiorly. The dissection was completed after the identification of the obturator nerve. Following the U-turn technique, all pelvic lymph nodes could be dissected as a single specimen. We tend to extract the pelvic lymph nodes through the vagina in laparoscopic bags. However, the assistant port can be used for the same reason.

Postoperative care

Our patients followed enhanced recovery protocol and are allowed free fluids the day of the operation and have breakfast the next morning (8). Early mobilization is encouraged based on our enhanced recovery protocols. For simple hysterectomies, we took the catheter out on the first postoperative day, usually at 6 am, and a scan was performed to check the bladder residual. Majority of our patients went home in the first 24 h postoperatively. In cases of radical hysterectomy, patients went home with a bladder catheter, which remains for 5–7 days and is then removed in an outpatient clinic.

Comment

The main advantages of the robotic approach are the wrist-like motion of the robotic arms, allowing difficult movements deep in the pelvis, a three-dimensional view, lower blood loss (even <50 mL), fewer wound complications, fewer urinary tract injuries, minimal rates of conversion to open, a reduced length of hospital stay (approximately 24 h), and a faster return to normal activities (5, 9). Surgeons' fatigue is minimized as he/she operates in the sitting position. The da Vinci® robot also decreases the learning curve for intracorporeal suturing. The main disadvantages include the high cost and the necessary learning curve, although the operative time continues to decrease with increasing experience (5, 10, 11). The overall costs of robotics are higher than those of the other approaches, depending on the instrumentation required. However, the standardization of the technique and an experienced team (including surgeon, assistant, and scrub nurse) can minimize the operative time and cost, while the increased efficiency provided by robotics to less-experienced surgeons would also overcome the increased equipment costs (11). When a comparison is performed with single-port laparoscopic hysterectomy and standard laparoscopic hysterectomy, we can see that the robotic-assisted technique, apart from offering obvious advantage to surgeons, such as comfort, can also sustain the abdominal wall, reducing the need of the pneumoperitoneum, in obese patients in particular. A decreased pneumoperitoneum is correlated with a decreased risk for venous gas embolism, decreased venous return to the heart, and cardiovascular collapse. Nevertheless, robotic and laparoscopic hysterectomy seems to present similar intraoperative and short-term postoperative outcomes (12). Moreover, the robotic single-port hysterectomy could also be a feasible and safe procedure, and ergonomic limitations are gradually corrected by the development of new instruments (13). However, more studies are necessary to assess the possible benefits of such an approach, such as better cosmetic results and use in obese patients or in patients with large uteri (14, 15). A recent SGO survey showed that there is a significant increase in the overall use and indications for robotic surgery (16). By suggesting our tips and tricks to literature, we aim to minimize the cost to achieve the effective utilization of instruments and operative time and to achieve a quicker learning curve.

Conclusion

Robotic-assisted hysterectomy is an equally effective alternative to the standard open or laparoscopic approach. The standardization of the technique and recognition of critical anatomical landmarks are essential for optimal oncological and clinical outcomes in both simple and radical robotic-assisted hysterectomy. Furthermore, the standardization of the technique using tips and tricks can, without doubt, shorten the learning curve of the operation in such a way that the surgeon can achieve cost-effective use of the equipment.

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Three-dimensional ultrasonography by means of HDlive rendering in the first trimester of pregnancy: A pictorial review

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Abstract

Our objective was to describe early embryo/fetus anatomy and abnormalities provided by three and four-dimensional (3D/4D) ultrasound using HDlive rendering technology in the first trimester of pregnancy. Normal and pathologic embryonic and fetal volume data set with postprocessing using HDlive rendering mode. Virtual fetoscopic imaging of the normal and pathologic fetus even at early stage of development with increasing maternal-fetal bonding process. HDlive represents a novel and valuable lightening system for 3D/4D ultrasound application that may aid the prenatal interpretation of early congenital malformations although limitations and cautions are still needed for inclusion in obstetric clinical practice. (J Turk Ger Gynecol Assoc 2016; 17: 110-9)

Keywords: First trimester of pregnancy, fetal malformations, three-dimensional ultrasound, HDlive rendering

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Introduction

Ultrasonography is the leading examination for the detection of fetal abnormalities during pregnancy, particularly in the second and third trimesters. Over the last 15 years, three- and four-dimensional (3D/4D) ultrasound has undergone dramatic technical improvements resulting in an enhanced diagnostic accuracy of ultrasonography in daily obstetrics practice (1). The technical advancement of 3D/4D ultrasound includes the improved evaluation of complex anatomical structures using the multiplanar approach (2) or volumetric measurements of fetal organ by means of virtual organ computer-aided analysis (3). In addition, anatomical details can be reformatted using the Omniview technique (4-6) and surface analysis of minor defects can be performed using the newly developed lightening technique called *High Definition* live (HDlive) (7, 8).

The estimated fetal weight can be enhanced by means of 3D ultrasound, and an angiographic study using Doppler ultrasound can be applied to the study of placental function, namely placental biopsy (9), to improve the antenatal diagnosis and to detect intrauterine growth restriction. Hence, the application of 3D/4D ultrasound has been proven to have contributed to an increased diagnostic accuracy when applied to the study of the central nervous system (neuroscan), cardiac anatomy, and orofacial malformations (10, 11). The introduction of the HDlive rendering offers a natural and realistic view of the fetus through the use of a new skin-like color (12) that makes the ultrasound images more discernible to both parents and clinicians than those obtained by conventional two-dimensional (2D) or 3D ultrasound. HDlive rendering can be applied during the entire pregnancy duration, although more natural and realistic views of the fetus are obtained when scanning in the first trimester. In this pictorial review, we aim to present our series of normal and pathologic embryo-fetus development by means of HDlive rendering.

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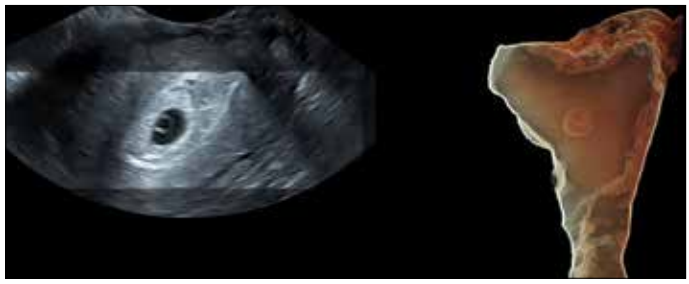

Figure 1. Topic gestation of 5 weeks using the HDlive rendering in the silhouette mode

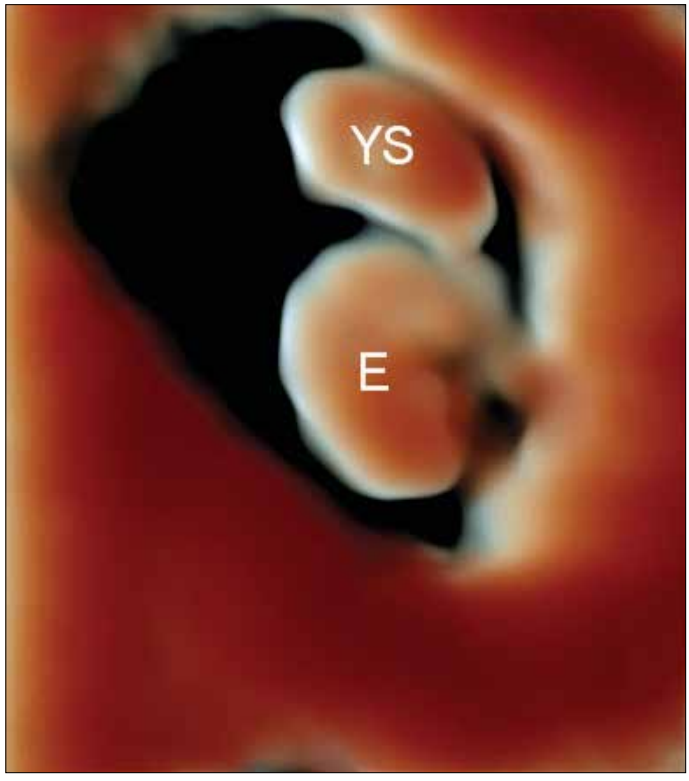

Figure 2. HDlive showing a single normal embryo and yolk sac at 6 weeks 6 days of gestation. YS: yolk sac; E: embryo

Methods

We conducted a retrospective cross-sectional study that involved four reference centers of prenatal diagnosis (Guastalla Civil Hospital; AUSL Reggio Emilia, Italy; "Carlo Poma" Hospital; Mantua, Italy; IRCCS Galliera Hospital, Genoa, Italy; and Monash Ultrasound for Women; Melbourne, Victoria, Australia). Normal and pathologic embryos/fetuses detected in the routine first trimester scan using 3D/4D ultrasound were reviewed from their database. This study was approved by the Local Ethic Committee from these institutions. Ultrasound examination was performed using a Voluson E8 apparatus (General Eletric, Healthcare; Zipf, Austria) (Guastalla Civil Hospital, AUSL Reggio Emilia, "Carlo Poma" Hospital; Mantua, Italy; and IRCCS Galliera Hospital; Genoa, Italy) and a Voluson E10 [Monash Ultrasound for Women; Melbourne, Victoria, Australia (GE; Milwaukee, WI; USA] equipped with transabdominal volumetric RAB4-8L and transvaginal RIC5-9W volumetric probes. All examinations were performed by four experienced sonographers in 3D/4D ultrasonography. Ultrasound volume was acquired during maternal apnea and fetal resting to reduce motion artifacts. Volume data set was acquired using the 3D static mode and was stored in an optical disk for subsequent post-processing offline analysis using the 4D view software version 13.0 (General Eletric, Heathcare; Zipf Austria).

The volume data set underwent post-processing analysis using HDlive rendering. All embryos/fetuses with malformations were followed until delivery and/or termination of pregnancy, and the prenatal diagnosis was confirmed on physical examination, diagnostic imaging, or autopsy.

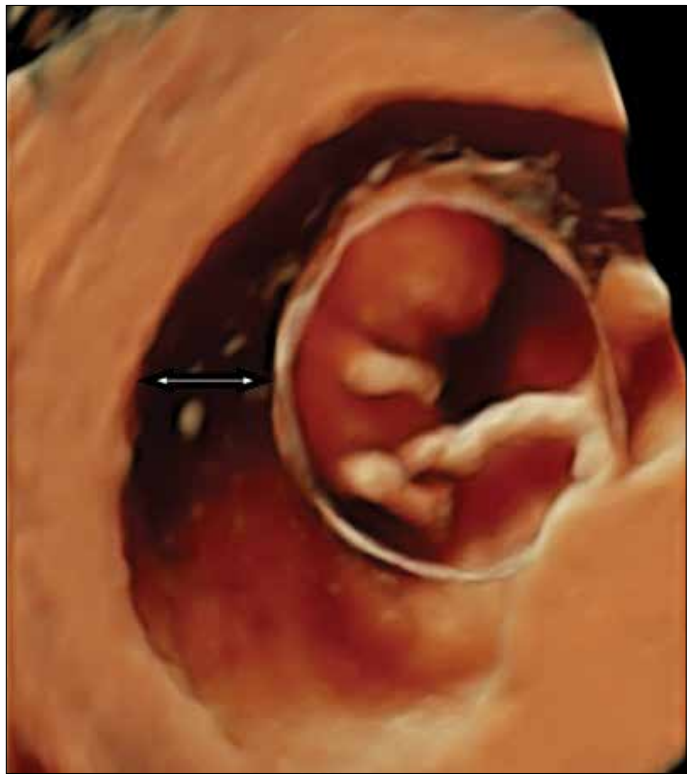

Figure 3. Fetus at 9 weeks 1 day of gestation. The extracelomic cavity is clearly seen (double arrow). HDlive has the potential to enhance visualization, aiding an invasive procedure such as transvaginal celocentesis performed for early embryo-fetal karyotyping

Results and Discussion

The HDlive mode is a recently developed technique for 3D/4D ultrasound that provides a natural and anatomically realistic appearance of the normal embryo/fetus and that can represent a diagnostic improvement in pathologic cases, particularly during the first trimester of pregnancy. HDlive has been proven to be useful not only for the examination of surface details due to its "*humanized*" rendering but also for imaging of the umbilical cord and amniotic membranes. The application of HDlive to normal embryo-fetal development from 6 to 12 weeks of gestation is depicted in Figures 1-4. The morphology of the yolk sac can be easily identified in normal and in pathological cases (13). Furthermore, at 12 weeks of gestation, HDlive offers

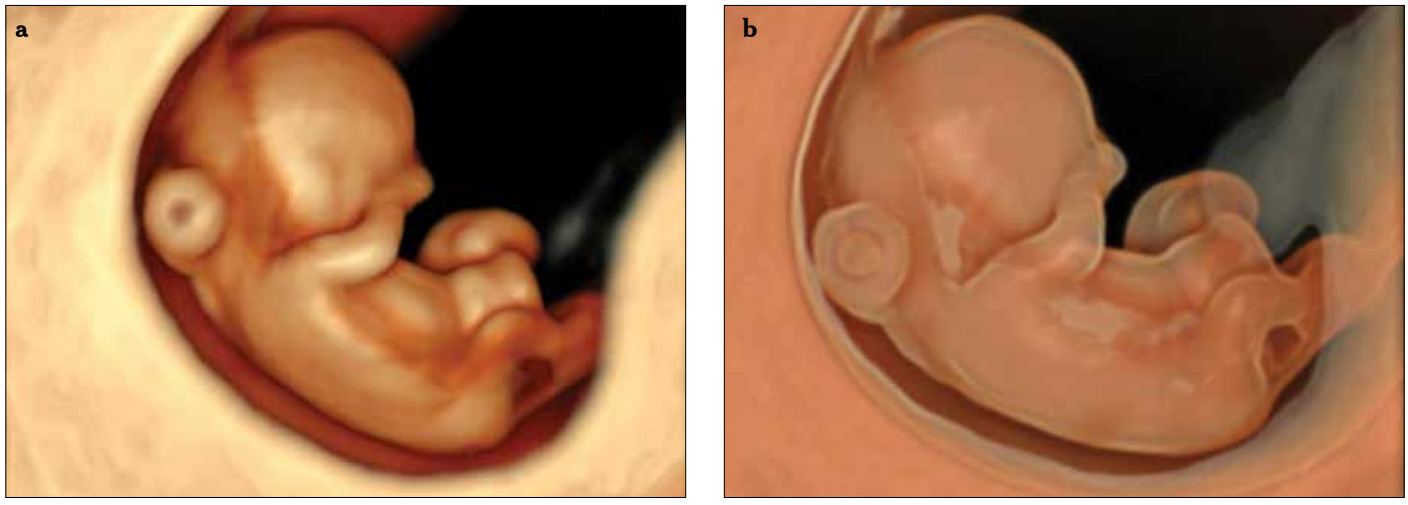

Figure 4. a, b. HDlive rendering mode of fetus at 10 weeks 2 days of gestation (a). Image obtained using the silhouette mode (b).

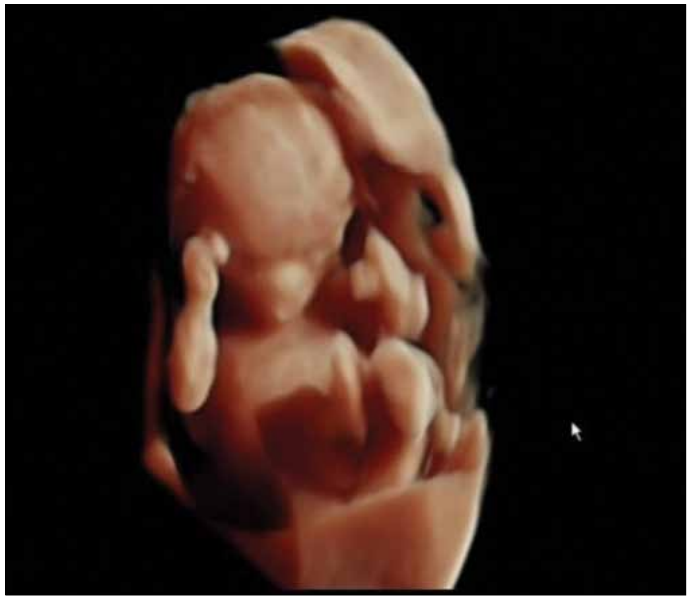

Figure 5. HDlive rendering mode of a normal fetus at 12 weeks of gestation

a clear-cut image of the superficial and small structures such as lips, eyelid, and ears or of extremities such as fingers in a manner that resembles a "*virtual fetoscopy*" (Figure 5). Bonilla-Musoles et al. (14) have demonstrated the role of HDlive when applied to fetuses at <16 weeks of gestation. Normal and abnormal placentas, similar to those observed in fetal hydrops, or placental shelf (and protruding into the amniotic cavity), subchorionic maternal lakes, or umbilical cord anomalies such as cord entanglement, cord cyst, and varix can be well identified by this novel lightening technique (15). Kanenishi et al. (16) have described a case of intra-amniotic umbilical vein varix with thrombosis at 35 weeks of gestation. The HDlive rendering clearly demonstrated a fragile massive thrombosis inside the varix. A histological examination performed after delivery proved the prenatal diagnosis. Recently, AboEllail et al. (17) described a case of circumvallate placenta at 16 weeks 5 days of

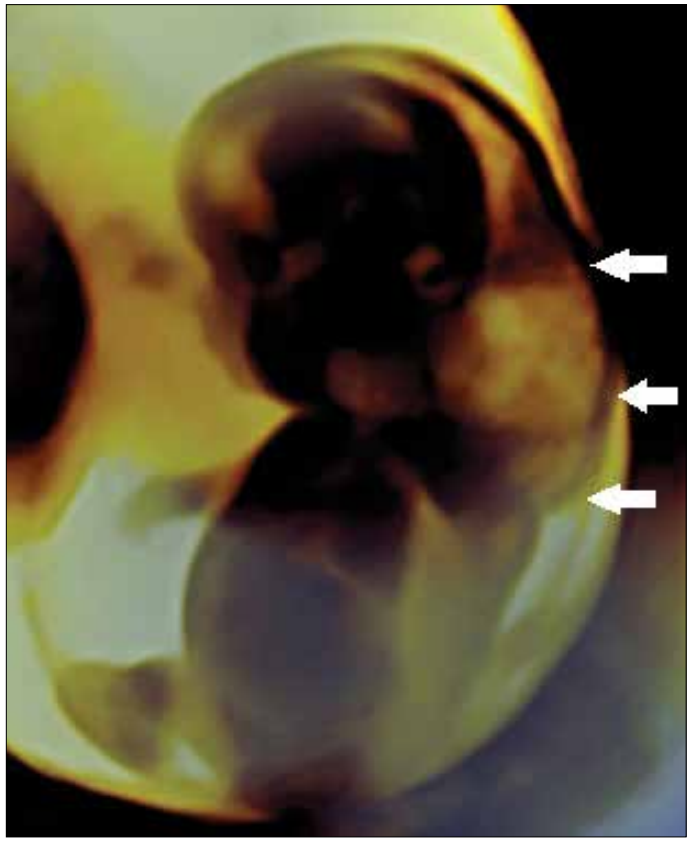

Figure 6. HDlive rendering mode of a fetus at 10 weeks 2 days of gestation showing a cystic hygroma (white arrows)

gestation using HDlive rendering in the silhouette mode. HDlive in the silhouette mode depicted the thickened, curved edges of the placenta and the central depressed region to which the umbilical cord was attached. The macroscopic examination of the placenta after delivery confirmed the prenatal diagnosis associated with a true knot of the umbilical cord. Furthermore, AboEllail et al. (18) have described the used of HDlive in a case of giant fetal hemangioma.

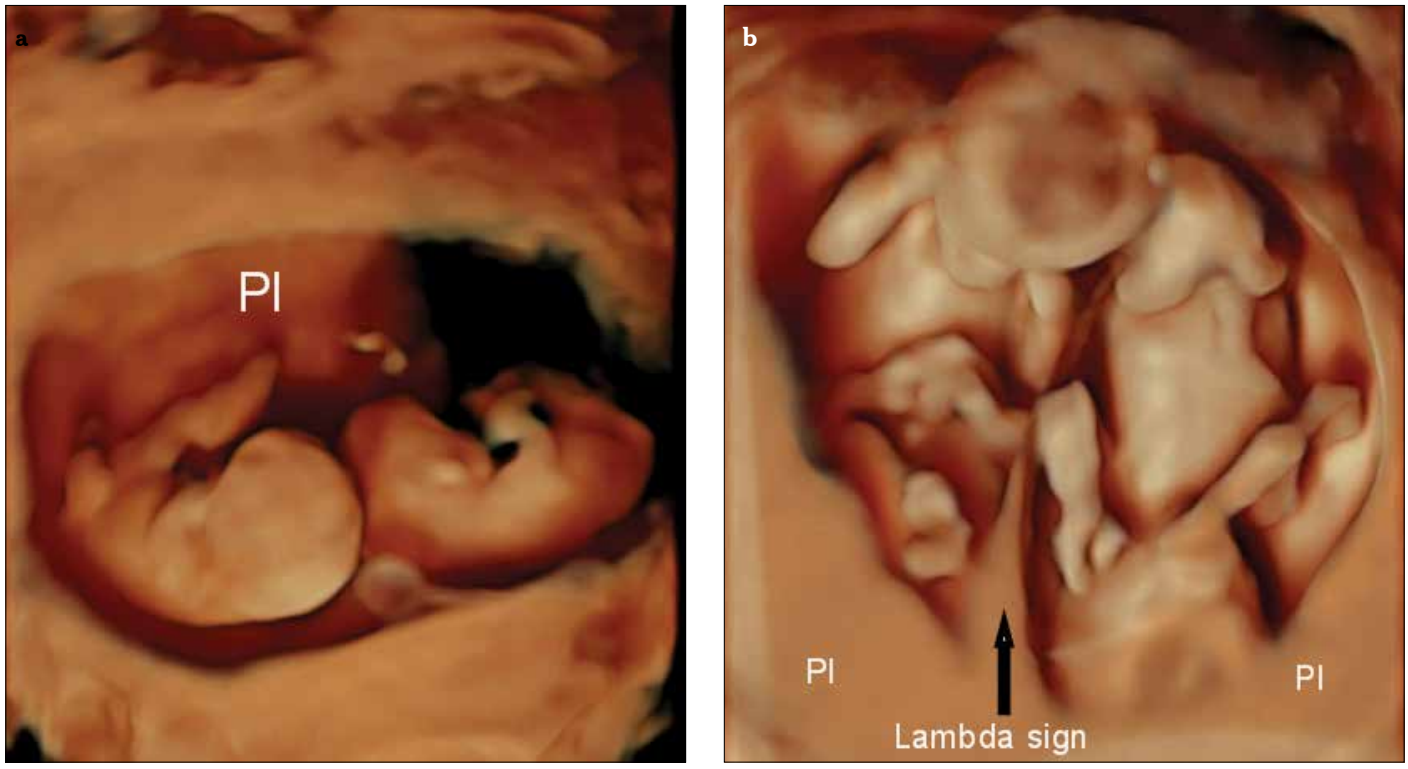

Figure 7. a, b. Monochorionic monoamniotic twins at 11 weeks 2 days of gestation (a) and dichorionic diamniotic twin pregnancy at 13 weeks of gestation. The "lambda sign" is clearly visible (b)

Two articles have reported on sonographic imaging of a normal embryo and fetus, and an abnormal fetus, placenta, and umbilical cord using 3D HDlive in the rendering mode (19, 20). HDlive may be superior to normal 2D ultrasound in the characterization of a chorionic bump, which is an irregular convex bulge passing from the choriodecidual surface into the first-trimester gestational sac. A chorionic bump may clinically represent a warning sign when detected in the first trimester scan as women with such an ultrasound marker have almost double the risk of miscarriages compared with controls (15, 21, 22). A cystic hygroma is highly defined using this lightening technique as can be seen in Figure 6. This finding has also been reported by Hata et al. (23, 24).

Pooh and Kurjak (10) have demonstrated that HDlive rendering easily allowed the image of ear position in the first trimester and also the documentation of feet/toe morphological abnormalities not only in the first trimester but also in various foot/toe appearances such as sandal gap, hypertonic contracture, foot edema, and rocker bottom feet in the second trimester.

In addition, HDlive can be used in multiple pregnancy with a clear definition of the "lambda sign" while evaluating chorionicity (Figure 7). Currently, only two cases of prenatal 3D ultrasound diagnosis of the twin reversed arterial perfusion (TRAP) sequence in the first trimester (at 11 weeks and 4 days and at 13 weeks) have been described (25-27). In these two cases, conventional 3D ultrasound assisted in confirming the diagnosis and evaluating the extent and severity of structural anomalies in the acardiac fetus. Notwithstanding, near-fetoscopic images of the TRAP sequence in the first trimester were obtained by employing the 3D HDlive rendering mode. In particular, 3D HDlive rendering images of the acardiac fetus seemed to be more readily discernible than those obtained by 2D and conventional 3D sonography because the 3D HDlive rendering provided a natural and anatomically realistic appearance of the acardiac fetus (25-27).

HDlive has been shown to improve our understanding of neurobehavioral development in twins and assist in the investigation of inter-twin temperament and relationship (28, 29). It can aid the prenatal diagnosis of orofacial malformations such as cleft lip as it enables enhanced anatomical details of the facial surface and structures even at a very early stage of embryo-fetal development (Figure 8). HDlive may be the diagnostic investigation and supplement conventional 2D/3D ultrasound in cases of acrania/exencephaly (30) or in case of minor or subtle fetal face abnormalities such as flattened nose and broad nasal bridge (31, 32) or be highly illustrative in case of severe midline defects (33, 34), particularly when surface rendering of the fetal face is applied after 28 weeks of gestation (34). Again, abdominal wall defects such as exomphalos and gastroschisis are well documented and illustrative (Figure 9, 10). The snapshot of digit aplasia, which is not easy to demonstrate, using conventional 2D/3D ultrasound, has been clearly defined (Figure 11). Nonetheless, the diagnostic enhancement of HDlive may allow the early detection of characteristic abnormal phenotypes such those observed in common trisomy and/or in syndromic fetuses (32) (Figure 12). HDlive allowed obtaining an extraordinary image quality in a case of sirenomelia, a midline defect of blastogenesis with an incidence of approximately 1 in 60,000 newborn infants. Sirenomelia results

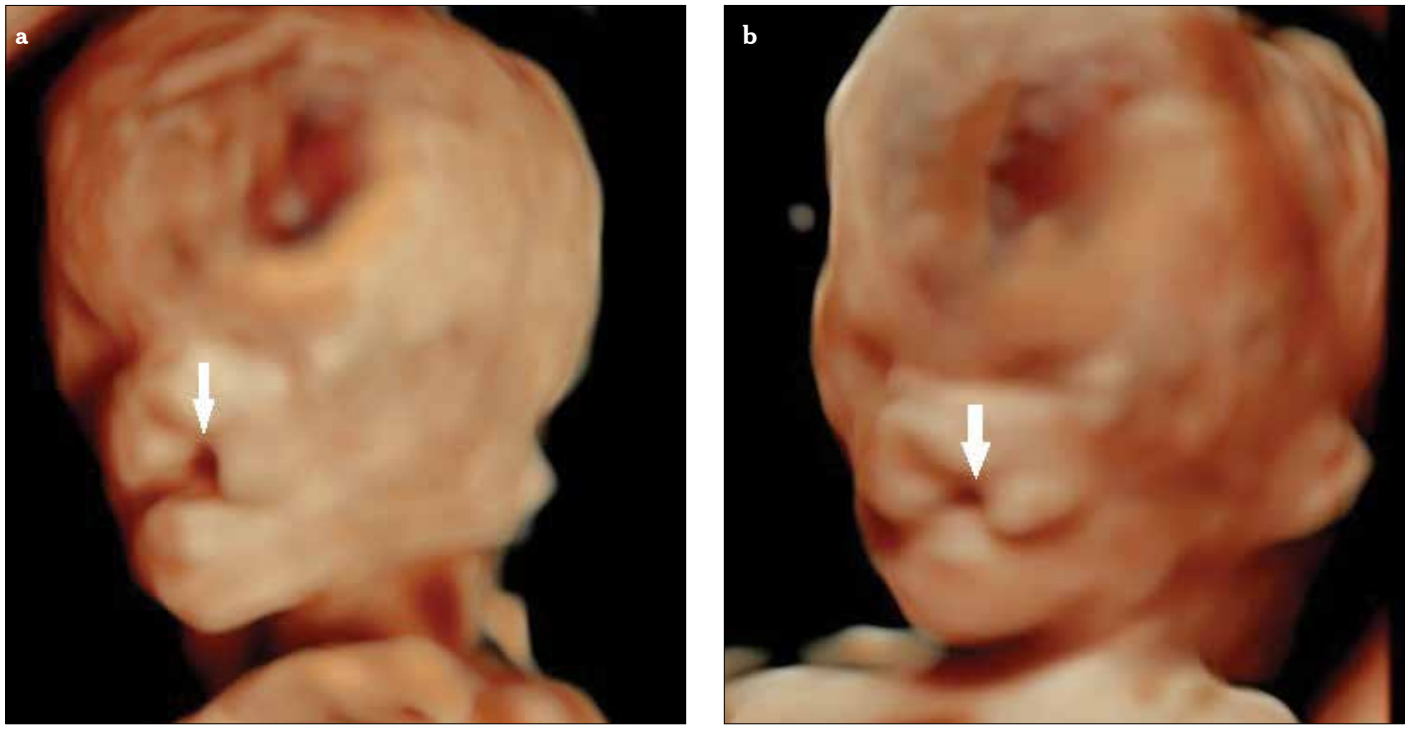

Figure 8. a, b. HDlive rendering mode in a fetus at 13 weeks of gestation presenting (a) median and (b) left cleft lip (white arrows)

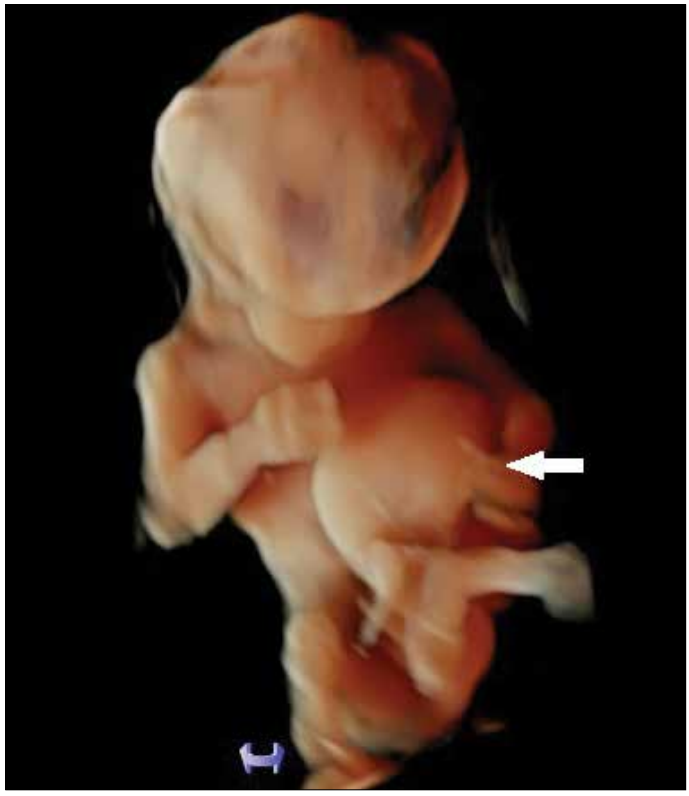

Figure 9. HDlive rendering mode in a fetus at 13 weeks of gestation with exomphalos (white arrow)

in a severe developmental defect of the posterior axis caudal blastema occurring in the primitive streak stage during week 3 of gestation (35). In this pictorial review, we report our case of a

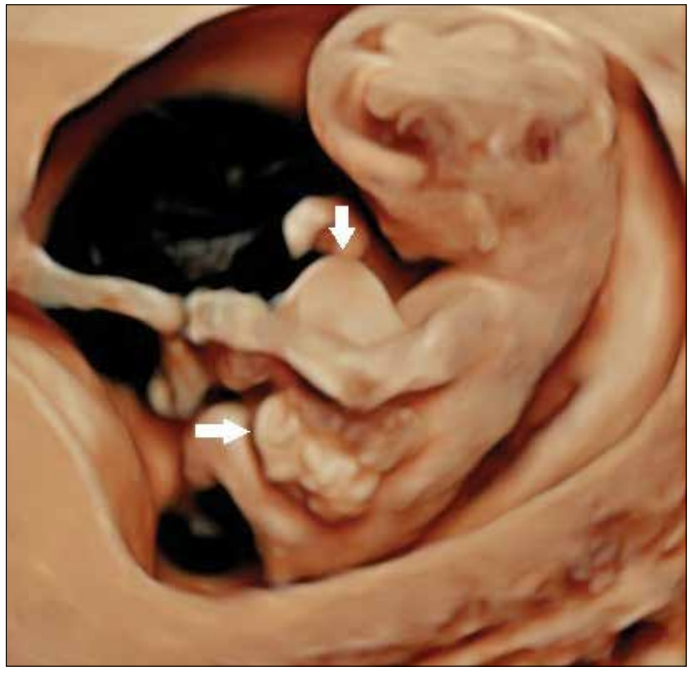

Figure 10. HDlive rendering mode in a fetus at 13 weeks of gestation with gastroschisis (white arrows)

fetus with sirenomelia showing single femur, fused lower limbs, and a single inverted foot with oligodactyly (sympus unipus) (36) that was diagnosed at 12 weeks 1 day of gestation in a fetus with a 46, XX karyotype. A single umbilical artery directly originating from the aorta and a caudal "tail" were the associated ultrasound findings (Figure 13). HDlive has been shown to perform well even in case of skeletal dysplasia in a fetus at 12 weeks 1 day of gesta-

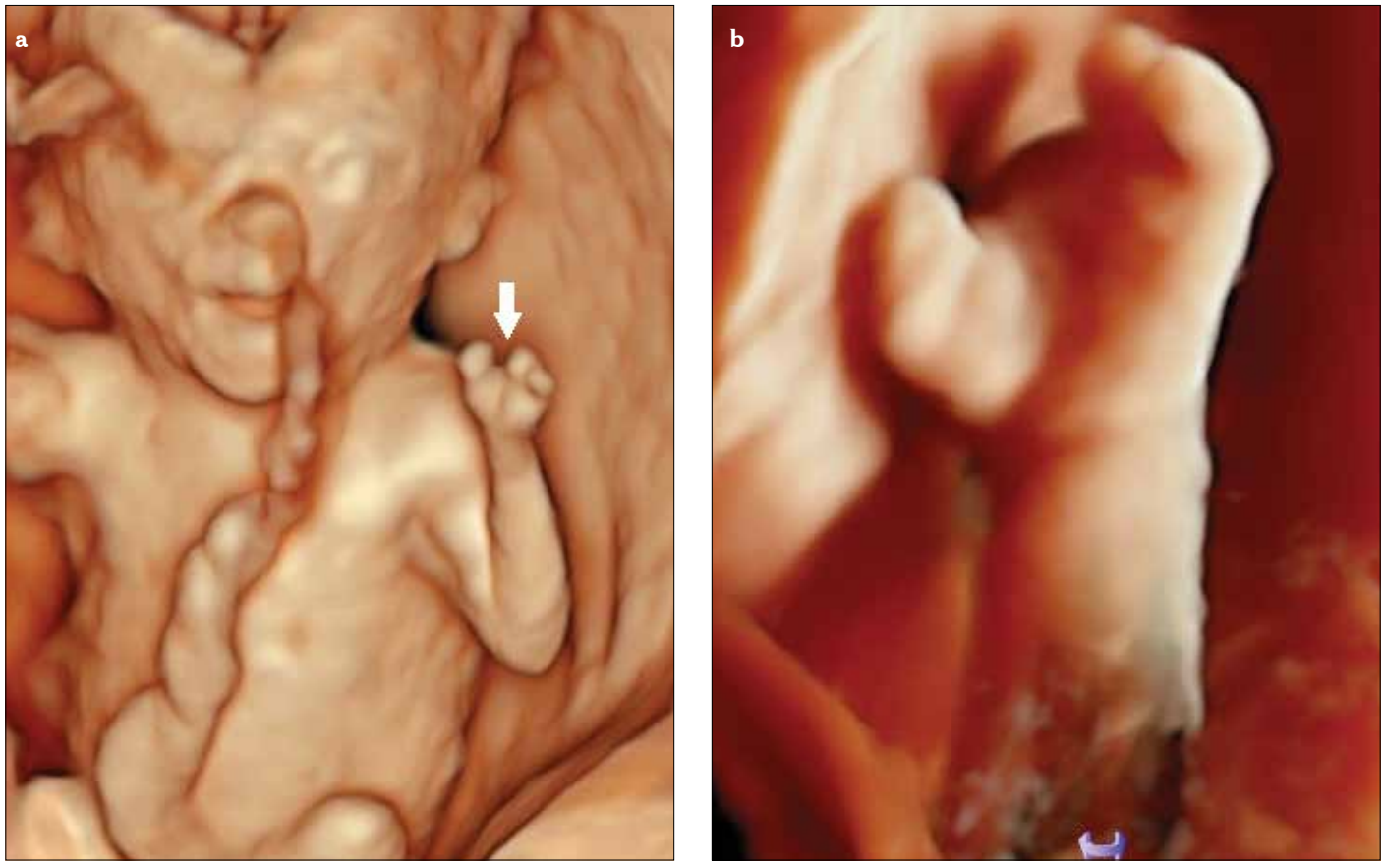

Figure 11. a, b. HDlive rendering in a fetus at 12 weeks 5 days of gestation with unilateral absence of the second and third fingers of the left hand (a) (white arrow). Detail of the left hand (b)

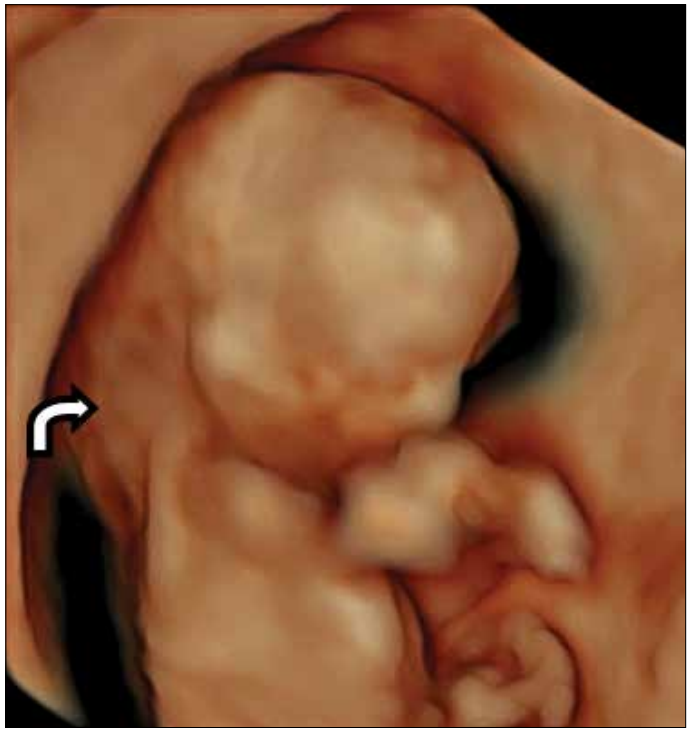

Figure 12. Increased nuchal translucency (curved arrow) in a fetus with trisomy 21

tion presenting with frontal bossing and a flat profile in the 2D scan at the time of the first trimester combined test for common trisomy, where HDlive allowed the better detection of agenesis of the left leg and exadactyly (Figure 14). Furthermore, HDlive has been useful in characterizing a split of the skull base with diplomyelia at the cranial level and an underlying diastematomyelia in a 12 week 2 day fetus with a 46, XY karyotype (Figure 15). Diastematomyelia is a rare form of spinal dysraphism characterized by a sagittal cleft in the spinal cord, conus medullaris, and/or filum terminale with splaying of the posterior vertebral elements (37). The defect may be isolated or associated with myelocele, myelomeningocele, and segmental vertebral body anomalies (38-39).

Moreover, it has been demonstrated that HDlive with spatio-temporal image correlation (STIC) that allows a new realistic image modality of endocardial cushion that may result in diagnostic enhancement in pathologic cases (11). AboEllail et al. (40) have reported the diagnostic value of HDlive in a case of atrioventricular septal defect, bilateral ventricular hypertrophy, and pericardial effusion. HDlive depicted a common atrioventricular (AV) valve and suspected that the free, floating leaflets of AV valve were not attached to the interventricular septum. Moreover, the contemporary use of STIC allowed the recognition of the anatomical relationship among the common AV valve, chordae, and papillary muscles.

Very recently, Pooh (41) has demonstrated that HDlive greatly improves our angiographic imaging of intracorporeal vessels

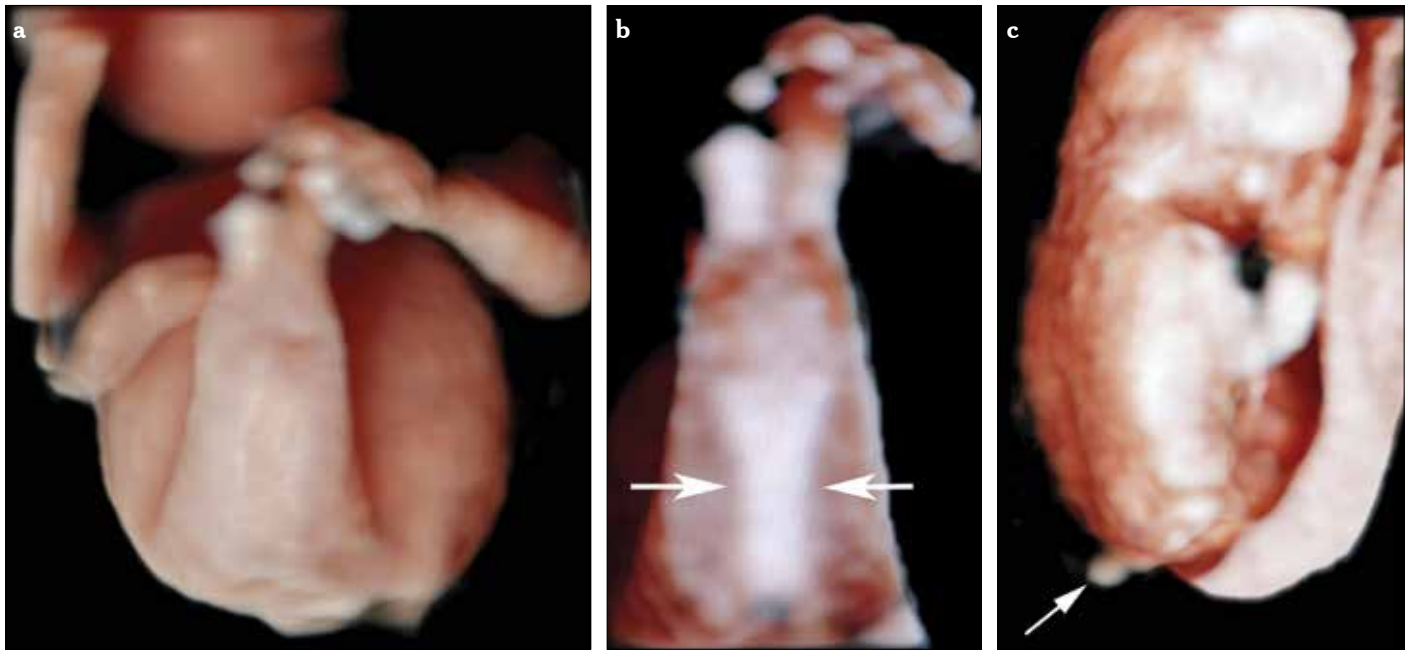

Figure 13. a-c. HDlive rendering showing sirenomelia (a) with single femur, fused lower limbs, and a single supinovarus foot with oligodactyly (symelya unipus) diagnosed at 12 weeks 1 day of gestation in a fetus with a 46, XX karyotype (b). The caudal "tail" was also detected (c) (white arrows)

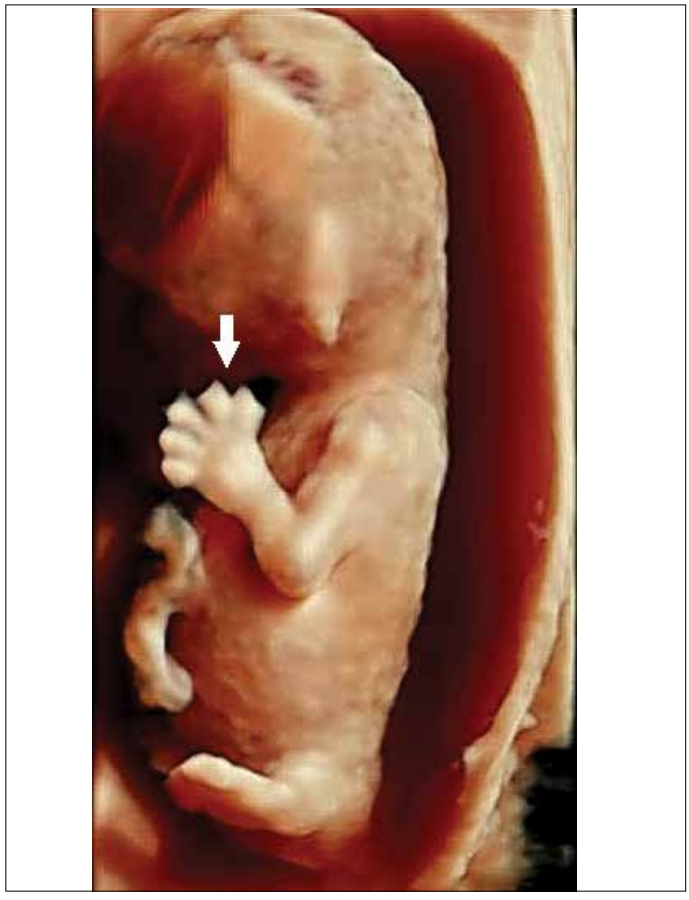

Figure 14. HDlive rendering in a fetus at 12 weeks 1 day of gestation showing skeletal dysplasia characterized by unilateral agenesis of the left leg and exadactyly (white arrow)

by demonstrating a rich pulmonary vascularity in a fetus at 13 weeks of gestation using HDlive silhouette/flow imaging with bidirectional power Doppler. Higher advancement in embryologic imaging has been reached by HDlive silhouette/flow mode, a novel developed 3D ultrasound algorithm that creates a gradient on organ boundaries where an abrupt change of the acoustic impedance exists within the tissue. By the HDlive silhouette mode, an inner cystic structure with fluid collection can be seen through the outer surface structure of the body in a "see-through fashion." Hyperechogenic structures such as those forming the skeleton can be extracted with emphasized bony imaging (42). The application of the HDlive silhouette mode or Radiance System Architecture has been proven to greatly contribute to the diagnostic definition in a high number of embryo-fetal malformations as documented by Bonilla-Musoles et al. (43) and AboEllail et al. (44).

The natural appearance of the image as created by HDlive rendering may help create a closer relationship between parents and their children and enhance maternal–fetal bonding. The term bonding is used in the literature to indicate feelings that people have toward their fetus, and through the realistic imaging of the fetus, the HDlive technology seems to increase, maternal and fetal attachment. Fetal blinking, mouthing, swallowing, yawning, tongue expulsion, and sucking are all fetal activities that have been clearly documented using the HDlive rendering mode. Moreover, various realistic fetal emotional expressions such as smiling- and crying-like movements have also been reported with this technique (45).

3D HDlive in the rendering mode has been applied not only to depict superficial anatomical details but also in cases of multycystic dysplastic kidney disease, where it has been shown that the peripheral circular arrangement of the cysts (the so-called

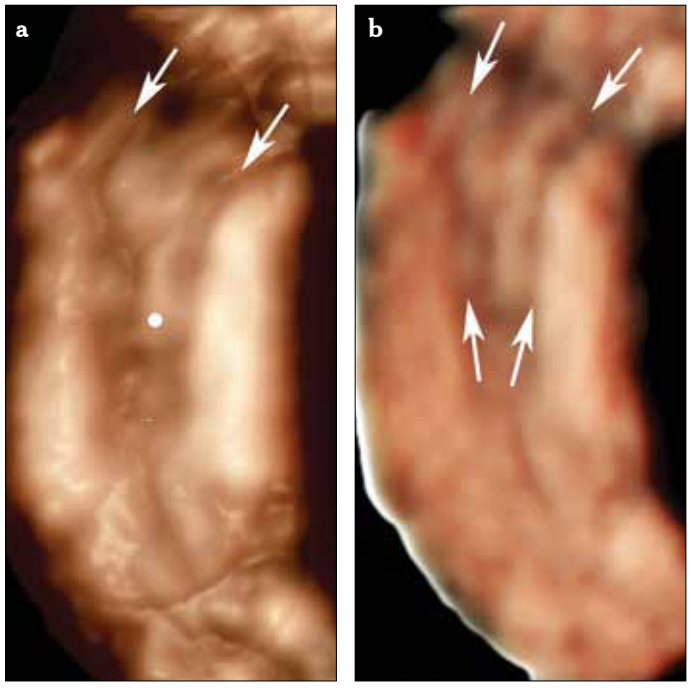

Figure 15. a, b. HDlive rendering of the split of the skull base with diplomyelia at the cranial level (white arrows) (a) and an underlying diastematomyelia (white arrows) (b) in a fetus at 12 weeks 2 days of gestation with a 46, XY karyotype

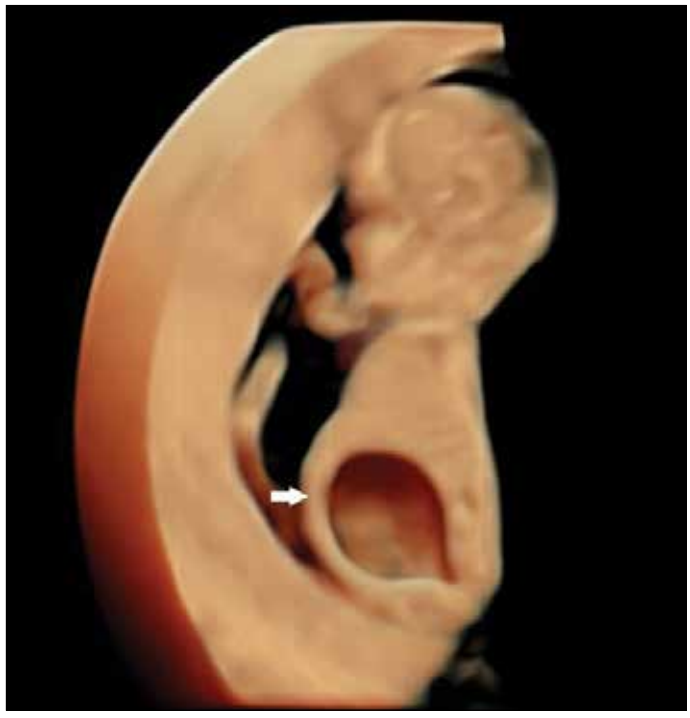

Figure 16. HDlive rendering in a fetus at 13 weeks 1 day of gestation showing a megacyst (white arrow) due to lower urinary tract obstruction

"necklace appearance") seemed to be more clearly understandable than that obtained by conventional 2D sonography (46).

This observation is in agreement with that by Cajusay-Velasco and Hata. (47) who used HDlive in the assessment of fetal intra-cranial, intra-thoracic, and intra-abdominal anomalies. Notwithstanding, in our series, HDlive has been demonstrated to perform well in a case of a megacyst due to lower urinary tract obstruction during the first trimester scan (Figure 16).

AboEllail et al. (48) have described a meconium peritonitis using HDlive rendering in a primigravida at 32 weeks 2 days of gestation with polyhydramnios and fetal bowel dilatation. HDlive rendering showed that the dilated intestinal loops were irregularly thickened and that there was meconium within the dilated intestine. HDlive with inversion mode rendering clearly showed the continuation between cystic dilatation and the small intestine. The X-ray of the newborn revealed the abdominal calcifications, thus proving the prenatal diagnosis of meconium peritonitis. In another recent case report, AboEllail et al. (49) have described a case of jejunal atresia at 28 weeks of gestation using HDlive using the "*silhouette mode*". HDlive rendering showed the inner stomach connected by the pylorus to the duodenum and clearly demonstrated the dilated stomach with the absence of peristalsis, while HDlive in the inversion mode identified an excessive duodenal peristalsis.

X-ray imaging performed after delivery documented a triple-bubble sign, and gastrografin enema revealed a microcolon. X-ray imaging performed after delivery showed a triple-bubble sign, and gastrografin enema revealed a microcolon. Recently, Kaji et al. (50) demonstrated a case of anorectal atresia in a fetus at 31 weeks 1 day of gestation, showing a normal perineum with the absence of an anal dimple. The postnatal clinical examination result proved the prenatal diagnosis of anorectal atresia with a rectovestibular fistula. X-ray imaging confirmed an intermediate-type of anorectal atresia and esophageal atresia.

Ishibashi et al. (51) used HDlive for the visualization of the bicornuate uterus with hydrometra and bilateral hydrosalpinx in the diagnosis of a persistent cloaca. However, 3D surface rendering and HDlive did not provide unique information for the prenatal diagnosis of persistent cloaca if there was no associated fetal ascites.

Although HDlive improves the quality of ultrasound imaging and new developments such as the "crystal view" have been reported (52-54), the true potential of this technique is yet to be scientifically determined (55). There are some limitations in using the HDlive technology that are caused by maternal and embryonic movement that can reduce the image quality. Another limitation is represented by the presence of a small amount of fluid around the embryo and the curvature of the gestational sac (56).

In summary, we demonstrated our experience in the application of HDlive rendering in normal embryo-fetus development and pathologic cases. Our clinical series confirm the previous observation that HDlive rendering generates realistic images by means of an appropriate control of lighting and shadowing effects (54). We believe that HDlive will be incorporated into 3D/4D ultrasound to enhance the sonographic detection of abnormal phenotypes, thus improving parental bonding and genetic counseling.

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Pessary use in pregnant women with short cervix

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Abstract

The purpose of this case series is to provide preliminary evidence on the efficacy of pessary application in women with short cervix and at risk for preterm labor. Between May 2015 and July 2015, four pregnant women were followed-up with Arabin pessaries. The gestational age at the time of diagnosis was between the 23th and 29th weeks. Pessary application was associated with a prolongation of pregnancy lasting between 28 and 98 days. The gestational age at the time of delivery was between the 33rd and 39th weeks. Pessary use is non-invasive for the prolongation of pregnancy in pregnant women with shortened cervix. The major advantage of pessary use is its easy application without requiring anesthesia. (J Turk Ger Gynecol Assoc 2016; 17: 120-2)

Keywords: Cervical pessary, preterm birth, short cervix

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Introduction

Preterm labor and birth, i.e., birth before the 37th week of gestation, is a major cause of perinatal morbidity and mortality in the developed world (1). Shortening cervical length is identified as a risk factor for preterm birth. Transvaginal measurements of cervix that reveals shortening (below 25 mm) or cervical funneling sign is an important indicator of threatened preterm birth. The management of such cases usually consists of progesterone administration or cervical cerclage with progesterone (2). However, conclusive evidence on the effectiveness of these treatments is lacking. Cervical pessary is a non-invasive alternative to cervical cerclage and it has the advantage of not requiring anesthesia (3). In the late 1970s, Hans Arabin in Germany designed a silicon pessary applied to the cervix and that works by changing the uterocervical angle and displacing it more posteriorly (Cerclage Pessary, Dr. Arabin GmbH and Co. KG; Witten, Germany). Compared with other pessaries, Arabin's pessary is flexible and more easily applied to the cervix (4).

Case Series

In Ankara University, Department of Obstetrics and Gynecology, between 2007 and 2014, we treated 136 patients for shortened cervix and threatened premature labor with mechanical and invasive methods. In total, 132 of these patients were treated with cervical cerclage and four with cervical pessary. Patients who did not accept cervical cerclage and cervical pessary were treated with medication and bed rest. Pessary application was restricted to patients who did not accept cervical cerclage treatment. We present the cases of the four patients with cervical pessary application for the prevention of preterm birth. The patients had no history of prior preterm birth, and progesterone treatments were withheld. Cervical and vaginal swabs were taken from all patients, and cultures were reported to have normal flora. Written consents were taken prior to treatment. There were no complications related to pessary application, and the patients were not administered tocolytics, progesterone, and antibiotics during pregnancy.

Case 1

A 23-year-old primigravida woman was referred to our clinic for cervical insufficiency. The woman was at her 29th week of gestation, and the amniotic membrane protruded into vaginal vault. Her digital examination showed a cervical dilatation of 3 cm. A pessary was applied. She was re-admitted to the hospital during her 33rd week of gestation due to uterine contractions. The cervical dilatation at the time was 6 cm, and the pessary was removed. A 2110 g female baby was vaginally delivered with Apgar scores of 7 and 9. The time from pessary application to delivery was 28 days (Table 1).

Case 2

A 29-year-old, gravida 2, parity 1 woman was referred to our clinic at the 23rd week of gestation with an initial diagnosis of cervical insufficiency. Her initial assessment revealed a shortened cervix (14 mm) and a closed cervical os. A cervical pessary was placed in the 23rd week of gestation. The patient remained asymptomatic until the 33rd week of gestation. She was readmitted at 33 weeks and 4 days due to rupture of membranes, and the pessary was removed. After pessary removal, digital examination showed a cervical dilatation of 7 cm, and a 1610 g female baby was vaginally delivered with Apgar scores of 7 and 9. The time from pessary application to delivery was 72 days (Table 1).

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Table 1. Baseline characteristics and delivery data of pessary cases

Case	Age	Parity	GA at admission (week, day)	Cervical assessment	GA at pessary application	GA at pessary removal	GA at delivery	Time from pessary application to delivery	Birth weight
1	23	0	29 w, 0 d	Dilatation: 3 cm	29 w, 0 d	33 w, 0 d	33 w	28 days	2110 g
2	29	1	23 w, 1 d	Cervical length: 14 mm	23 w, 2 d	33 w, 4 d	33 w, 4 d	72 days	1610 g
3	29	1	27 w, 4 d	Cervical length: 15 mm, funneling sign positive	27 w, 5 d	39 w, 0 d	39 w, 0 d	76 days	3300 g
4	31	2	23 w, 1 d	Cervical length: 15 mm, Cervical dilatation: 5 cm	23 w, 3 d	37 w, 2 d	37 w, 3 d	98 days	3200 g

GA: gestational age

Case 3

A 29-year-old, gravida 2, parity 0 woman was referred to our clinic for cervical insufficiency at 27 weeks and 4 days. Her initial assessment revealed a shortened cervix of 15 mm with a funneling sign. A cervical pessary was placed on the same day. She remained asymptomatic until term. The pessary was removed at the 39th week of gestation due to the rupture of membranes. The cervical dilatation at admission was 5 cm, and 3300 g female baby was vaginally delivered with Apgar scores of 8 and 9. The time from pessary application to delivery was 76 days (Table 1).

Case 4

A 31-year-old, gravida 3, parity 2 woman was referred to our clinic for cervical insufficiency at 23 weeks and 4 days. Her initial assessment revealed a cervical dilatation of 5 cm and transvaginal measured cervical length of 15 mm. A cervical pessary was placed at 23 weeks and 4 days. She remained symptom free until the 37th week of gestation. She was readmitted at 37 weeks and 3 days due to uterine contractions. The digital examination of her cervix revealed an unchanged dilatation of 5 cm, and the pessary was removed due to regular uterine contraction and also as the patient reached term. A 3200 g female baby was vaginally delivered with Apgar scores 8 and 9. The time from pessary application to delivery was 98 days (Table 1).

Discussion

Preterm birth is a major cause of neonatal mortality and morbidity in developed countries. It is implicated as a cause of both short- and long-term morbidity in infants (1), and the prevention of preterm birth is the most effective way of decreasing morbidity rates. Shortened cervical length is an important predictor of spontaneous preterm birth, and several treatment strategies have been proposed. Alfirevic et al. (5) reported that cervical cerclage, vaginal progesterone, and cervical pessary appear to have a similar effectiveness as management strategies in women with singleton pregnancy, previous spontaneous preterm birth, and short cervix. Cervical cerclage is the most common mechanical prevention method for the prevention of preterm birth. However, the procedure is not without complications (6). Cervical pessaries have been shown to be efficacious in preventing preterm birth, especially in a high-risk population of women with a shortened cervical length (7-10). On the other hand, a randomized controlled trial showed that the prophylactic use of cerclage pessaries did not reduce the rate of preterm delivery before 34 weeks (11). Cervical pessary is an inexpensive and less invasive option to cervical cerclage. Pessary application and removal do not require anesthesia. Pessary use, instead of cervical suturing, can decrease hospital stays and reduce healthcare costs. In low-resource countries, cervical pessaries are an alternative method for the prevention of preterm birth. Few complications arising from pessary use have been reported, including increased vaginal discharge. However no major infectious morbidity, i.e., chorioamnionitis, has been reported. It appears to be cost effective with minimal side effects, and Quaas et al. (9) recommended the Arabin pessary as a favorable alternative to surgical cerclage with either prophylactic or therapeutic intent.

Pessary use for the treatment of cervical insufficiency is uncommon. A cervical cerclage was used in almost 130 patients in our clinic during the last 10 years, while we applied a cervical pessary to only four patients for the prevention of preterm labor. A cervical pessary resulted in a modest prolongation of pregnancy in all four patients.

Although our experience is derived from four cases, cervical pessaries seem to be safe and feasible for the prevention of preterm labor. The efficacy of cervical cerclages in the prevention of preterm birth in women with short cervix is unproven. If the efficacy of pessaries in preventing preterm birth is confirmed, they may become a safe alternative to traditional surgical cerclage when surgery is undesired by the patient or doctor. In the absence of randomized cohorts, the decision regarding which treatment options to choose should take into account the preferences of patients and clinicians.

Ethics Committee Approval: *N/A.*

Informed Consent: *Written informed consent was obtained from patients who participated in this case.*

Peer-review: *Externally peer-reviewed.*

***Author Contributions**: Concept - T.Y., B.K.; Design - T.Y., B.K.; Supervision - T.Y., F.S.; Funding - T.Y., B.K.; Materials - T.Y., E.K.; Data Collection and/or Processing - T.Y., E.K.; Analysis and/or Interpretation - T.Y., B.K.; Literature Review - T.Y., B.K.; Writer - T.Y., B.K., E.K.; Critical Review - T.Y., F.S.*

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What is your diagnosis?

A 20-year-old primigravida was referred due to increased risk of trisomy 21 in the triple test result at 21 weeks of gestation, which was 1/59. Sonographic examination revealed hypoplastic nasal bone (NB=4.3 mm) and hyperechogenic bowel with mildly dilated segments (bowel diameter=4.5 mm) (Figure 1). Past medical histories of both parents were insignificant. There was no consanguineous marriage. Amniocentesis was performed. Quantitative fluorescence polymerase chain reaction (PCR), karyotype, and cystic fibrosis panel were normal. Due to hyperechogenic bowel and mildly dilated segments of the bowel, close surveillance was planned. At 24 weeks and 25 weeks, these segments of the bowel continued to dilate and increased to 9.8 mm and 11.2 mm, respectively. At 25 weeks of gestation, anorectal complex was observed intact. Dilatation of the bowel progressed and reached to 23.8 mm at 29 weeks of gestation, which was also coincided with an increase in the amount of amniotic fluid (Figure 2). Besides dilated bowel segments, a cystic structure with hyperechogenic inner fluid than the other dilated bowel segments had become noticeable during the examination at 29 weeks. Due to the risk of volvulus, weekly follow-up visits were scheduled. From 29 to 37 weeks, the size of the cyst and amount of amniotic fluid continued to increase, although the diameter of the dilated bowel remained relatively stable (Figure 3). Within last 4 weeks before delivery, in addition to the cyst and bowel dilatation, ascites began to accumulate in the fetal abdomen. In this period, Doppler of the middle cerebral artery and umbilical artery remained normal, however a-waves in the ductus venosus started to deepen and fetal ascites progressively increased. We, therefore, decided to perform paracentesis via ex utero intrapartum treatment (EXIT) procedure in the intrapartum period. At 37 weeks, planned cesarean under deep general anesthesia was performed. Male infant weighing 2910 g was delivered. Umbilical cord was not clamped, and oxytocin administration was withheld. Newborn was laid between the mothers legs. Under the ultrasound guidance, paracentesis was performed and 240 mL of green fluid was aspirated, which effectively ruled out hemorrhagic ascites. The newborn was neither intubated nor required mechanical ventilation, and after a brief stay in the neonatal intensive care unit, he was operated. After an uneventful postoperative course, he was discharged. At 2 years of age, he was faring well.

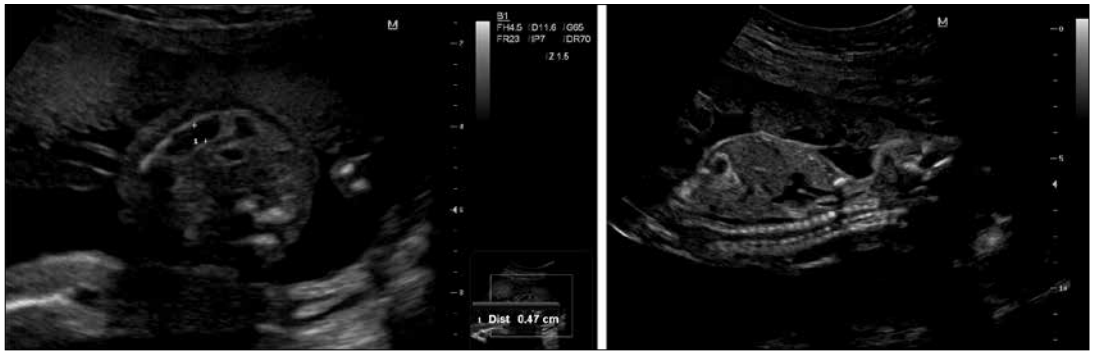

Figure 1. Hyperechogenic and dilated bowel loops at 21 weeks gestation

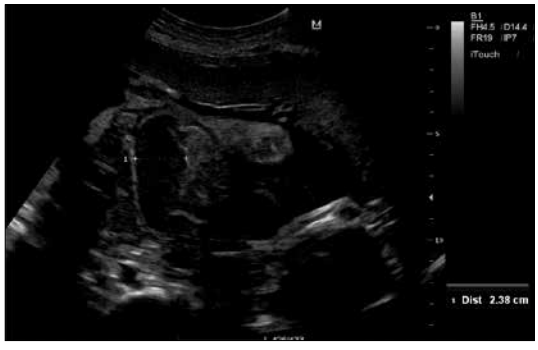

Figure 2. Dilated bowel at 29 weeks of gestation

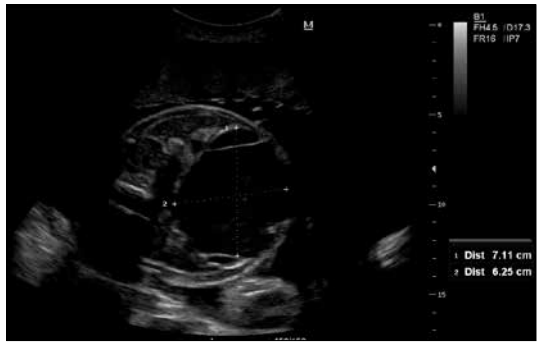

Figure 3. Cystic structure at 34 weeks of gestation

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Answer

Intra-operative findings and pathological examination of the specimen revealed diagnoses of ileal atresia, intestinal perforation, intestinal malrotation, Ladd band, and intestinal duplication cyst, which overlap with the prenatal ultrasound findings. Incidence of intestinal obstruction in the neonatal period is around 1/2000 (1). Causes include duodenal atresia/stenosis, malrotation and midgut volvulus, intestinal atresia, meconium ileus/peritonitis, and, last but not least, enteric duplication cyst. Each of these underlying pathologies has varying incidences and frequently accompanied with another pathology. Incidence of malrotation has been estimated to be around 15:1,000,000 in children less than 1 year old (2). Associated abnormalities can be found in 30–60% of the malrotation cases, which include intestinal atresia and obstruction, Meckel diverticulum, intussusception, Hirschsprung's disease, anorectal malformations, mesenteric cysts, anomalies of the extrahepatic biliary system, cardiac anomalies, and trisomy 21 (2, 3) (4). However, intestinal duplication cysts rarely coincide with malrotation (5). We systematically searched PubMed from inception through January 2016 to discover relevant cases of intestinal malrotation and intestinal duplication cyst that were prenatally diagnosed, followed up, and managed in the immediate postpartum period. In order to achieve this, we had used the first query in Table 1. This query yielded seven results among which only one was relevant (5). This related article had reported diagnosis and management of a 2-year-old boy (5). Accordingly, and to best of our knowledge, prenatal diagnosis, follow-up, and management of malrotation with intestinal atresia/perforation and accompanying intestinal duplication cyst have not been reported in the English literature.

With the second, third, and fourth queries in Table 1, we systematically searched the literature for the antenatal ultrasonographic signs of fetal intestinal malrotation and volvulus, meconium pseudocyst, and intestinal duplication cyst, which may help in differential diagnosis. Differentiation of volvulus from a duplication cyst and meconium pseudocyst may be of utmost importance because of the life-threatening complications of the former pathology. Imaging findings for malrotation and volvulus included midline stomach, small bowel on right side of the midline, classic mesenteric whirlpool sign (twisted mesentery around the mesenteric vascular stalk), coffee-bean sign, and three parallel fixed bowel loops on transverse scan (4, 6-8). Reported imaging findings for intestinal duplication cyst included thin- or thick-walled cystic structure, double wall appearance/sign, pseudo-double bubble appearance, abdominal cyst with peristalsis, echogenic mesenteric tissue consisting of fat close to the cyst, and intra-cystic echogenic contents (9-12). Reported characteristics of meconium pseudocyst included thick membrane containing multiple calcium deposits and plaques (13). Although it is hard to differentiate duplication cyst from a meconium pseudocyst, malrotation and volvulus can be differentiated from the former two according to the reported ultrasonographic findings. From clinical standpoint, the diagnosis of volvulus is imperative, because if not treated surgically within 6 hours, it may cause necrosis of the entire small bowel and right colon (7). In this context, Doppler examination and effort to demonstrate mesenteric whirlpool sign should be attempted in every case in doubt. However, there will always be cases where we cannot reach a definitive diagnosis, and close follow-up of these cases with serial ultrasound scans and fetal well-being evaluation are clearly required. Fetal demise in case of volvulus occurs secondary to bowel infarction, hemorrhagic ascites, and fetal anemia (14, 15). Accordingly, to predict fetal demise secondary to hemorrhagic ascites, middle cerebral artery Doppler and non-stress testing becomes critical (14, 15). Increased peak systolic velocity in middle cerebral artery Doppler examination shows anemic condition of the fetus secondary to bowel infarction and hemorrhage. In cases with abnormal fetal heart rate pattern and ascites for which the diagnosis cannot be established, in utero paracentesis may provide both diagnostic information and treatment benefit in terms of correcting abnormal fetal heart rate pattern (16). In utero paracentesis may also facilitate vaginal delivery by preventing dystocia via decreasing fetal abdominal distention (16). Moreover, in utero paracentesis may reduce respiratory compromise after birth (16). For the identification of hemorrhagic change in the bowel wall, combined use of ultrasonography and ultrafast magnetic resonance imaging was also reported (17). On T2-weighted single-shot fast-spin echo magnetic resonance images, enlarged loops that exhibit a lower signal intensity than the surrounding bowel loops suggests bowel infarction and intraluminal hemorrhage (17). When the middle cerebral artery Doppler indicates anemic fetus or in utero paracentesis shows hemorrhagic ascites emergency in utero transfusion may be required (15). In the overlooked cases, when fetus escapes the sudden insult and the condition becomes relatively chronic, hemorrhagic volvulus may result in fetal hydrops (15, 18).

For the previously mentioned benefits and indications of in utero intervention, paracentesis may also be performed safely in the immediate intrapartum period as an EXIT procedure as in the current case. The first EXIT procedure was utilized for a fetus with cervical teratoma to secure the airway with bronchoscopy for 10 min (19). In the subsequent attempts of the EXIT procedure, it was noticed that when the fetus was completely delivered, the volume of the uterus decreased significantly; as a result, placental circulation can only continue for a short duration of time (20, 21). With further attempts, durations of 20–60 min were obtained when the fetus was partially delivered (22). Due to the short duration of time required by the paracentesis procedure, intervention can be easily performed after complete delivery of the fetal body. Tense ascites, enlarging abdominal organs or structures, and secondary abdominal distention impact the fetal thoracic structures, which can adversely affect the neonatal respiratory functions. As in our case, intrapartum paracentesis via the EXIT procedure under ultrasonographic guidance can be easily and safely performed to relieve pressure on diaphragm and to ensure stable neonatal respiratory functions which may be important in a newborn awaiting surgery. Avoiding mechanical ventilation during this critical period is imperative for these fragile newborns which can deteriorate quickly. In addition, aspiration of green bile colored fluid during paracentesis can rapidly rule out hemorrhagic ascites secondary to bowel infarction and establish diagnosis of bowel perforation in this critical transition period.

Conclusion

In cases of fetal bowel obstruction with increased abdominal pressure secondary to meconium peritonitis, ascites and/or enlarging duplication cyst paracentesis can be utilized either in utero or ex utero. When utilized in the immediate intrapartum period para-

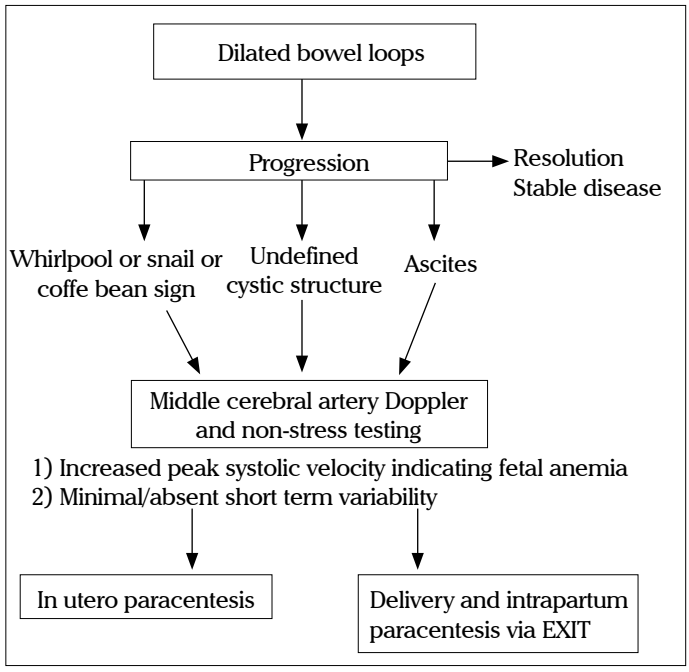

Figure 4. Antenatal management of fetal bowel obstruction

centesis can be safely performed via the EXIT procedure to prevent deleterious effects of the abdominal distention on the respiratory functions of the newborn and provide fast and reliable diagnostic information. Based on the available relevant literature a management algorithm is proposed for this very specific patient group (Figure 4). When ultrasound indicate hyperechogenic bowel and dilated bowel segments, we should keep mind that 53% of those will have an abnormality after birth (23). Consequently, antenatal follow-up and postpartum close surveillance of these fetuses should continue.

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CONGRESS CALENDAR

INTERNATIONAL MEETINGS

June 15-18, 2016	**European Congress of Perinatal Medicine** Maastricht, the Netherlands https://www.eiseverywhere.com
July 3-6, 2016	**ESHRE-17th Annual meeting of the European Society of Human Reproduction and Embryology** Helsinki, Finland http://eshre2016.org
September 8-10, 2016	**ESGO-European Society of Gynaecological Oncology** Antalya, Turkey http://www.esgo.org
October 2-5, 2016	**ESGE-25th Annual Congress of the ESGE** Brussels http://www.esgecongress.eu
October 15-19, 2016	**ASRM- 72nd Annual Meeting of the ASRM** Salt Lake City, UT, USA https://www.asrm.org
October 26-28, 2016	**Update in Obstetrics, Gynecology and Reproductive Medicine** Barcelona, Spain http://www.comtecmed.com
November 10-13, 2016	**The 24th World Congress on Controversies in Obstetrics, Gynecology & Infertility (COGI)** Amsterdam, Netherlands http://congressmed.com/cogi
September 21-24, 2017	**The 19th World Congress on Gestational Trophoblastic Diseases** İstanbul, Turkey www.worldcongressgtd2017istanbul.com

NATIONAL MEETINGS

5-9 October 2016	**14. Ulusal TJOD Kongresi** Antalya Kaya Palazzo Otel http://www.tjodkongre2016.org
17 - 20 November, 2016	**7. Ulusal Üreme Endokrinoloji ve İnfertilite Kongresi (TSRM 2016)** Antalya, Turkey http://2016.tsrm.org.tr/
30 November - 4 December, 2016	**15. Ulusal Jinekolojik Onkoloji Kongresi** Titanik Deluxe Otel, Antalya http://www.trsgo.org

JTGGA CME/CPD CREDITING

Questions on the article titled "*Effect of chorionic villus sampling on the occurrence of preeclampsia and gestational hypertension: An updated systematic review and meta-analysis*" within the scope of CME/CPD

1. What percentage of pregnancies are affected by preeclampsia?
   a. 1-2%
   b. 5-8%
   c. 12-14%
   d. 20-25%
   e. 30-35%

2. Which of the following prenatal tests affects the maternal AFP levels?
   a. Midtrimester amniocentesis
   b. Maternal Cell free fetal DNA tests
   c. Quadruple test
   d. Chorionic villus sampling
   e. Amniodrainage

3. Which of the following is not associated with chorionic villus sampling?
   a. Miscarriage
   b. Limb reduction
   c. Preeclampsia
   d. Vaginal spotting or bleeding
   e. Uterine cramping

4. Which of the following tests is used for the evaluation of heterogeneity in meta-analysis?
   a. Student's t-test
   b. Chi-square test
   c. Analysis of variance
   d. I2 statistic
   e. None of the above

5. Which of the following is true for the studies evaluated the association between preeclampsia and chorionic villus sampling?
   a. None of them were randomized prospective trials
   b. Both patients that were performed amniocentesis and patients with no invasive procedure were used for control group comparison
   c. Prevalence rate of preeclampsia were considerably lower in these studies compared to recent prospective studies
   d. None of the above
   e. All of the above

6. Which of the following is a risk factor for preeclampsia and gestational hypertension?
   a. Amniocentesis
   b. Chorionic villus sampling
   c. Cordocentesis
   d. Nulliparity
   e. Amniodrainage

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Questions on the article titled "*Effect of chorionic villus sampling on the occurrence of preeclampsia and gestational hypertension: An updated systematic review and meta-analysis*" within the scope of CME/CPD

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2nd Question				
A	B	C	D	E

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A	B	C	D	E

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A	B	C	D	E

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