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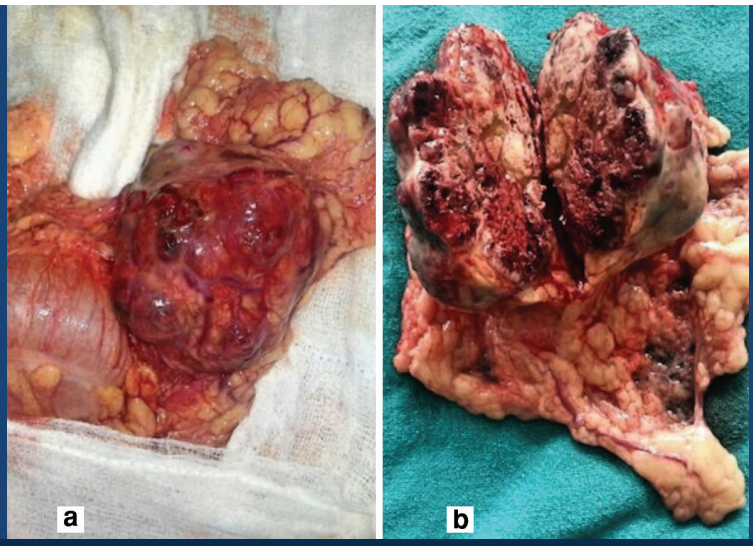

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Aims and Scope

Journal of the Turkish-German Gynecological Association is the official, open access publication of the Turkish-German Gynecological Education and Research Foundation and Turkish-German Gynecological Association and is published quarterly on March, June, September and December.

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 are also published.

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PRISMA for preferred reporting items for systematic reviews and meta-analyses (Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 2009; 6(7): e1000097.) (http://www.prisma-statement.org/),

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/),

STROBE statement-checklist of items that should be included in reports of observational studies (http://www.strobe-statement.org/),

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MOOSE guidelines for meta-analysis and systemic reviews of observational studies (Stroup DF, Berlin JA, Morton SC, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting Meta-analysis of observational Studies in Epidemiology (MOOSE) group. JAMA 2000; 283: 2008-12).

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A separate title page should be submitted with all submissions and should include the title of the article, name(s), affiliations and major degree(s) of the author(s) and source(s) of the work or study, a short title (running head) of no more than 50 characters. The name, address, telephone (including the mobile phone number) and fax numbers and e-mail address of the corresponding author should be listed on the title page.

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Original articles should have the following sections.

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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.

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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

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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

Dear Colleagues,

It is my great pleasure to meet with you again in the third issue of the Journal of the Turkish - German Gynecological Association *(J Turk Ger Gynecol Assoc)*.

Today I want to give some information about **ORCID**. ORCID provides a persistent digital identifier that distinguishes you from every other researcher and, through integration in key research workflows such as manuscript and grant submission, supports automated linkages between you and your professional activities ensuring that your work is recognized.

ORCID (Open Researcher and Contributor ID) is a nonproprietary alphanumeric code to uniquely identify scientific and other academic authors and contributors. This addresses the problem that a particular author's contributions to the scientific literature or publications in the humanities can be hard to recognize as most personal names are not unique, they can change (such as with marriage), have cultural differences in name order, contain inconsistent use of first-name abbreviations and employ different writing systems. It provides a persistent identity for humans, similar to that created for content-related entities on digital networks by digital object identifiers (DOIs).

The ORCID organization offers an open and independent registry intended to be the *de facto* standard for contributor identification in research and academic publishing. On 16 October 2012, ORCID launched its registry services and started issuing user identifiers. The aim of ORCID is to aid "the transition from science to e-Science, wherein scholarly publications can be mined to spot links and ideas hidden in the ever-growing volume of scholarly literature". Another suggested use is to provide each researcher with a constantly updated 'digital curriculum vitae' providing a picture of his or her contributions to science going far beyond the simple publication list. The idea is that other organizations will use the open-access ORCID database to build their own services.

It has been noted in an editorial in *Nature* that ORCID, in addition to tagging the contributions that scientists make to papers, "could also be assigned to data sets they helped to generate, comments on their colleagues' blog posts or unpublished draft papers, edits of Wikipedia entries and much else besides". In April 2014, ORCID announced plans to work with the Consortia Advancing Standards in Research Administration Information to record and acknowledge contributions to peer review. In an open letter dated 1 January 2016 eight publishers, including the Royal Society, the American Geophysical Union, Hindawi, the Institute of Electrical and Electronics Engineers, PLOS, and Science, committed to requiring all authors in their journals to have an ORCID ID. From now on, we ask from all authors an ORCID ID too. Therefore I recommend all of you to get your unique ORCID identifier from https://orcid.org.

Dear Young Researchers,

Publishing an article in an academic journal can be a frustrating process that demands a substantial commitment of time and hard work. Nevertheless, establishing a record of publication is essential if you intend to pursue a career as an academic or scientific researcher. I want to give five suggestions will help you turn the odds in your favor and make the publishing process less daunting.

1. Target an Appropriate Journal
2. Say Something New
3. Edit Your Work Extensively

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4. Reference Strategically
5. Make it Difficult for Reviewers to Say "No"

When choosing a journal, you want to keep in mind two factors: *review times* and policies on *multiple submissions*. You should expect most reviews to take several months at a minimum. Meanwhile, most journals do not accept an article for review that is simultaneously being reviewed by another journal.

As a result, the journal you target is particularly important because it's not practical to submit your work to many publications. If you aren't interested in waiting 6-months or longer to hear back from several journals (one after the other), start out by targeting a publication that's more likely to give your article the green light. You'll have a better chance of publishing in a top journal with this experience under your belt.

Dear Colleagues,

In this issue, we are dealing with very interesting research articles and reviews. We worked hard to deliver you the journal with the best manuscripts in time. In this issue, you will read several good papers from all over the world from India to Germany and USA.

I would also like to remind you the sixth Social Responsibility Project of Turkish German Gynecological Education and Research Foundation (TGGF), which will be held on September 8-9, 2017, in Antakya-Turkey. The project held in this beautiful city is traditionally organized from four steps; public awareness meeting with participation of the locals, the scientific meeting with participation of health professionals, performing of the advanced operations and medical examination/screening to local women, and finally a medical device donation to a local hospital. We believe our project could be considered a success if only one maternal death is prevented. Since it is these small steps which may one day make the difference. We would be excited to have our colleagues join us in this intense scientific activity.

I would like to remind you that our journal has been indexed in **PubMed Central**. We are looking forward to receiving your valuable submissions and thank you in advance for your contributions.

Sincerely,

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

Liposome-encapsulated diacyl glycerol and inositol triphosphate-induced delayed oocyte activation and poor development of parthenotes

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Abstract

Objective: To explore the ability of diacyl glycerol (DAG) and inositol triphosphate (IP3), two major secondary messengers in the calcium signaling pathway, in activating oocytes.

Material and Methods: Oocyte cumulus complex obtained from superovulated Swiss albino mice were incubated in M16 medium with liposome-encapsulated 1,2-Dipalmitoyl-sn-glycerol (LEDAG) and/or IP3 for 3 h. Strontium chloride was used as positive control. The activation potential, ploidy status, and blastocyst rate was calculated.

Results: Both DAG and IP3, individually, induced activation in ~98% of oocytes, which was significantly higher ($p<0.01$) than activation induced by strontium chloride (60%). Delayed pronucleus formation and a higher percentage of diploid parthenotes was observed in oocytes activated with LEDAG and/or IP3. However, these embryos failed to progress beyond the 6-8–cell stage. Only when the medium was supplemented with LEDAG (5 μg/mL) and IP3 (10 μg/mL) could activated oocytes progress till the blastocyst stage (5.26%), which was lower than the blastocyst rate in the positive controls (13.91%).

Conclusion: The results of the present study indicate that DAG and IP3 can induce delayed oocyte activation and poor development of parthenotes *in vitro*. (J Turk Ger Gynecol Assoc 2017; 18: 102-9)

Keywords: Oocyte activation, diacyl glycerol, inositol triphosphate, liposomes, embryo development

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Introduction

Fertilization is a complex process which involves a series of well-defined morphologic and biochemical events in both spermatozoa and oocytes (1). The entry of sperm into the oocyte initiates a signaling cascade, which hydrolyzes the membrane-bound phosphoinositidyl 4,5 bis phosphate (PIP2) to inositol triphosphate (IP3) and diacyl glycerol (DAG). DAG causes the activation of protein kinase C (PKC) (2), and IP3 binds to IP3 receptors present on the surface of the endoplasmic reticulum (ER) to release Ca^{2+} into the cytoplasm (3), which initiates multiple downstream events required for zygote formation.

The development of an embryo without the involvement of spermatozoa (paternal factors) is known as parthenogenesis. Parthenogenetic embryos play an important role in understanding the paternal and maternal contribution to the development of embryos, act as an alternative source of normally fertilized embryos for quality control experiments in assisted reproductive technology (ART) laboratories, and as a source of embryonic stem cells in the field of regenerative medicine. Even though artificial oocyte activation can be achieved *in vitro* using various physical, electrical, and chemical means (4-6), *in vitro* development of parthenotes is characterized by increased embryonic arrest, a high degree of fragmentation, developmental delay of embryos (7, 8), decreased blastocyst rate, and low cell number in blastocyst.

The oocyte activation process and the subsequent development of parthenotes is driven by the calcium

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signaling pathway (7, 9). Artificial oocyte activation agents generate calcium spikes of lower amplitude, which last for a short duration when compared with spermatozoa-induced calcium waves (10). The present investigation was aimed to study whether using DAG and IP3 exogenously as activating agents could improve the developmental potential of activated oocytes. An earlier study showed that microinjection of the secondary messengers in the calcium signaling pathway (DAG and IP3) evoked a calcium spike that was more physiologic and similar to that of natural stimuli (11). However, whether it would be sufficient to drive complete activation of oocytes and support embryo development has not yet been reported. Previous studies used microinjection for the delivery of the secondary messengers for oocyte activation, which requires technical expertise and a sophisticated instrument. In the present study, we used liposome-encapsulated DAG and IP3 individually and in combination to study their oocyte activation potential and the subsequent development of activated oocytes under *in vitro* conditions.

Material and Methods

Animal handling

Inbred Swiss albino female mice (8-10 weeks) maintained in the Central Animal Research Facility, Kasturba Medical College, Manipal University, under standard conditions of temperature (25±2 ºC), humidity (45-55%) and light (12:12 h of light and dark) were used for the study. The authors assert that all procedures contributing to this work complied with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals. Prior approval to carry out the experiment was obtained from the Institutional Animal Ethics Committee of Kasturba Medical College, Manipal (IAEC/KMC/64/2013).

Preparation of liposomes encapsulated with 1,2-Dipalmitoyl-sn-glycerol

1,2-Dipalmitoyl-sn-glycerol (5 mg, Catalogue no. D9135, Sigma) and soy phosphatidyl choline (45 mg) were dissolved in 15 mL of chloroform in a round-bottomed flask connected to a rotary flash evaporator (ROTAVAP, Buchhi, Switzerland), and chloroform was removed under reduced pressure at 40 °C. A thin film of the lipid mixture and 1,2-Dipalmitoyl-sn-glycerol was obtained after dessication on the inner wall of the flask. Ten milliliters of Milli Q water was added to the flask and agitated using a magnetic bead for 30 min to obtain a milky white liposomal dispersion. The preparation was frozen at -80 °C, lyophilized for 36 h, and the product formed (white powder) was stored in the dark at 4 °C till further use.

Parthenogenetic activation

Oocyte cumulus complexes (OCC) were obtained by superovulating 8-10–week-old inbred Swiss albino female mice by intraperitoneal (i.p.) administration of 5 IU pregnant mare's serum gonadotropin (PMSG; Catalogue no. G4877; Sigma, St Louis, MO, USA) followed by 10 IU human chorionic gonadotropin (hCG; Ovutrig-HP, VHB Life Science Inc. GenBiotech, Mumbai, India) 48 h later. The OCCs were collected from the oviduct at 13 h after hCG injection in M2 medium. The oocyte activation was carried out by incubating the OCCs in M16 medium with 10 mM strontium chloride ($SrCl_2$; Catalogue no; 107865, Merck) in varying concentrations of liposome-encapsulated diacyl glycerol (LEDAG, 1, 5, 10, 50, 100, 150, 200, 500 μg/mL) and varying concentrations of inositol triphosphate (IP3, Catalogue no. I7012; Sigma, 5, 10, 25, 50, 100, 200 μg/mL) at 37 °C and 5% CO_2 for 3 h. $SrCl_2$ served as a positive control. After 3 h incubation with the activating agents, OCCs were denuded by brief incubation (1 min) in hyaluronidase solution (1 mg/mL in M2 medium) and observed under a phase contrast microscope to check for oocyte activation. Activated oocytes with a single pronucleus and two polar bodies (1PN/2PB) were considered as haploid parthenotes, and those with two pronuclei and one polar body (2PN/1PB) were scored as diploid parthenotes. Parthenotes were washed and cultured *in vitro* in M16 medium to assess their developmental potential and blastocyst rate.

Statistical analysis

The data pertaining to the activation potential and developmental potential of embryos were represented as percentage and the validity of the data was analysed using the Chi square test using GraphPad InStat 3.0 statistical package (GraphPad Inc, USA). Differences were considered to be statistically significant if $p<0.05$.

Results

Activation potential of liposome encapsulated Dipalmitoyl-sn-glycerol and inositol triphosphate

Liposome encapsulated Dipalmitoyl-sn-glycerol (LEDAG): Strontium chloride (positive control) induced activation in 60% of oocytes, whereas liposome alone (vehicle control) induced activation in 40% of oocytes. At all concentrations of LEDAG used in the study (1, 5, 10, 50, 100, 200 and 500 μg/mL), the activation rate was ~98% (Figure 1). However, unlike in the oocytes activated with $SrCl_2$, where pronuclei were observed as early as 3 h after exposure, it took at least 48 h for pronucleus formation in the LEDAG group, indicating delayed activation. Incubating oocytes *in vitro* without any activating agents for up to 48 h did not cause any oocyte activation, suggesting that the

activation observed in the LEDAG group was not due to ageing of oocytes under *in vitro* conditions.

Inositol triphosphate (IP3): The lowest concentration of IP3 (5 μg/mL) used in the study resulted in 75% activation, whereas at 10 and 25 μg/mL concentration, 100% activation was observed (Figure 2). With further increase in IP3 concentrations (50, 100, 200 μg/mL), though the activation rate was marginally reduced, it was significantly higher ($p<0.01$) than in the positive control, and lower concentrations of IP3 (25 μg/mL). As observed with LEDAG, exposure to IP3 also resulted in delayed pronucleus formation.

Combination of LEDAG and IP3: Varying combinations of IP3 and LEDAG were used to test whether combinations of these agents reduced the time required for pronucleus formation. However, LEDAG and IP3 when used in combination, despite having no dose-dependent effect on activation, ~80-90% of oocytes were activated in all different combinations used (Figure 3).

The maximum activation of oocytes was observed in 50 μg/mL of LEDAG and 10 μg/mL of IP3; therefore, only these concentrations were used to check the ploidy status and degeneration/ fragmentation rate of the oocytes.

Degeneration and fragmentation rate of oocytes activated with LEDAG and IP3: The degeneration and fragmentation rates were found as 5.13% and 9.2%, respectively, in the positive control group (Sr_2Cl) (Table 1). Neither in the vehicle control group nor in LEDAG or IP3 group were there any degenerated oocytes. However, 4.47% of oocytes underwent fragmentation in the vehicle control group, similar to the LEDAG (5.15%) and IP3 (5.26%) groups, which was lower than in the positive control group.

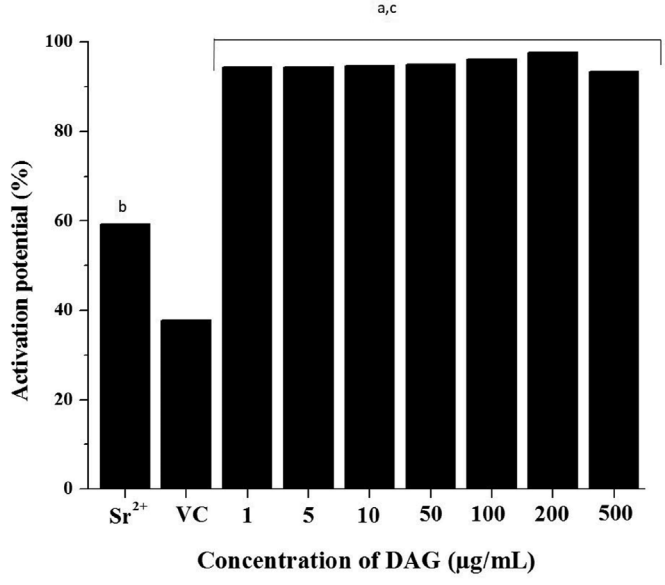

Figure 1. Effect of varying concentrations of diacyl glycerol on activation potential of mouse oocytes. Bar represents percentage of oocytes forming pronuclei with activation.
[a]p<0.001 Sr^{2+} vs. diacyl glycerol all concentrations; [b]p<0.0001 VC vs. diacyl glycerol all concentrations; [c]p<0.05 VC vs. Sr^{2+} (n=50, 37, 18, 18, 92, 59, 77, 29, 81, 89 for strontium chloride, vehicle control, 1, 5, 10, 50, 100, 150, 200, 500 μg/mL respectively)

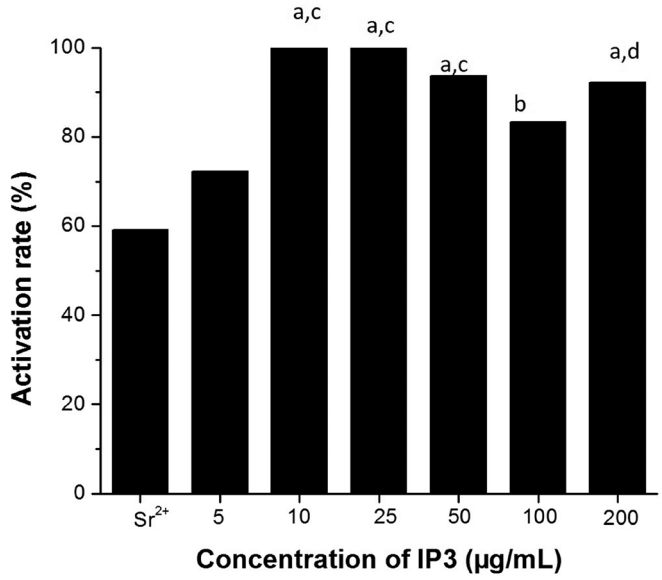

Figure 2. Effect of varying concentrations of inositol triphosphate on activation potential of mouse oocytes. Bar represents percentage of oocytes with pronuclei post activation
[a]p<0.0001, [b]p<0.001 vs. Sr^{2+}; [c]p<0.0001, [d]p<0.001 vs. 5 μg/mL of IP3 (n=50, 79, 77, 28, 67, 59, 20 for strontium chloride, 5, 10, 25, 50, 100, 200 μg/mL respectively)

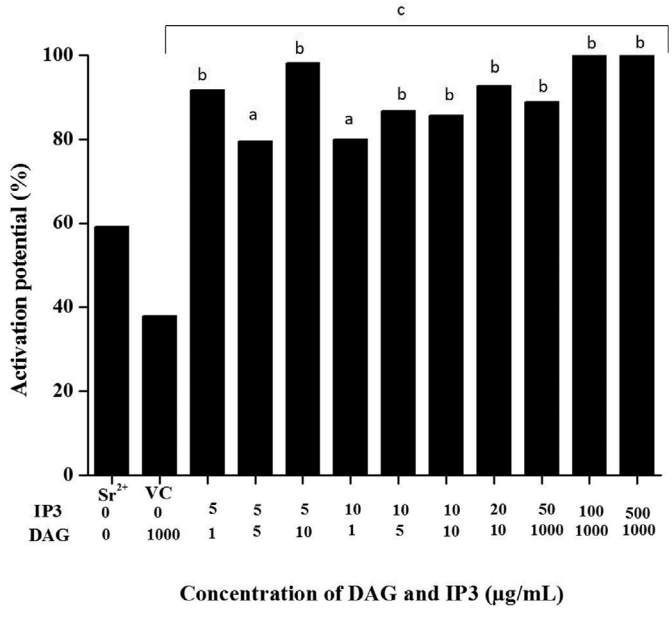

Figure 3. Effect of varying concentrations of inositol triphosphate on activation potential of mouse oocytes
[a]p<0.01 vs. Sr^{2+}; [b]p<0.0001 vs. strontium chloride; [c]p<0.0001 vs. vehicle control

Ploidy status of oocytes activated with LEDAG and IP3: Oocytes from the positive control group had clear pronuclei formation at 3 h after incubation with $SrCl_2$. Parthenotes derived from strontium chloride are usually haploid in nature, which was confirmed from the results of the present study (100% haploid parthenotes) (Table 1). However, both in the LEDAG and IP3 groups, pronucleus formation was observed only at 48 h after incubation, indicating a delayed activation process. In addition, a significantly higher percentage of diploid parthenotes were observed in the LEDAG (76.92%) and IP3 group (43.75%) compared with strontium ($p < 0.001$). Liposomes alone resulted in the formation of diploid parthenotes in ~ 4% of oocytes.

Developmental potential of oocytes activated with LEDAG and IP3

LEDAG: In the $SrCl_2$ group, within 24 h of activation, 92.92% of parthenotes progressed to the 2-cell stage (Table 2). The 2-cell rate in the LEDAG group was in the range of 57-85% and had no concentration-dependent effect (63.16, 84.21, 66.67, 62.67, 82.43, 62.03, and 57.83% in 1, 5, 10, 50, 100, 150, 200 and 500 μg/mL, respectively). Even in the vehicle control group, 60% of parthenotes progressed to the 2-cell stage, indicating that the phospholipids present in soy lecithin or cholesterol itself could induce parthenogenesis. Except in lower concentrations of LEDAG (1 and 5 μg/mL), none of the embryos progressed beyond the 6-8–cell stage. Even in these two concentrations, the embryos were arrested at the morula stage (5.26 and 13.16% in 1 and 5 μg/mL, respectively). This indicates that the use DAG alone is inefficient at driving the whole process of embryogenesis in activated oocytes.

IP3: A dose-dependent increase in the 2-cell rate was observed in groups activated with IP3 till 50 μg/mL above which a decrease in the 2 cell rate was observed (80 and 85% in 100 and 200 μg/mL, respectively) (Table 3). The embryos in lower

Table 1. Degeneration and fragmentation rate of oocyte after activation with various agents

Oocyte activating agents	Degeneration rate (%)	Fragmentation rate (%)	Ploidy status	
			Haploid (%)	Diploid (%)
Strontium chloride	5.13	9.02	100.00	0
Vehicle control	0	4.47	96.10	03.90
Diacyl glycerol (50 μg/mL)	0	5.15	23.08[a,b]	76.92
Inositol triphosphate (10 μg/mL)	0	5.26	56.25[a,b]	43.75

[a]p<0.001 vs. strontium chloride; [b]p<0.001 vs. vehicle control

Table 2. Developmental potential of oocytes activated with 1,2-Dipalmitoyl-sn-glycerol

Develop-mental stage	Control (Sr^{2+})	Vehicle control	Concentration of DAG (μg/mL)							
			1	5	10	50	100	150	200	500
2 cell (%)	92.92	60.00[d]	63.16[c,d]	84.21[b]	66.67[c,d]	62.67[c,d]	82.43[b]	82.76[b]	62.03[c,d]	57.83[c,d]
4 cell (%)	31.67	21.43[e]	23.68[e]	57.89[b]	35.29[d]	29.33[d]	35.14	27.59[e]	24.05[e]	26.51[e]
6-8 cell (%)	23.33	0.00	21.05	34.21	9.80[c,e]	10.67[a,d]	13.51[d]	3.45[c,e]	11.39[a,d]	8.43[b,e]
Morula (%)	15.51	0.00	5.26[a]	13.16	0.00	0.00	0.00	0.00	0.00	0.00
Blastocyst (%)	13.91	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

[a]p<0.05; [b]p<0.01; [c]p<0.001 vs. strontium chloride; [d]p<0.01; [e]p<0.001 vs. 5 μg/mL diacyl glycerol
DAG: diacyl glycerol

Table 3. Developmental potential of oocytes activated with inositol triphosphate

Developmental stage	Control (Sr^{2+})	Concentration of IP3 (μg/mL)					
		5	10	20	50	100	200
2 cell (%)	92.92	30.30[b,c,d]	61.19[b]	55.17[b]	100.00	80.00	85.00
4 cell (%)	31.67	20.00[c]	63.41[b]	56.25[a]	40.00	75.00[b]	47.06
6-8 cell (%)	23.33	0.00	42.31[a]	22.22	0.00	0.00	0.00
Morula (%)	15.51	0.00	0.00	0.00	0.00	0.00	0.00
Blastocyst	13.91	0.00	0.00	0.00	0.00	0.00	0.00

[a]p<0.01; [b]p<0.001 vs. strontium chloride; [c]p<0.01 vs. 10, 20 μg/mL; [d]p<0.001 vs. 100, 200 μg/mL
IP3: inositol triphosphate

concentrations (10 and 20 μg/mL) progressed till 6-8 cell stage (42.31 and 22.22%), whereas others were arrested at the 4-cell stage. Oocytes activated with IP3 failed to progress beyond 6-8 cell stage at all the concentration used, suggesting that even though the presence of IP3 in activation medium can induce high oocyte activation (delayed), they cannot support the development of early embryos.

Combination of LEDAG and IP3: Various concentrations of DAG (1, 5, 10 μg/mL) and IP3 (1, 5, 10, 20 μg/mL) were assessed at different combinations to see whether the activation and developmental potential of parthenotes improved in presence of the two secondary messengers together. In oocytes activated with $SrCl_2$, around 23% of parthenotes progressed to the 6-8–cell stage, with a blastocyst rate of 13.91% (Table 4). Oocytes activated with LEDAG alone developed only till the 4-cell stage, whereas 50% of oocytes activated with IP3 developed till the 6-8–cell stage. Except in activation medium containing 10 μg/mL IP3 and 5 μg/mL of LEDAG, the embryos failed to progress to the blastocyst stage in all other combinations. In this group, the blastocyst rate was found to be 5.26%, which was lower than in the positive control.

Discussion

In this study, we explored the ability of major secondary messengers in calcium signaling pathway, diacyl glycerol and inositol triphosphate as oocyte activating agents. In previous studies, the microinjection approach was used to deliver secondary messengers for oocyte activation (12, 13), which itself is limited by the presence of Ca^{2+} in the injection medium (14, 15), as well as pressure of injection (16). In the present study, IP3 was directly dissolved in the activation medium, and liposomes were used for the delivery of DAG due to their insoluble nature in aqueous solutions.

The use of DAG and IP3, alone and in combination, efficiently activated oocytes, with a lower percentage of degeneration and fragmentation in activated oocytes. Earlier studies observed that microinjection of DAG and IP3 into oocytes could elicit a similar pattern of calcium oscillation as observed during normal fertilization (2, 11-13). The presence of liposomes could induce activation in oocytes indicates that phospholipids present in soy lecithin and cholesterol also have the potential to cause artificial oocyte activation. To support this, earlier reports available in the literature showed that cholesterol could cause oocyte activation by increasing the intracellular calcium concentration (17), and phospholipids could cause oocyte activation through activation of protein kinase C (18).

One of the major limitations of using these agents for oocyte activation was the delay observed in the process of pronucleus formation, which took almost 48 h, unlike in strontium chloride, which takes only 3 h. Strontium chloride is known to cause long-lasting Ca^{2+} transients during artificial oocyte activation (13). In contrast, the microinjection of IP3 and its agonist was reported to result in calcium oscillations in a higher frequency

Table 4. Developmental potential of oocytes activated with varying combinations of inositol triphosphate and diacyl glycerol

Concentrations of IP3 and DAG		No. of oocytes	Developmental stage of embryo (%)			
IP3 (μg/mL)	DAG (μg/mL)		2 cell (%)	4 cell (%)	6-8 cell (%)	Blastocyst (%)
Sr^{2+}		50	92.92	31.67	23.33	13.91
VC		37	60.00	21.43	0	
5	1	24	47.62[d]	40.00[f]	0.00	0.00
5	5	28	63.16[d,h]	58.33[c,e,n]	04.17[d,g]	0.00
5	10	34	73.68[c,i]	50.00[b,g]	10.71[b,f]	0.00
10	1	36	19.44[b,j]	28.57	0.00	0.00
10	5	40	58.80[b]	47.50[a,f]	42.10[b,e,s]	05.26
10	10	32	56.25[b]	44.44[f]	0.00	0.00
20	10	30	38.64[b,k,l,m]	17.65[i,g,n]	38.64[a,e,s]	0.00
50	1	52	50.00[b]	11.54[g,q,r]	0.00	0.00
100	1	52	61.54[b]	40.63[f]	0.00	0.00
500	1	62	67.74[b,i]	28.57	02.38[b,g]	0.00

[a]p<0.05; [b]p<0.01; [c]p<0.001; [d]p<0.001 vs. Sr^{2+}; [e]p<0.01; [f]p<0.001; [g]p<0.001 vs. VC; [h]p<0.05; [i]p<0.01 vs. 5 and 1 μg/mL of IP3 and DAG respectively; [j]p<0.001 vs. all other combinations; [k]p<0.01 vs. 5 and 5, 100, and 1, 500 and 1 μg/mL of IP3 and DAG respectively; [l]p<0.001 vs. 5 and 10 μg/mL of IP3 and DAG respectively; [m]p<0.01, [n]p<0.001 vs. 10 and 5, 10 and 10 μg/mL of IP3 and DAG respectively; [o]p<0.01 vs. 5 and 1, 100 and 1 μg/mL of IP3 and DAG respectively; [p]p<0.0001 vs. 5 and 5, 5 and 10, 10 and 5, 10 and 10 μg/mL of IP3 and DAG respectively; [q]p<0.01 vs. 10 and 1, 500 and 1 μg/mL of IP3 and DAG respectively; [r]p<0.0001 vs. all other combinations; [s]p<0.0001 vs. 5 and 5, 5 and 10, 500 and 1 μg/mL of IP3 and DAG respectively.
DAG: diacyl glycerol; IP3: inositol triphosphate

than during normal fertilization (9, 13, 19-21), leading to a rapid desensitization of calcium stores (19, 20).

Strontium-induced oocyte activation is mediated through TRPM ion channels activating store-operated calcium (SOC) entry to refill endoplasmic stores to generate the transient calcium wave (22). Even though DAG activates TRPM channels directly (23-25), it fails to activate its downstream signaling molecules (24, 26) and SOC (23). Even uptake of Sr^{2+} and Ca^{2+} was hindered by the addition of DAG due to inactive SOC (23). A similar result was also observed in our study; the addition of Sr^{2+} after DAG activation failed to rescue delayed activation caused by DAG (data not shown). We hypothesize that the Ca^{2+} entry during DAG induced oocyte activation is through activating plasma membrane SOCs (23), which results in slow Ca^{2+} entry to oocytes and hence the delayed activation process. In the present study, we used 1,2-Dipalmitoyl-sn-glycerol, which has saturated fatty acids. It may be interesting to see whether the type of fatty acid present in DAG has any significant difference in calcium metabolism because wide varieties of saturated and unsaturated fatty acids are found in the cell membrane of the oocyte. Similarly, it is difficult to explain why IP3 causes a similar delay in activation and poor embryo development. It could be due to the shorter half-life of IP3 (27) and therefore, may require frequent additions of IP3 to induce the sufficient calcium spike required for the development of parthenotes. In addition, whether the soluble IP3 present in the medium can efficiently enter the oocyte must be ascertained in further experiments.

DAG and IP3, individually, were not capable of driving the development of parthenotes till the blastocyst stage. A similar observation was made by Schoenbeck et al. (11), who found that microinjection of DAG (1,2-Dioctanoyl-sn-glycerol) activated porcine oocytes efficiently but resulted in an arrest at the 4-cell to 6-8–cell stage. The poor development observed in the present study could also be related to the increased oocyte ageing because pronucleus formation itself takes almost 48 h from the collection of OCCs. Aged oocytes are known to have poor developmental potential *in vitro* (28, 29). However, when both these agents are used together, the intracellular calcium rise may be much higher than when they are used alone. The proposed mode of action of DAG and IP3 in oocyte activation is depicted in Figure 4.

During artificial activation of oocyte, cytochalasin D, a microtubule inhibitor, is usually used to derive the diploid

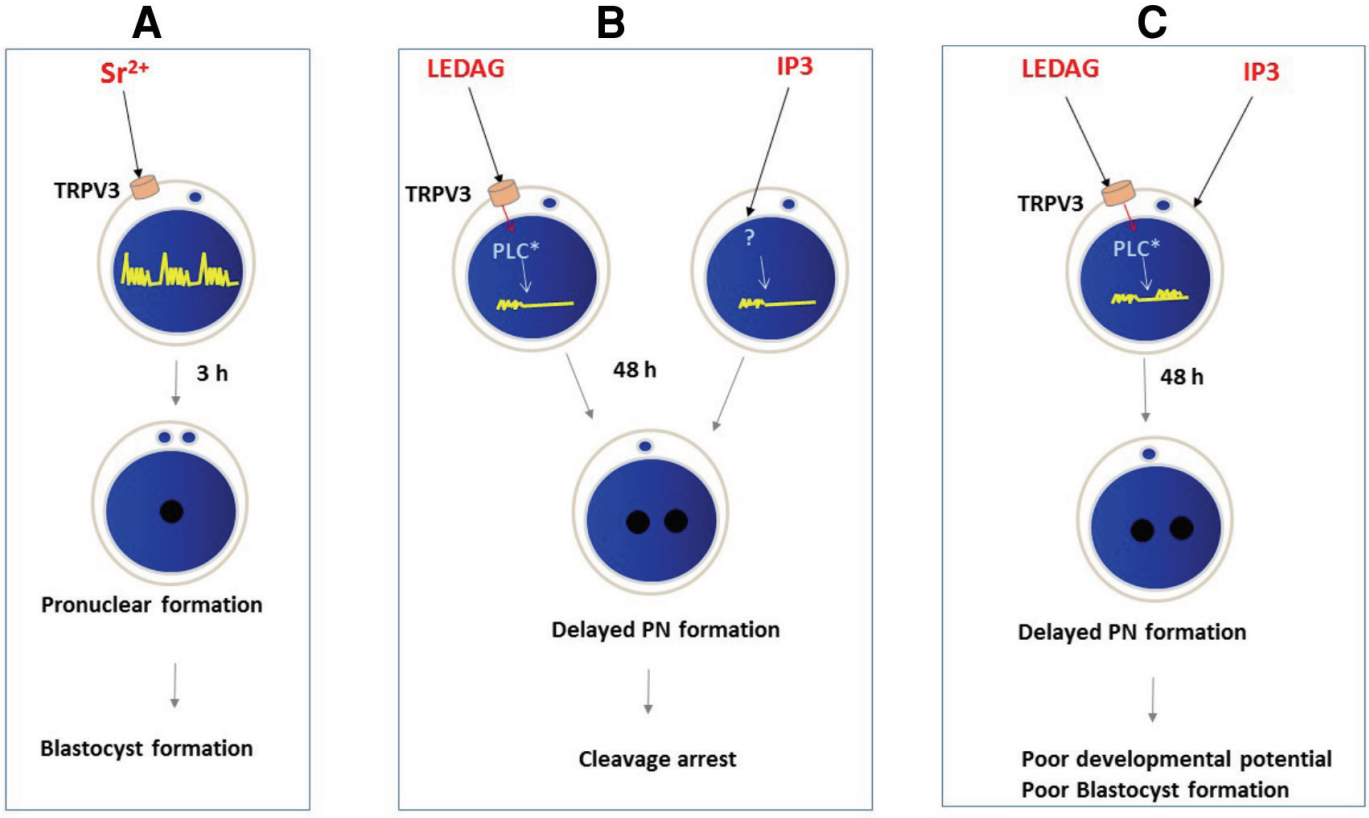

Figure 4. Mode of action of diacyl glycerol and inositol triphosphate in oocyte activation. A) Mode of action of strontium chloride-induced oocyte activation as proposed by Carvacho et al. (22). B) and C) Expected mode of action of diacyl glycerol and inositol triphosphate alone and in combination in inducing oocyte activation

LEDAG: Liposome-encapsulated diacyl glycerol; Sr^{2+}: Strontium; PN: Pronucleus; PLC*: Inactivated phospho lipase C; TRPV3: Transient receptor potential cation channel, subfamily V, member 3

parthenogenetic embryos (30, 31). Both DAG and IP3 were shown to increase the percentage of diploid parthenotes obtained after activation. In mouse oocytes, the use of protein kinase C as artificial oocyte activating agents are reported to form embryos without the extrusion of the polar body (2, 32, 33). Earlier studies proved that DAG and IP3 could activate protein kinase C (34, 35). Therefore, use of DAG and IP3, alone or in combination, can help to derive diploid parthenotes without using any microtubule inhibitors. The toxicity exerted by microtubule inhibitors on the development of embryos (36, 37) can be minimized because they are part of the calcium signaling pathway. This is further supported by the low degeneration rate and fragmentation of activated oocytes observed in our study.

In conclusion, liposomes can be used as an approach to deliver DAG across the oolemma. DAG and IP3, when used individually or in combination, induce delayed oocyte activation and poor embryo development. These agents may have potential application in developing diploid parthenotes when used along with efficient activating agents such as strontium because they are shown to give rise to 50% of diploid parthenotes without using any cytokinesis inhibitors during activation. Further studies are essential to understand the exact mechanism of action of these two agents in inducing delayed oocyte activation when supplemented exogenously.

Ethics Committee Approval: *Ethics committee approval was received for this study from the Institutional Animal Ethical Committee of Kasturba Medical College, Manipal (IAEC/KMC/64/2013).*

Informed Consent: *Not Applicable.*

Peer-review: *Externally peer-reviewed.*

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Revaluating the survival effects of International Federation of Gynecology and Obstetrics 1988 stage IIIA criteria for endometrial cancer

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Abstract

Objective: This study aimed to define factors that affected survival in the International Federation of Gynecology and Obstetrics (FIGO) 1988 stage IIIA endometrial cancer (EC).

Material and Methods: The study included patients with EC who underwent surgery between 1992 and 2013. Patients with adnexal metastases, uterine serosal involvement or positive peritoneal cytology (stage IIIA disease according to the former 1988 FIGO staging system) were selected for further analysis. Clinical and pathologic factors associated with progression-free survival (PFS) were evaluated using univariate and multivariate statistical tests.

Results: Seventy-seven patients with stage IIIA disease according to the 1988 FIGO staging system were included. The median follow-up was 37 months (range, 1-175 months) and recurrence was detected in 19 patients. Univariate analysis revealed that the presence of uterine serosal invasion and advanced histologic grade (grade 1-2 vs. grade 3) were associated with diminished PFS ($p=0.001$, $p=0.047$). The presence of adnexal involvement and positive peritoneal cytology had no statistically significant influence on PFS ($p=0.643$ and $p=0.795$, respectively).

Conclusion: In patients with stage IIIA EC according to the FIGO 1988 staging system, only uterine serosal involvement was related with adverse oncologic outcomes, not adnexal involvement or presence of positive cytology. (J Turk Ger Gynecol Assoc 2017; 18: 110-5)

Keywords: Endometrial cancer, peritoneal cytology, surgical staging

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Introduction

Endometrial cancer (EC) is the most frequent gynecologic tumor and the fourth most common malignancy in women (1). EC is mostly seen in the sixth and seventh decades and 95% of patients are aged over 40 years (2). According to International Federation of Gynecology and Obstetrics (FIGO), 83% of patients are diagnosed at stage I and II (3). Endometrioid-type constitutes 75% of the histologic types. Conversely, non-endometrioid types are high-grade aggressive tumors and mostly diagnosed at advanced stages (4, 5).

After 1988, in accordance with FIGO's proposition, EC has been staged surgically. The surgical stage is the most significant factor that determines prognosis (6). According to the staging system developed in 1988, peritoneal cytology was considered as an important variable, and positive peritoneal cytology, adnexal metastasis, and serosal invasion were defined as stage IIIA (7). Various studies showed that peritoneal cytology has important implications for prognosis (7-9). On the contrary, other investigators showed that positive cytology does not determine survival in the absence of serosal invasion and adnexal metastasis (10-12). After several studies on the prognostic value of peritoneal cytology, FIGO revised the EC staging system in 2009 and peritoneal cytology was excluded from the staging criteria (13).

In this study, we purposed to evaluate the prognostic value of peritoneal cytology in 1988 FIGO stage IIIA for endometrial malignancy and to determine factors that affect survival in this stage.

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Material and Methods

This study included patients with EC who underwent surgery in our unit between 1992 and 2013. Patients with ovarian and tubal metastases, uterine serosal involvement or peritoneal cytology (stage IIIA disease according to the former 1988 FIGO staging system) were selected for further analysis. Patients with sarcoma or synchronized gynecologic tumors were excluded from the study. Patients with FIGO 1988 stage IIIA EC were re-staged according to FIGO 2009 criteria. Demographics, intraoperative findings, and surgico-pathologic results were collected from patient files, pathology results, and electronic database of the department of gynecological oncology.

All patients underwent hysterectomy and bilateral salpingo-oophorectomy and peritoneal cytologic sampling. Systematic lymphadenectomy was performed for patients in whom frozen section revealed a non-endometrioid adenocarcinoma, stage III disease, deep myometrial invasion, cervical involvement, tumor size >2 cm or extrauterine spread. We defined recurrence between the levator muscle and linea terminalis as pelvic recurrence, recurrence between the linea terminalis and diaphragm muscle as upper-abdominal recurrence, and all other recurrences as extra-abdominal recurrence. Ascites and peritonitis carcinomatosa were considered as upper abdominal recurrence. Liver parenchyma, skin and bone recurrence were considered as extra-abdominal recurrence. Progression-free survival (PFS) was defined as the time between primary treatment and recurrence or last visit. The time until death of disease or until the last visit was defined as disease-specific survival (DSS).

SPSS (SPSS Inc, Chicago IL, USA) version 15.0 was used for statistical analyses. Kaplan-Meier analysis was used for the computation of PFS and DSS. Survival curves were checked using the log-rank test. Prognostic factors were analyzed using the Cox regression model. Factors with a p value below 0.25 in the univariate analysis were included in the multivariate analysis. The cut-off for statistical significance was set at $p<0.05$. As this work represents a retrospective chart review, local ethics committee permission was not sought. However, all patients signed informed consent thereby allowing our institution to use their clinical data.

Results

Seventy-seven patients with stage IIIA EC according to the 1988 FIGO staging system were available for analyses. Data of 1413 patients who had at least extrafascial hysterectomy + bilateral salpingo-oophorectomy were obtained from the gynecological oncology clinic electronic database and patient's files, retrospectively. Among these 1413 patients, 77 (5.4%) were at stage IIIA according to FIGO 1988 criteria. The median age was 59 years (range, 29-81 years). The pathologic characteristics of the patients are represented in Table 1. Sixty-six (85.7%) patients had endometrioid histology and more than half had deep myometrial invasion and serosal invasion. The mean tumor size was 36.4 mm (range, 16-60 mm).

Table 2 shows details of the pathologic features of the patients that were used to assign stage IIIA according to FIGO 1988. Isolated positive peritoneal cytology without serosal and adnexal involvement was positive in 11 (14.3%) patients. Isolated serosal invasion and isolated adnexal involvement were the cause of stage IIIA disease in 10 (13%) and 40 (51.9%) patients, respectively. Eleven patients who had isolated positive peritoneal cytology were re-evaluated

Table 1. Pathologic characteristics of patients with stage IIIA disease according to the International Federation of Gynecology and Obstetrics 1988 staging system (n=77)

Parameters	n	%
Tumor type		
Endometrioid	66	85.7
Serous	5	6.5
Undifferentiated	2	2.6
Mucinous	1	1.3
Mixed	3	3.9
FIGO Grade		
I	25	32.5
II	23	29.9
III	29	37.7
Depth of myometrial invasion		
No invasion	2	2.6
<1/2	26	33.8
≥1/2	32	41.6
Serosal invasion	17	22.1
Peritoneal cytology		
Negative	55	71.4
Positive	22	28.6
Adnexal metastases		
Negative	23	29.9
Positive	54	70.1
Lymphovascular space invasion		
Negative	49	63.6
Positive	28	36.4
Cervical invasion		
Negative	52	67.5
Positive	25	32.5
FIGO: International Federation of Gynecology and Obstetrics		

using FIGO 2009 staging criteria and 6 patients were found to have stage IA disease, 4 patients were defined as stage IB, and 1 was evaluated as having stage II disease. Sixty-four patients underwent systematic lymph node dissection. Lymphadenectomy could not be performed in 13 patients due to medical co-morbidities. The median number of harvested nodes was 58 (range, 11-103). There was no significant association between positivity of peritoneal cytology and surgico-pathologic factors such as adnexal metastasis, serosal involvement, tumor grade, cervical involvement, and depth of myometrial invasion.

The decision of adjuvant treatment and type of adjuvant therapy was given in gynecologic oncology council according to the risk factors of patients, including grade, myometrial invasion, serosal involvement, and cervical involvement. Seventy-one patients received adjuvant treatment. Of these, 14 patients received chemotherapy, 50 were irradiated, and 7 received combination chemo-radiotherapy. Sixty-one (97.2%) patients had complete clinical response after adjuvant treatment, and 2 (2.8%) had progressive disease under treatment. After the disease progressed, these 2 patients refused further treatment and were lost to follow-up.

The median follow-up was 37 months (range, 1-175 months) and recurrence was detected in 19 (24.7%) patients. Five patients had extra-abdominal recurrence, 4 had pelvic, 4 had upper abdominal, 4 had both pelvic and upper abdominal, and 2 had both pelvic and extra-abdominal recurrence. The estimated 5-year PFS was 68.6%. There were no deaths due to disease during follow-up; therefore, factors that determined DSS were not studied in this paper.

Univariate analysis revealed that the presence of uterine serosal invasion and advanced histologic grade (grade 1 and 2 vs. grade 3) were associated with diminished PFS (p=0.001, p=0.047, respectively) (Table 3). The presence of adnexal involvement and positive peritoneal cytology had no statistically significant influence on PFS (p=0.643, p=0.795, respectively).

Table 2. Pathologic features that necessitated the assignment of stage IIIA according to the International Federation of Gynecology and Obstetrics 1988 staging system

Parameters	n	%
Isolated peritoneal cytology	11	14.3
Isolated serosal invasion	10	13
Isolated adnexal metastases	40	51.9
Peritoneal cytology and serosal invasion	2	2.6
Peritoneal cytology and adnexal metastasis	9	11.7
Serosal invasion and adnexal metastasis	5	6.5
Peritoneal cytology and serosal invasion and adnexal metastasis	-	-

Subgroup analysis showed that there was no statistically significant difference between patients with negative cytology and those with isolated positive cytology in terms of PFS (Figure 1). The 5-year PFS was 68.3% and 69.1% in patients with negative and isolated positive cytology, respectively. Age, tumor histology, depth of myometrial invasion (patients with uterine serosal invasion were excluded), cervical involvement, lymphovascular space invasion, and type of adjuvant treatment were not related with PFS (Table 3).

Uterine serosal involvement, grade, cervical stromal invasion, and tumor type were used for the multivariate analysis model. Uterine serosal involvement was determined as the only independent adverse prognostic factor for recurrence (hazard ratio: 5.015, 95% confidence interval: [1.850-13.592]; p=0.002) (Table 4, Figure 2).

Table 3. Univariate analysis comparing factors associated with progression-free survival

Prognostic factor	5-year PFS, (%)	p value
Age		
<59	70.4	0.619
≥59	61.9	
Peritoneal cytology		
Negative	68.3	0.795
Positive	69.1	
Adnexal metastasis		
Negative	67.9	0.643
Positive	69.6	
Serosal invasion		
Negative	75.5	0.001
Positive	38.3	
Depth of myometrial invasion		
<1/2	85.7	0.070
≥1/2	65.9	
Grade		
I-II	74.2	0.047
III	57.4	
Tumor type		
Endometrioid	70.5	0.227
Non-endometrioid	50.1	
Lymphovascular space invasion		
Negative	69.4	0.348
Positive	67.1	
Cervical stromal invasion		
Negative	71.7	0.215
Positive	55.4	
PFS: progression-free survival		

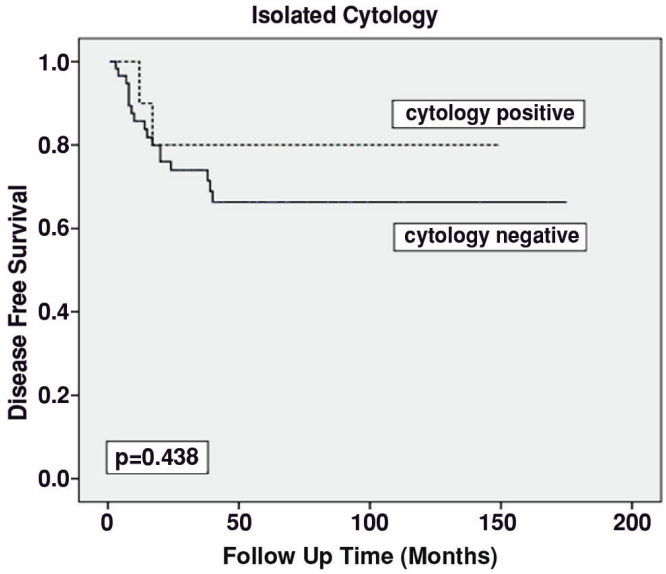

Figure 1. Survival effect of peritoneal cytology

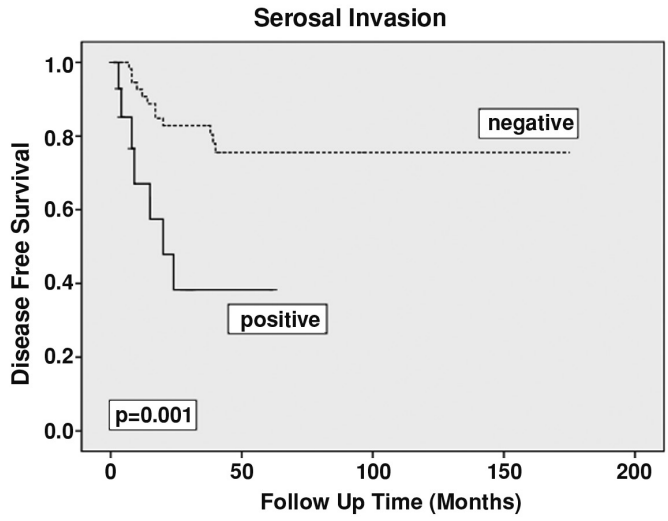

Figure 2. Survival effect of serosal invasion

Table 4. Multivariate analysis of factors determining recurrence

Parameters	p value	Hazard ratio	Confidence interval
Serosal invasion (negative vs. positive)	0.002	5.015	1.850-13.592
Grade (grade I and 2 vs. grade III)	0.081	2.293	0.903-5.825
Cervical invasion (negative vs. positive)	0.203	1.959	0.696-5.516
Tumor histology (endometrioid vs. non endometrioid)	0.287	2.002	0.558-7.181

Discussion

Despite many studies on the subject, the prognostic value of peritoneal cytology in EC has not yet been proven. There are studies that describe the prognostic value of peritoneal cytology in EC (7-9). In addition, previous studies demonstrated that peritoneal cytology is an independent prognostic factor (14-17). Garg et al. (8) defined peritoneal cytology as an independent prognostic factor in patients with EC limited to the uterus. The study contained 14,704 patients with tumors of variable histology that were limited to the uterus and revealed that peritoneal cytology was associated with diminished survival (8). In a study of 196 patients who had stage IIIA EC according to FIGO 2009 criteria, Milgrom et al. (18) showed that positive peritoneal cytology was related with cervical stromal invasion, ovarian and tubal involvement, and non-endometrioid type tumors. In our study, cervical invasion, adnexal involvement, serosal invasion, depth of myometrial invasion, and FIGO stage did not seem to have an effect on peritoneal cytology. This difference is related with the fact that we defined our study according to FIGO 1988 criteria, whereas Milgrom et al. (18) used FIGO 2009 criteria. In our study, there were only 11 patients with stage IIIA disease according to FIGO 1988 criteria because of isolated positive peritoneal cytology.

In our study, the 5-year PFS was 68%. Uterine serosal invasion was the only independent prognostic factor associated with PFS, whereas peritoneal positive cytology, adnexal metastasis, age, tumor histology, myometrial invasion depth, and lymphovascular space invasion were not associated with PFS. Milgrom et al. (18) showed that the 5-year PFS for stage IIIA patients according to 2009 criteria was 63%. In their study, the 5-year PFS was found as 39% in patients with positive peritoneal cytology and 69% for patients with negative peritoneal cytology ($p=0.001$). In another study, Garg et al. (8) reported that deep myometrial invasion, aggressive tumor type, absence of pelvic lymphadenectomy, and not receiving adjuvant radiotherapy were poor prognostic factors regarding PFS ($p=0.014$, $p<0.001$, $p=0.005$, $p<0.001$, respectively). They found that patients with positive peritoneal cytology had higher recurrence in the paraaortic lymph node region and peritoneal surface than those with negative peritoneal cytology; however, there was no difference regarding pelvic recurrence rates between these two patient groups. They also determined no meaningful higher recurrence rate in lymph node regions in patients

with positive peritoneal cytology (p=0.438) (19). In our study, we found no retroperitoneal recurrence in our cohort. The absence of lymphatic recurrence may be explained by our institution's aggressive lymphadenectomy approach to EC, which was to perform complete lymphadenectomy exclusively and not performing lymph node sampling. In the present study, the median number of extracted lymph nodes was 58, the median number of the pelvic lymph nodes was 42.5, and 15.5 for the paraaortic region.

In patients with FIGO 1988 stage IIIA EC, we found that uterine serosal involvement was the only prognostic factor associated with recurrence. Serosal involvement increased the recurrence rate 5-fold. Slomovitz et al. (20) reported that non-endometrioid type and adnexal/serosal involvement was associated with diminished survival in their study in patients with FIGO 1988 stage IIIA EC. They found no effect of peritoneal cytology on survival (20). Similarly, Preyer et al. (12) reported that adnexal and serosal involvement in patients with stage IIIA EC according to FIGO 1988 was related with poor survival rates. However, they determined no effect of peritoneal cytology on survival.

The fundamental restriction of our study is its retrospective nature. Although the number of patients seems to be limited, it is an extensive number of patients when it comes to stage IIIA disease. In addition, this was a single-center study and the practice of complete lymphadenectomy maintains the homogeneity of the group.

In conclusion, recurrence is seen in one quarter of patients with stage IIIA EC according to FIGO 1988 criteria. Only uterine serosal involvement was related with adverse oncologic outcomes, not adnexal involvement or presence of positive cytology.

Ethics Committee Approval: *As this work represents a retrospective chart review, the local ethics committee permission was not sought.*

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

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept – O.T., T.T.; Design – O.T., A.K.; Supervision – D.B., G.T.; Materials – G.K., G.K.; Data Collection and/or Processing – G.K., G.K., O.T.; Analysis and/or Interpretation – A.K., D.B.; Literature Review – O.T.; Writer – O.T.; Critical Review –T.T., D.B.*

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Risk factors for appendiceal involvement in women with epithelial ovarian cancer

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Abstract

Objective: To evaluate the risk factors for appendiceal involvement in women with epithelial ovarian cancer (EOC) who underwent appendectomy at the time of initial surgery.

Material and Methods: Patients with a final diagnosis of EOC who underwent appendectomy at the time of initial surgery were evaluated retrospectively. Risk factors related to the presence of appendiceal involvement were analyzed.

Results: A total of 210 patients underwent appendectomy during staging surgery. Appendiceal involvement was detected in 61 patients. No women with apparent clinical early-stage tumors had evidence of isolated metastatic disease to the appendix; therefore, no upstaging was detected due to solitary appendiceal involvement in this group of patients. For all patients, univariate analysis of the appendiceal involvement revealed age, stage, grade, extragenital organ involvement (omentum, bowel, peritoneum), positive cytology, and lymph node metastasis as significant factors ($p<0.05$). In the multivariate analysis, appendiceal involvement was significantly affected by age and omental involvement. Older age (>50 years) [odds ratio (OR) 2.8; 95% confidence interval (CI): (1.24-6.37); $p=0.014$] and presence of omental involvement [OR: 3.2; 95% CI: (1.22-8.59); $p=0.018$) seemed to be independent risk factors for appendiceal involvement in women with EOC.

Conclusion: Our findings indicate that routine appendectomy at the time of surgery for apparent early-stage EOC is not warranted. Nevertheless, the surgeon can take the initiative in regards to performing appendectomy because the morbidity rates due to this procedure are negligible. Older age (>50 years) and presence of omental involvement seem to increase the risk of appendiceal involvement by 2.8 and 3.2 times, respectively. (J Turk Ger Gynecol Assoc 2017; 18: 116-21)

Keywords: Epithelial ovarian cancer, appendectomy, risk factors

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Introduction

The 2014 statements of International Federation of Gynecology and Obstetrics (FIGO) recommends surgical staging for epithelial ovarian cancer (EOC) based on findings during exploration (1). The standard staging procedure for EOC includes exploratory laparotomy, extrafascial hysterectomy, bilateral salpingo-oophorectomy, pelvic and para-aortic lymph node (LN) dissection, ascites sampling/peritoneal washing, multiple peritoneal biopsies, and omentectomy.

It has been reported that the appendix was a potential site of involvement in patients with EOC (2). The presence of solitary appendiceal involvement upstages the disease to stage III. Therefore, appendectomy can be added to the staging surgery for both accurate staging and optimal cytoreduction. The majority of studies do not routinely recommend appendectomy in clinical early stage disease, i.e. stage I and II. The only indication for routine appendectomy is in patients with mucinous histology, even in early stages, in order to exclude a possible appendiceal carcinoma. However, routine removal of the appendix in other histologiesis also recommended by some authors so as to achieve complete staging and cytoreduction (3, 4).

In the present study, we aimed to evaluate the risk factors for appendiceal involvement in patients with EOC who underwent appendectomy during staging surgery by analyzing the histopathologic findings in appendectomy specimens. Complications related to appendectomy were also assessed.

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Material and Methods

Following the institutional review board approval, the pathological reports, medical records, and operation notes of patients who underwent staging surgery with a pre-diagnosis of EOC between January 2008 and December 2013 were retrospectively evaluated. All patients provided informed consent regarding the research and use of their medical information at admission.

All patients were staged according to the FIGO staging system for ovarian carcinoma. Data regarding age, menopausal status, disease stage, grade, histologic subtype, cytology, status of extragenital organ involvement, LN metastasis and complications related to appendectomy (e.g., peri-appendiceal abscess, intestinal obstruction, and peritonitis) were extracted from the records. Patients whose final pathology was any benign disease, primary appendiceal carcinoma, primary peritoneal cancer or metastatic disease were excluded. Appendiceal involvement was considered microscopic if the appendix was noted to be grossly normal by the operating surgeon and pathologist but histologic sections were positive for disease. If the appendix was observed abnormal during the surgery, the involvement was considered gross.

The primary end point of the present study was the determination of risk factors for appendiceal involvement in women with EOC.

Statistical analyses were performed using the Statistical Package for the Social Sciences software (version 18, SPSS, Inc. Chicago, IL, USA). Data are expressed as median and range for continuous variables and binary variables are reported as counts and percentages. A simple logistic regression analysis was performed to determine the correlation of patient and tumor characteristics with appendiceal involvement. A p value <0.05 was considered to indicate statistical significance. Variables with a p value less than 0.05 were included in the multiple logistic regression analysis. The impact of each factor on appendiceal metastasis was evaluated.

Results

A total of 210 patients with a final diagnosis of EOC who underwent appendectomy at the time of initial surgery were evaluated retrospectively. The median age of the patients was 51 years (range, 28-81 years). Patient and disease characteristics are shown in Table 1.

All patients with appendiceal involvement also had extra-pelvic disease, which already upstaged them to stage III or IV. The histopathologic results of patients who had appendiceal involvement are shown in Table 2. No evidence of macroscopic or microscopic metastasis in the appendix was observed in patients with early-stage EOC during intraoperative and postoperative evaluation. The rate of appendiceal involvement was significantly higher in patients with advanced-stage disease. For the entire cohort, univariate analysis revealed age, stage, grade, extra-genital organ involvement, positive cytology, and LN metastasis as significant risk factors for appendiceal involvement. However, no correlation was found between histopathology and appendiceal involvement. Univariate analysis of risk factors associated with appendiceal involvement in women with EOC (n=210) is shown in Table 2.

When women with advanced-stage disease were evaluated alone, age, presence of positive cytology, and involvement of the omentum and bowel were detected as significant factors via univariate analysis; however, stage, grade, histopathology, LN metastasis, and other organ involvement had no effect on appendiceal involvement (Table 3).

Table 1. Results of patients who underwent appendectomy at the time of initial surgery

Variables	Values
Age years, median, range	51 (28-81)
≤50 years, n (%)	97 (46%)
>50 years, n (%)	113 (54%)
Stage	
I, n (%)	58 (28%)
II, n (%)	12 (6%)
III, n (%)	133 (63%)
IV, n (%)	7 (3%)
Histopathology	
Serous, n (%)	109 (52%)
Endometrioid, n (%)	39 (18%)
Mucinous, n (%)	16 (8%)
Clear cell, n (%)	17 (8%)
Mixed, n (%)	29 (14%)
Grade	
Grade 1, n (%)	73 (35%)
Grade 2, n (%)	59 (28%)
Grade 3, n (%)	78 (37%)
Extragenital organ involvement	
Omentum, n (%)	93 (44%)
Bowel*, n (%)	65 (31%)
Urinary bladder, n (%)	18 (9%)
Peritoneum, n (%)	54 (26%)
Liver#, n (%)	9 (4%)
Spleen#, n (%)	7 (3%)
Appendix*, n (%)	61 (29%)
Positive cytology	102 (49%)
Lymph node metastasis	105 (50%)

n: number of patients, *: serosal and/or mucosal, #: capsule and/or parenchymal

In the multivariate analysis, older age (>50 years) [odds ratio (OR): 2.8; 95% confidence interval (CI): (1.24-6.37); p=0.014] and the presence of omental involvement [OR: 3.2; 95% CI: (1.22-8.59); p=0.018] were found as independent risk factors for appendiceal involvement in women with EOC (Tables 4 and 5).

No intraoperative or postoperative complications directly related with appendectomy were detected.

Table 2. Univariate analysis of risk factors for appendiceal involvement in women with epithelial ovarian cancer (n=210)

Variables	Cases n (%)	p
Age		
≤50 years	17/97 (17.5%)	**0.001**
>50 years	44/113 (38.9%)	
Stage		
I	0/58	
II	0/12	**<0.001**
III	56/133 (42.1%)	
IV	5/7 (71.4%)	
Histopathology		
Serous	40/109 (36.7%)	
Endometrioid	6/39 (15.3%)	
Mucinous	3/16 (18.7%)	**0.035**
Clear cell	2/17 (11.7%)	
Mixed	10/29 (34.4%)	
Grade		
Grade I	5/73 (6.8%)	
Grade II	18/59 (30.5%)	**<0.001**
Grade III	38/78 (48.7%)	
Extragenital organ involvement		
Yes	61/124 (49.2%)	**<0.001**
No	0/86	
Omentum		
Yes	52/93 (55.9%)	**<0.001**
No	9/117 (7.6%)	
Bowel*		
Yes	35/65 (53.8%)	**<0.001**
No	26/145 (17.9%)	
Urinary bladder*		
Yes	8/18 (44.4%)	0.132
No	53/192 (27.6%)	
Peritoneum		
Yes	26/54 (48.1%)	**<0.001**
No	35/156 (22.4%)	
Cytology		
Positive	50/102 (49%)	**<0.001**
Negative	11/108 (10.1%)	
Lymph node metastasis		
Yes	48/105 (45.7%)	**<0.001**
No	13/105 (12.3%)	

n: number of patients, *: serosal and/or mucosal

Table 3. Univariate analysis of risk factors for appendiceal involvement in women with advanced-stage epithelial ovarian cancer (n=140)

Variables	Cases n (%)	p
Age		
≤50 years	17/56 (30.3%)	**0.01**
>50 years	44/84 (52.3%)	
Histopathology		
Serous	40/86 (46.5%)	
Endometrioid	6/18 (33.3%)	
Mucinous	3/9 (33.3%)	0.657
Clear cell	2/7 (28.5%)	
Mixed	10/20 (50%)	
Grade		
Grade I	5/15 (33.3%)	
Grade II	18/47 (32.3%)	0.366
Grade III	38/78 (48.7%)	
Extragenital organ involvement		
Yes	61/121 (50.4%)	**<0.001**
No	0/19	
Omentum		
Yes	52/93 (55.9%)	**<0.001**
No	9/47 (19.1%)	
Bowel*		
Yes	35/65 (53.8%)	**0.022**
No	26/75 (34.6%)	
Urinary bladder*		
Yes	8/18 (44.4%)	0.936
No	53/122 (43.4%)	
Peritoneum		
Yes	26/51 (50.9%)	0.181
No	35/89 (39.3%)	
Cytology		
Positive	50/96 (52.1%)	**0.004**
Negative	11/43 (25.5%)	
Lymph node metastasis		
Yes	48/105 (45.7%)	0.376
No	13/35 (37.1%)	

n: number of patients, *: serosal and/or mucosal

Table 4. Multivariate analysis of risk factors for appendiceal involvement in women with epithelial ovarian cancer (n=210)

	OR	CI (95%)	p
Age			
≤50 years	1	1.23-7.02	0.015
>50 years	2.9		
Omental metastasis			
Absent	1	1.3-10.1	0.017
Present	3.6		

OR: odds ratio; CI: confidence interval

Table 5. Multivariate analysis of risk factors for appendiceal involvement in women with advanced-stage epithelial ovarian cancer (n=140)

	OR	CI (95%)	p
Age			
≤50 years	1	1.24 - 6.37	0.014
>50 years	2.8		
Omental metastasis			
Absent	1	1.22 - 8.59	0.018
Present	3.2		
OR: odds ratio; CI: confidence interval			

Discussion

The main target in the treatment for EOC is accurate surgical staging and maximal cytoreduction. This procedure is extremely important and necessary in order to increase the rates of disease control and survival.

Ovarian cancer initially spreads inside the abdominopelvic cavity. Although patients with clinically early-stage disease rarely tend to have appendiceal involvement, evaluation of the appendix is necessary during surgery because appendiceal involvement leads to upstaging and demands adjuvant treatment. The rate of appendiceal metastasis has been shown to be low; therefore, routine performance of appendectomy in women with early-stage EOC is still controversial. Routine performance of appendectomy is justified by some authors in all stages of EOC in order to achieve complete staging (3, 4). On the contrary, other studies suggested that appendectomy was not warranted in early-stage EOC because the risk of involvement was extremely low (2, 5-8).

Westermann et al. (2) retrospectively evaluated the results of 53 patients with EOC who underwent appendectomy during staging surgery and reported the rate of appendiceal involvement as 34%. Four of these patients had normal appendixes at macroscopic evaluation. However, it is impossible to assess the actual rate of appendiceal involvement in early-stage disease because the authors did not mention the stage of the patients.

The first study that reported an appendiceal involvement rate and made a distinction between early and advanced-stage disease was published by Malfetano (5). In that study, the rate of appendiceal involvement was 51% and 70% in all patients and in women with advanced-stage disease, respectively. Appendiceal involvement did not lead to upstaging in any patients with early-stage disease, and was not the solitary site of involvement in women with stage III disease. Fontanelli et al. (6) reported the rate of appendiceal involvement as 23% in 160 patients with EOC. The authors stated that 91% of these 23 patients had serous histology and grade 2-3 disease, adding that no appendiceal involvement was observed in early-stage EOC. Ramirez et al. (7) reported the results of 57 patients with stage I-II ovarian cancer and stated that no appendiceal involvement or appendectomy-related complications were observed. The largest study on appendiceal involvement in EOC was published by Lee et al. (9) which reported the results of 149 women who had no clinical disease outside the pelvis. They reported no appendiceal involvement or upstaging due to isolated appendiceal involvement in these patients. As a conclusion of these studies, routine performance of appendectomy is not recommended in patients with stage I-II EOC.

On the other hand, there are two studies that reported microscopic appendiceal involvement in patients with apparent early-stage EOC. The first study was reported by Rose et al. (3) who performed appendectomy in 80 patients during staging surgery and found the appendiceal involvement rate as 31%. Nevertheless, they stated that in a total of 47 patients with early-stage disease, microscopic involvement of the appendix was detected in 2 (4.3%) patients, but added that these patients already had stage III disease due to omental involvement. None of the patients with early-stage disease were upstaged due to isolated appendiceal involvement; however, appendiceal involvement was detected in 70% of patients who already had advanced-stage disease. In addition, the appendiceal involvement rate was higher in patients with serous carcinoma compared with those with mucinous carcinoma (48% vs. 8%, respectively). Although the results of this study were consistent with earlier series, the authors recommended routinely removing the appendix owing to the low morbidity rate related to appendectomy. Subsequently, the authors reported a case with isolated microscopic appendiceal involvement in a patient with clinical early-stage EOC (10). The second study that recommended the routine removal of the appendix was published by Ayhan et al. (4). In that study, the rate of appendiceal involvement was reported as 9.8% in 102 patients with clinical stage I-II disease. In a total of 10 patients with microscopic appendiceal involvement, 5 patients were upstaged due to this finding. The overall rate of appendiceal involvement was reported as 37% and the rate of upstaging due to isolated appendiceal involvement was 5% in patients with clinical early-stage disease. Therefore, the authors recommended that routine appendectomy should be performed during staging surgery in all patients with EOC, even if they have clinical early-stage disease.

In the present study, 210 women who underwent appendectomy at initial surgery were evaluated. None of the patients with an apparent clinical early-stage tumor had evidence of isolated appendiceal involvement; as such, upstaging due to solitary involvement of the appendix was not detected in this group of patients, consistent with the results of earlier series.

Nevertheless, routine appendectomy is recommended particularly in patients with ovarian mucinous cystadenocarcinoma owing to the fact that it is frequently observed as a metastatic tumor from the gastrointestinal system in which the appendix may contain the primary lesion (11, 12). However, the rate of appendiceal involvement was 18.7% in women with mucinous cystadenocarcinoma in the current study. The relatively low rate of appendiceal involvement in mucinous histology can be attributed to the limited number of patients with mucinous histology (n=18) in our cohort. Although our findings do not completely support the concept of routine performance of appendectomy in women with mucinous EOC, appendectomy should be a routine component of comprehensive surgical staging in all patients with mucinous histology based on the evidence in the literature. However, there are also studies that reported higher rates of appendiceal involvement in patients with advanced-stage serous adenocarcinoma (3, 6, 13).

Tumor grade was detected as a prognostic factor for appendiceal involvement. In the study by Ayhan et al. (4) the rates of appendiceal involvement in women with grade 1, 2, and 3 disease was reported as 18.2%, 33.8%, and 48.2%, respectively. However, grade was not detected as a significant factor for appendiceal involvement via multivariate analysis, stage being the only factor that was statistically significant. Furthermore, patients with grade 2 and 3 EOC had a higher rate of appendiceal involvement in the study by Malfetano (5).

In the present study, univariate analysis revealed age, stage, grade, extra-genital organ involvement, presence of positive cytology, and LN metastasis as significant factors for appendiceal involvement in all patients, but histopathology was not found to be a risk factor for the involvement of the appendix. When patients with advanced-stage disease were evaluated alone, age, presence of positive cytology, and involvement of the omentum and bowel were found as significant factors in univariate analysis, but stage, grade, histopathology, LN metastasis, and other organ involvement had no effect on the risk of appendiceal involvement. In the multivariate analysis, the risk of appendiceal involvement was significantly affected by age and omental involvement. In addition to these findings, we observed no complications directly related with appendectomy in the present study.

Although this study represents one of the largest series on the evaluation of appendiceal involvement in EOC, the retrospective design of this study may be a significant source of bias. However, to our knowledge, this is the first study in the literature to mention omental involvement as a risk factor for appendiceal involvement in EOC.

The only definitive indication for appendectomy in early-stage EOC seems to be mucinous histopathology in order to exclude ovarian metastasis of primary appendiceal carcinoma in light of data in the literature.

Conclusion

Our findings indicate that routine appendectomy at the time of surgery for apparent early-stage EOC is not warranted. Nevertheless, surgeons can take the initiative in regards to performing appendectomy because the morbidity rate due to this procedure is negligible. Older age (>50 years) and the presence of omental involvement seem to increase the risk of appendiceal involvement in EOC.

Ethics Committee Approval: *Following the institutional review board approval, the pathological reports, medical records, and operation notes of patients who underwent staging surgery with a pre-diagnosis of EOC between January 2008 and December 2013 were retrospectively evaluated.*

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

Peer-review: *Externally peer-reviewed.*

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Platelet-to-lymphocyte ratio: A new inflammatory marker for the diagnosis of preterm premature rupture of membranes

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Abstract

Objective: Preterm premature rupture of membranes (PPROM) is closely related with maternal and fetal complications. Therefore, early diagnosis is extremely important to provide maternal and fetal well-being. Many inflammatory markers have been evaluated for their ability to diagnose membrane rupture at early stages. We aimed to investigate the relationship between the platelet-to-lymphocyte ratio (PLR) and preterm premature membrane rupture.

Material and Methods: In this study, 121 pregnant women with PPROM and 96 age-matched pregnant women with spontaneous preterm labor who were admitted to our hospital between January 2014 and December 2015 were enrolled. Demographic data, complete blood cell count results, and neonatal outcomes were recorded.

Results: The neutrophil and platelet counts were higher in the PPROM group (9948.4±3393.2 vs. 7466.1±1698.5/mm^3 and 244.5±60 vs. 210.6±64.8/mm^3, respectively, $p<0.001$). The PLR and neutrophil-to-lymphocyte ratios (NLR) were both significantly higher in the PPROM group ($p<0.001$). Correlation analysis revealed that the PLR was positively correlated with the NLR ($r=0.10$, $p=0.031$). The ability of the PLR to diagnose preterm premature rupture of membranes was evaluated using an ROC curve. The sensitivity and specificity of the PLR was 57.8% and 73.7%, respectively, at a threshold >117.14 ($p<0.001$).

Conclusion: The PLR might be a cost effective, easy to use, and practical marker for the early diagnosis of PPROM, which can help to determine the appropriate waiting time for delivery and provide maternal and fetal well-being. (J Turk Ger Gynecol Assoc 2017; 18: 122-6)

Keywords: Inflammatory markers, platelet-to-lymphocyte ratio, preterm premature rupture of membranes

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Introduction

Preterm premature rupture of membranes (PPROM), which is defined as spontaneous rupture of fetal membranes before labor begins before 37 weeks' gestation, affects approximately 3% of all pregnancies (1). It is closely related with significant maternal and fetal morbidity and mortality. PPROM is one of the most common causes of preterm delivery, and is associated with maternal and neonatal infections (2, 3). The risk of chorioamnionitis is approximately 6-10% and increases to 40% if it prolongs over 24 hours (4). Moreover, neonatal infection risk is two times greater in patients without chorioamnionitis (5). Infection risk increases with PPROM, and neonatal hypoxia

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and jaundice are also more common in this condition (6). Early diagnosis is very important to provide maternal and fetal well-being because of these serious complications (7). Even though the pathophysiologic mechanism of PPROM has not been clearly defined and is multifactorial; inflammation plays a crucial role in the rupture of membranes (8). The role of inflammation in PPROM has been evaluated in many studies, and a significant association between various inflammatory markers and PPROM has been reported (9-11). Many inflammatory markers were recently evaluated for their ability to diagnose membrane rupture at early stages.

In chronic inflammatory processes, megakaryocytic series proliferate increasingly and lymphocyte counts tend to decrease due to severe apoptosis. As a consequence, markers obtained from total blood counts such as the platelet-to-lymphocyte ratio (PLR) can be affected in severe chronic inflammatory diseases (12).

PLR is a widely available, effective, and simple marker. It has been proposed as a predictive and prognostic parameter for many kinds of diseases such as cardiovascular diseases and malignancies (13, 14). Also, it has been shown to be related with gestational diabetes mellitus, acute appendicitis, preeclampsia, recurrent pregnancy loss, and preterm labor in pregnant women (15-18). There are scant data about the relation between PLR and presence of PPROM in the literature. Therefore, we investigated the role of PLR for predicting PPROM at early stages.

Material and Methods

Study population and data collection

This is a prospective case-control study, in which 121 pregnant women with PPROM and 96 age- matched pregnant women with spontaneous preterm labor between January 2014 and December 2015 were enrolled. It was conducted at a university-affiliated research and training hospital.

Age, gestational week, gravida, parity, delivery mode, birth weight, APGAR score, neonatal intensive care unit (NICU) admission rate, presence of neonatal sepsis, and development of respiratory distress syndrome (RDS) were recorded from medical records. In addition, results of a complete series of routine laboratory investigations including complete blood cell counts were recorded. Blood samples were taken from all study participants on admission and complete blood counts were analyzed using a Coulter LH 780 Hematology Analyzer (Beckman Coulter Ireland INC, Mervue, Galway, Ireland). The neutrophil-to-lymphocyte ratio (NLR) was calculated by dividing the neutrophil count by the lymphocyte count, and PLR was calculated as the number of platelets divided by the lymphocyte count, both of which were obtained from the same blood samples.

Inclusion criteria

The inclusion criteria included PPROM diagnosed as defined between 24-37 gestational weeks of pregnancy, and eligible for recording complete blood samples and other clinical perinatal findings.

Exclusion criteria

We excluded patients with multiple gestations, hematologic disorders, malignancies, hepatic disease, history of autoimmune disease, any inflammatory disease of pregnancy such as gestational diabetes mellitus and preeclampsia, any acute or chronic infectious or inflammatory diseases, pregnancies with fetal chromosomal anomalies, intrauterine growth restriction, any fetal infection, and women who underwent any invasive procedures such as amniocentesis.

Diagnosis of preterm premature rupture of membranes

PPROM was diagnosed if 1 and one of the other following were present; 1) all patients were asked for risk factors and any fluid leakage before 37 weeks' gestation and regular uterine contractions, 2) examination in dorsolithotomy position with a sterile speculum to verify the pooling of amniotic fluid in the fornices or active flowing of amniotic fluid from the cervix, 3) positive nitrazine test, 4) when necessary, confirming the presence of insulin-like growth factor binding proteins (ACTIM PROM test; MedixBiochemica, Kauniainen, Finland) in the vaginal fluid.

All participants gave informed consent and the local ethics committee approved the study.

Statistical analysis

SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. The Shapiro-Wilk test was used to determine whether the variables were distributed normally. Categorical variables were presented as frequencies and/or percentages, and continuous, normally distributed variables were stated as mean ± SD. Student's t-test or the Mann-Whitney U test were performed to compare normally distributed continuous numeric variables, and the Chi-square test was used to compare categorical variables between the two groups. In order to determine the sensitivity and specificity of PLR values to predict PPROM, receiver-operator curve (ROC) analysis was performed. The area under the curve (AUC) value, specificity, sensitivity were reported. A p value of ≤ 0.05 was considered statistically significant.

Results

Baseline demographic and clinical features of the patients were shown in Table 1. There was no difference between the

two study groups in terms of age, gravida, parity, gestational age and lymphocyte count (p>0.05). The neutrophil count was significantly higher in patients with PPROM as compared with controls (9948.4±3393.2 vs. 7466.1±1698.5/mm^3, p<0.001). Similarly, the platelet count was found to be significantly higher in the PPROM group (244.5±60 vs. 210.6±64.8 x1000/mm^3, p<0.001). NLR and PLR were both higher in the PPROM group (p<0.001). Correlation analysis revealed that PLR levels were positively correlated with NLR (r= 0.10, p=0.031).

The neonatal outcomes of pregnancies were presented in Table 2. The groups did not significantly differ with regard to birth weight, RDS, APGAR score, and NICU admissions (p>0.05). Sepsis was more common in the PPROM group (34.7% vs. 19.8%, p=0.02).

The ability of the PLR to diagnose PPROM was evaluated using ROC curve analysis. The AUC for PLR was 0.62 (p<0.001) (Figure 1). The sensitivity and specificity of the PLR was 57.8% and 73.7%, respectively, at a threshold >117.14. PLR values >117.14 were significantly related with increased risk of PPROM.

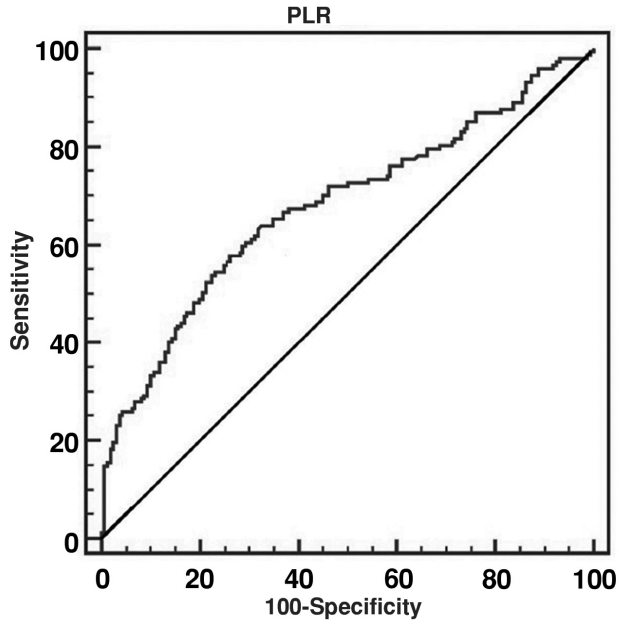

Figure 1. Receiver operating curve for PLR for the diagnosis of PPROM
PLR: platelet to lymphocyte ratio, PPROM: preterm premature rupture of membranes

Table 1. Demographic and clinical characteristics of patients

	PPROM group (n=121)	Control group (n=96)	p value
Age (years)	28.7±5.1	29.4±5.0	0.56[a]
Gravida (number)	3.9 ±2.7	3.4±1.8	0.10[b]
Parity (number)	2.0±1.1	1.9±1.1	0.84[b]
Gestational age (week)	33±2.1	34±1.8	0.91[a]
Neutrophil count (/mm^3)	9948.4±3393.2	7466.1±1698.5	**<0.001**[a]
Lymphocyte count (/mm^3)	1876.8±649.7	2044.6±694.1	0.10[a]
Platelet count (x1000/mm^3)	244.5±60.0	210.6±64.8	**<0.001**[a]
NLR	5.38± 3.3	3.85 ±2.4	**<0.001**[a]
PLR	126.3±68.9	106.9±49.4	**<0.001**[a]

NLR: neutrophil to lymphocyte ratio; PLR: platelet to lymphocyte ratio; PPROM: preterm premature rupture of membranes
[a]: Independent sample t test, [b]: Mann Whitney U test

Table 2. Neonatal outcomes of pregnancies

	PPROM group (n=121)	Control group (n=96)	p value
Birth weight (grams)	2120.4±1078	2345.2±924.6	0.11[a]
Sepsis (n,%)	42 (34.7%)	19 (19.8%)	**0.02**[b]
Respiratory distress syndrome (n,%)	20 (16.5%)	16 (16.7%)	0.98[b]
APGAR score (1st min)	6.9±1.2	7.1±1.5	0.28[a]
APGAR score (5th min)	7.8±1	8±1.2	0.18[a]
Neonatal intensive care unit admission (n,%)	67 (55.4%)	45 (46.9%)	0.21[b]

PPROM: preterm premature rupture of membranes
[a]: Independent sample t test, [b]: Chi-square test

Discussion

The main findings of our study are as follows: (1) NLR and PLR were both significantly higher in the PPROM group as compared with controls (2). With the exception of sepsis, a similar relation was found between the two groups according to the neonatal outcomes of pregnancies; sepsis was more common in the PPROM group (3). PLR values >117.14 were significantly related with an increased risk of PPROM.

PPROM, the exact pathophysiology of which is still controversial, leads to common and serious pregnancy complications such as RDS, intraventricular hemorrhage, necrotizing enterocolitis, sepsis, and sudden intrauterine death due to umbilical cord compression. Recent studies demonstrated that the major etiologic mechanism of PPROM was inflammation (19, 20). However, many inflammatory markers have been studied for their ability to diagnose PPROM accurately; a reliable marker for diagnosing PPROM that can demonstrate intraamniotic or placental inflammation is not evident.

Cytokines that participate in inflammatory reactions have been reported to be associated with PPROM. Satar et al. (21) reported that interleukin (IL)-8 levels were increased in PPROM in maternal serum and in the umbilical cord. Similarly, IL-6 was found elevated only in the umbilical cord, especially in PPROM with microbial invasion and histologic chorioamnionitis (21). In the study of Flídrová and Krejsek (22), cytokines such as tumor necrosis factor (TNF)-α, IL-8, IL-6, and IL-1, were reported to be increased in preterm birth and PPROM.

A study by Popowski et al. (23) demonstrated that C-reactive protein was elevated in patients with PPROM with clinical and histopathologic chorioamnionitis. Also, procalcitonin, proadrenomedullin, and serum amyloid A levels were reported to be related with chorioamnionitis before any clinical signs appear (24).

Another marker that can play a role in inflammatory processes is NLR. In systemic inflammatory conditions, leukocyte subtypes differentiate as an immune response. Neutrophil counts increase and lymphocyte counts decrease. As such, the NLR tends to alter in various systemic inflammatory diseases. Several studies demonstrated the prognostic and predictive value of increased NLR in cancers such as colorectal cancer, lung cancer, and hepatocellular carcinoma (25-27). Also, NLR was found significantly altered in many conditions of pregnancy. Kurtoglu et al. (28) reported high NLR values in preeclampsia. Likewise, NLR values were found altered in gestational diabetes, intrahepatic cholestasis, hyperemesis gravidarum, and acute appendicitis of pregnancy (15, 16, 29, 30). In the study of Köseoğlu et al. (24), NLR was higher in the PPROM group than in controls. The authors concluded that NLR was a useful marker for predicting PPROM (24). In our study, we found higher NLR levels in the PPROM group, consistent with the literature.

PLR is a widely-used marker, which has been demonstrated to predict thrombotic events, inflammatory diseases, and malignancies. Many previous studies reported a significant association between increased PLR and major adverse outcomes in cardiovascular diseases, and reduced survival in malignancies such as pancreatic, colorectal cancer, and endometrial cancer (13, 14, 31, 32). In pregnant women, PLR was investigated in gestational diabetes, acute pancreatitis, preeclampsia, and PPROM (10, 15, 17, 33, 34). In PPROM, Ekin et al. (10) found that the PLR showed no significant alteration between their oligohydramnios and normal amniotic fluid index groups. Another study that investigated the relationship between PLR and PPROM was designed regarding the latency period. PLR was not found to be significant between latency periods <72 hours and >72 hours (34). In our study, regardless of the latency period and amniotic fluid index, statistically significant PLR values were found in patients with PPROM.

In conclusion, we demonstrated an important relation between PLR values of more than 117.14 and the occurrence of PPROM. Moreover, it was found to be a significant independent discriminator for PPROM, a condition that leads to adverse maternal and neonatal events. PLR is a cost effective, easy to use, and practical marker that can be used for the early diagnosis of PPROM, which can help to provide maternal and fetal well-being.

Study limitations

There are some limitations of the present study. First, this study has a small sample size. Second, it lacks the measurement and correlation analysis of well-known inflammatory markers such as C-reactive protein.

Ethics Committee Approval: *Ethics committee approval was received for this study from the ethics committee of Başkent University Konya Training and Research Hospital (No: 019).*

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

Peer-review: *Externally peer-reviewed.*

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Lymph node dissection in atypical endometrial hyperplasia

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Abstract

Objective: The rate of concomitant endometrial carcinoma in patients with atypical endometrial hyperplasia is high. We aimed to investigate the role of lymphadenectomy in deciding adjuvant treatment in patients with concomitant atypical endometrial hyperplasia and endometrial carcinoma.

Material and Methods: Women with atypical endometrial hyperplasia were enrolled in this retrospective study. Lymph node dissection was performed in only some patients who gave informed consent if their surgeon elected to do so, or if the intraoperative findings necessitated. The final histopathologic evaluations of surgical specimens were compared with endometrial biopsy results.

Results: Eighty eligible patients were evaluated. Seventy-two (90%) patients had complex hyperplasia with atypia, and 8 (10%) patients had simple hyperplasia with atypia. Hysterectomy and bilateral salpingo-oophorectomy were performed to all patients; 37 also underwent lymph node dissection. Lymph node dissection was extended to the paraaortic region in 9 of 37 patients. The concomitant endometrial carcinoma rate was 50%. Two patients had lymph node metastasis. Among 40 cases of carcinoma, 17 had deep myometrial invasion and/or cervical or ovarian involvement or grade 2 tumors with superficial myometrial invasion on hysterectomy specimens; 27.5% of all carcinomas were stage Ib or higher.

Conclusion: The concomitant endometrial carcinoma rate was high in patients with atypical endometrial hyperplasia. Nearly half of these patients had risk factors for extrauterine spread. Lymph node dissection might be helpful to decide adjuvant treatment. (J Turk Ger Gynecol Assoc 2017; 18: 127-32)

Keywords: Atypical hyperplasia, concomitant, endometrial carcinoma, lymphadenectomy

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Introduction

The rate of concomitant endometrial cancer in patients with atypical endometrial hyperplasia (AEH) is high in hysterectomy specimens. Although some factors have been suggested to predict concomitant endometrial cancer such as older age, diabetes and obesity (1), there is no tool to predict concomitant malignancy precisely, and the vast majority of cases are diagnosed postoperatively in hysterectomy specimens. Moreover, intraoperative frozen section assessment with high accuracy is not available in most centers.

Postoperative histopathologic findings may be discordant to either pre- or intra-operative diagnoses. This issue makes the extent of surgery for AEH controversial, as it is in endometrial carcinoma. Hysterectomy may be insufficient in the event of concomitant carcinoma, especially if the patient has high risk factors, and lymph node status should be known to plan adjuvant treatment. Many reports underline the possibility of lymph node metastases, which cannot be ignored (2), and some surgeons recommend lymphadenectomy (3).

In the present study, we examined the rate of concomitant carcinoma, risk factors for extrauterine spread, and the role of lymph node dissection in deciding adjuvant treatment in patients with postoperative diagnosis of invasive carcinoma among patients who underwent surgery because of AEH, some including lymph node dissection.

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Material and Methods

The medical records from between January 2006 and December 2014 were reviewed and eighty patients who were diagnosed as having complex or simple AEH according to the World Health Organization classification system on endometrial biopsy were enrolled in the study. All endometrial biopsies were obtained with suction curettage (Pipelle) and all specimens were placed in formalin before the pathologic examination. Only patients who underwent hysterectomy without prior medical treatment for hyperplasia were eligible for this retrospective study. Patients with preoperative diagnoses of concomitant endometrial hyperplasia and endometrial carcinoma or who were treated with progestins were excluded. This study was designed as a retrospective data assessment; therefore, ethics committee approval was not required.

Electronic medical records, pathology reports, hospital and outpatient medical charts were reviewed. Data collected included age, preoperative diagnosis, medical treatment, type of operation, postoperative diagnosis, and final pathology.

Operations were performed by laparotomy (n=48) or laparoscopy (n=32) under general anesthesia. Lymph node dissection was performed if the surgeon elected to do so, or according to intraoperative findings such as an appearance suggestive of endometrial carcinoma during macroscopic observation of the opened uterus or suspicious metastatic lymph node(s). Frozen section evaluation of the uterus was not performed. Patients were informed about the risk of concomitant carcinoma and they gave informed consent for lymph node dissection if required.

The final histopathologic evaluation of surgical specimens and preoperative endometrial biopsy results were compared.

Results

Five hundred thirty-seven patients with endometrial hyperplasia were reviewed. One hundred twelve patients had AEH and 80 (71.4%) underwent surgery. The mean age of these 80 patients was 58.9 years. Seventy-two (90%) patients had complex hyperplasia with atypia, and 8 (10%) had simple hyperplasia with atypia. Forty-three (53.7%) patients underwent hysterectomy with bilateral salpingo-oophorectomy, and 37 (46.2%) had hysterectomy, bilateral salpingo-oophorectomy, and bilateral pelvic lymph node dissection; lymph node dissection was extended to the paraaortic region in 9 of these 37 patients.

The final pathology results of the surgical specimens and comparison of preoperative findings are shown in Table 1. Forty (50%) patients were diagnosed as having endometrioid-type endometrial carcinoma in the final pathologic examination of surgical specimens. The characteristics of 40 endometrial carcinoma cases according to the International Federation of Gynecology and Obstetrics 2009 Endometrial Carcinoma Staging System and lymph node metastases are given in Table 2. The histologic type in all patients with endometrial carcinoma was endometrioid. There were no grade 3 tumors.

Eight patients with simple AEH underwent surgical treatment. In the final histopathologic diagnosis, 4 patients had benign histology, 1 had simple hyperplasia without atypia, 2 had simple hyperplasia with atypia, and 1 of these 8 patients (12.5%) had stage Ia endometrioid-type endometrial carcinoma on hysterectomy specimens.

Fifteen (20.8%) of 72 patients with atypical complex endometrial hyperplasia had benign histology, 2 (2.7%) had simple hyperplasia without atypia, 4 (5.4%) had complex hyperplasia without atypia, and 39 (54.1%) had endometrioid carcinoma in their hysterectomy specimens.

Among the 37 patients who underwent lymph node dissection, 28 were diagnosed as having endometrial carcinoma, and the

Table 1. Comparison of pathological results of endometrial sampling and hysterectomy specimen of all cases (n=80)

		Hysterectomy Specimen					
		Benign	**Simple hyperplasia without atypia**	**Simple atypical hyperplasia**	**Complex hyperplasia without atypia**	**Complex atypical hyperplasia**	**Endometrial carcinoma**
Endometrial sampling	Complex atypical hyperplasia (n=72)	15 (20.8%)	2 (2.8%)	-	4 (5.6%)	12 (16.7%)	39 (54.1%)
	Simple atypical hyperplasia (n=8)	4 (50%)	2 (25%)	1 (12.5%)	-	-	1 (12.5%)
	Total (n=80)	19 (23.75%)	4 (5%)	1 (1.25%)	4 (5%)	12 (15%)	40 (50%)

remaining 9 had no invasive disease. As shown in Figure 1, the majority of patients were candidates for adjuvant treatment and underwent lymph node dissection. Four patients with grade 2 disease (3 with superficial myoinvasion and one with deep myoinvasion) received no lymph node dissection. These 4 patients were given adjuvant brachytherapy. Two of the 4 patients with stage Ib disease who underwent lymph node dissection were given brachytherapy; the other 2 were followed up without adjuvant treatment. Patients with stage II and IIIa disease were given pelvic radiotherapy. Two patients with stage IIIc disease had grade 2 tumors with deep myoinvasion and were given chemotherapy with extended radiotherapy.

Discussion

Some patients with AEH who are postoperatively diagnosed as having concomitant endometrial cancer carry high risk factors that necessitate knowledge about lymph node status in order

Table 2. Endometrial cancer cases (n=40)

		Lymphadenectomy (n=28)		No lymphadenectomy (n=12)	
		Grade 1	Grade 2	Grade 1	Grade 2
Stage	Ia	15	4	8	3
	Ib	0	4	0	1
	II	0	2	0	0
	IIIa	0	1	0	0
	IIIc2	0	2	0	0
All patients underwent total hysterectomy and bilateral salpingo-oophorectomy. There were no grade 3 or non-endometrioid tumors					

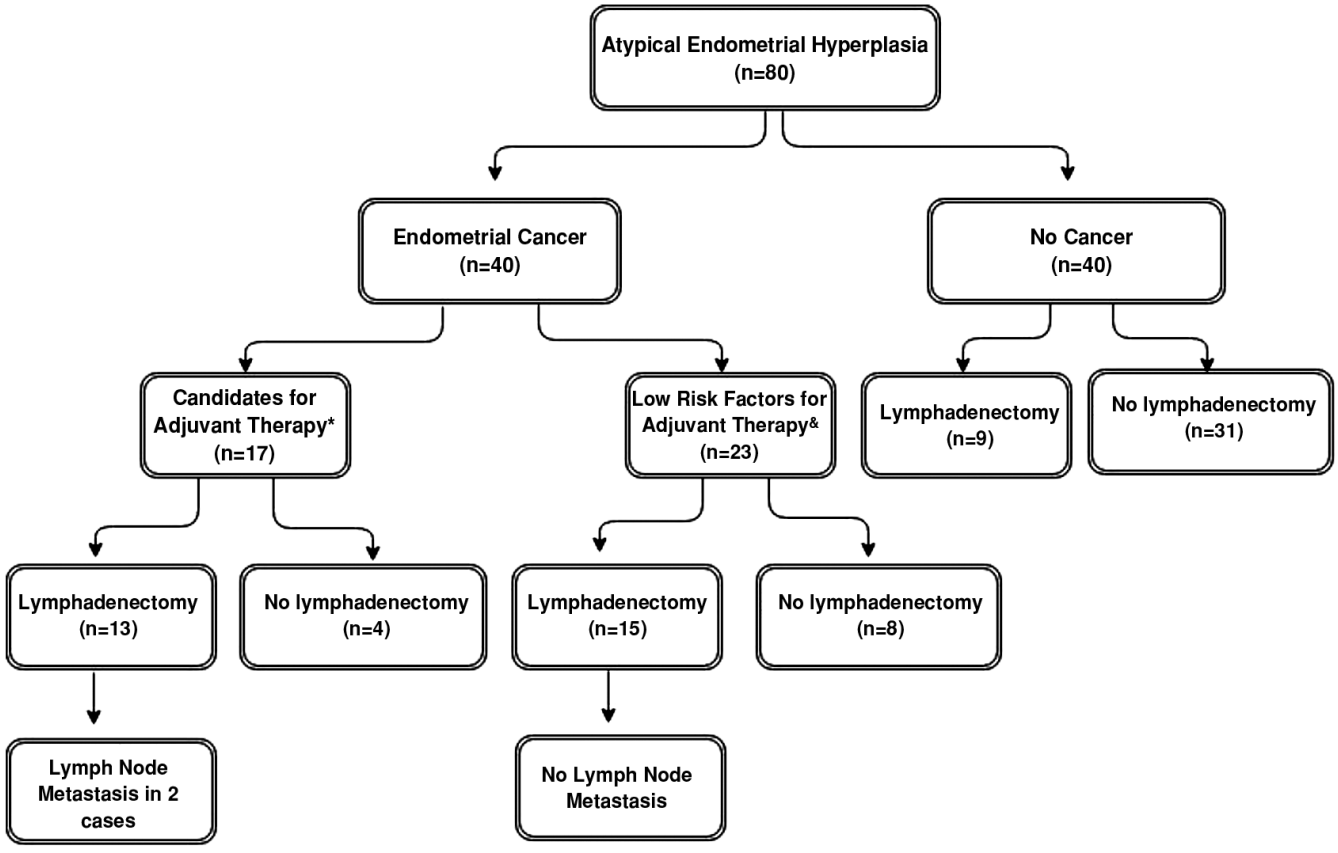

Figure 1. Brief summary of patient features

: Presence of at least one of the following on hysterectomy and salpingo-oophorectomy specimen: (i) Grade 2 with inner half myoinvasion, (ii) Deep myoinvasion (iii) Cervical stromal invasion (iv) Ovarian involvement

&: Grade 1 with inner half myoinvasion

to plan adjuvant therapies. Therefore, we evaluated the role of lymph node dissection in patients with AEH as a disease that carries a high risk for concomitant malignancy, which is preoperatively unpredictable in most patients.

In this study, we found that the prevalence of concomitant carcinoma in hysterectomy specimens was 50% in patients with AEH. This rate was 54.1% in patients with complex atypical hyperplasia and 12.5% with simple atypical hyperplasia. Among women with complex atypical hyperplasia diagnosed at endometrial biopsy, 17-52% were found to have concomitant endometrial carcinoma in previous studies (4-10). The concomitant carcinoma rate in our study is similar to these reports. The mean age of our patients was 58.9 years, which might explain the higher rate of concomitant endometrial carcinoma compared with other studies that were conducted on younger patient groups (8, 11, 12).

Invasive endometrial carcinoma concurrent with AEH is supposed to be grade 1 and associated with low risk factors, and hysterectomy was thought to be sufficient (13). However, some studies demonstrated that this was not the case. Whyte et al. (3) reported the rate of concurrent carcinoma as 29%, of which, 84% had myometrial invasion and 8% had cervical stromal invasion. In that series, one (5.5%) of 18 patients who underwent lymph node dissection had metastases. The authors concluded that the surgical staging decision, in addition to hysterectomy, was critical, especially in patients with complex atypical hyperplasia due to high rates of concomitant invasive cancer with high risk factors for extrauterine spread.

In a recent study, the ratio of lymph node metastases was estimated as 6.8% in patients with invasive endometrial carcinoma concomitant to AEH based on defined risk factors (2). This ratio decreases to 2.1% if all patients with AEH cases in the series are considered. Similarly, in our study, 2 of 28 (7.1%) patients with endometrial carcinoma who underwent lymph node dissection had lymph node metastasis. Therefore, the incidence of lymph node metastases in patients with concomitant endometrial carcinoma is nearly similar to clinical early-stage endometrial carcinoma.

Fifty percent of the women who were primarily diagnosed as having AEH had undiagnosed carcinoma. That kind of high coexistence rate may lead to inadequate surgical staging (8). In our study, all carcinomas had endometrioid histology and none of the tumors were poorly differentiated. All tumors were grade 1 or 2 (55% grade 1, 45% grade 2). In nearly 70% of carcinoma cases, tumor was confined to the inner half of the myometrium. On this account, lymphadenectomy for all AEH may not be advisable.

On the other hand, 17 patients with carcinoma had deep myometrial invasion, and/or cervical or ovarian involvement or grade 2 tumors with superficial myometrial invasion, and 27.5% of all carcinoma cases were stage Ib or higher. Thus, in 42.5% (17 of 40) of patients with endometrial carcinoma, and in 21% of all patients (17 of 80), lymph node dissection could have provided information about lymph node status in our study. Thirteen of these patients underwent lymph node dissection, and in the majority (except only 2), no lymph node metastasis was detected (Table 2). By these means, treatment plans were made more appropriately. Similarly, it was reported that lymph node dissection affected the adjuvant treatment decision in 28% of patients who were diagnosed as having endometrial carcinoma in uterine pathology without additional morbidity. The authors recommended lymph node dissection for AEH due to the high risk of concomitant carcinoma (3).

At this stage, routine lymphadenectomy in AEH cannot be recommended, it may be an unnecessarily aggressive approach. However, our results showed that extensive evaluation of patients with AEH is mandatory pre- and intraoperatively, according to the center's capability. Immunohistochemical studies using phosphatase and tensin homolog (PTEN) and ARID1a on curettage materials can be helpful to exclude concomitant endometrial carcinoma. Loss of these markers may support the existence of concomitant carcinoma (14, 15).

Until now, there is no method to precisely determine lymph node status in endometrial carcinoma, other than lymph node dissection. We routinely perform pelvic lymph node dissection in all patients with endometrial carcinoma because lymph node dissection is the only accurate way to detect lymph node metastasis. Half of patients with endometrial carcinoma with pelvic lymph node metastasis also have paraaortic lymph node involvement (16). Paraaortic lymph node dissection is added when preoperative high risk factors or intraoperative palpable/suspicious pelvic and/or paraaortic lymph nodes are present. In the current study, pelvic nodes looked suspicious macroscopically, and dissections were extended to paraaortic area in two cases with lymph node metastasis (stage IIIc2).

Discordance between pre-and post-operative pathologic diagnoses can be encountered in up to 30% of patients (17). In a series of patients with preoperative diagnosis of grade 1 endometrium carcinoma, surgical staging affected the decision of adjuvant treatment in 29% of cases; in other words, the patients were protected against under or over treatment (18). Another study reported that 15% of preoperative grade 1 cases were upgraded, and 18% had high-risk uterine pathology (19). Surgical staging allows the identification of patients who woud benefit from adjuvant therapy, as well as those who may be safely spared from the morbidity of these treatments.

Nevertheless, lymph node dissection brings with it some additional surgical risks. Some groups tried to identify a subset of patients who could be spared from surgical staging. In some reports, frozen section examination was

advised for determining the extent of the surgery; however, there have been conflicting reports about the accuracy of findings. Some authors stated that frozen section was useful for guiding intraoperative decision-making (20), whereas others found frozen section results as inconsistent with the final histopathologic examination results (21). Another study showed only 48% concordance between intraoperative frozen section and postoperative pathology in AEH, and the sensitivity for the diagnosis of endometrial carcinoma was reported as 33% (3). A model for predicting endometrial cancer based on age, body mass index, endometrial thickness, and postmenopausal status was proposed with 80% sensitivity and 70% specificity (22).

The Mayo Clinic suggests grouping patients with grade 1 and 2 tumors smaller than 2 cm diameter with myometrial invasion <50% as the low risk group and to skip lymphadenectomy (23). However, only specialized and experienced gynecologic pathologists can reliably define uterine risk factors in frozen sections. In practice, it is impossible for most oncology centers to employ such experts. Determining lymphadenectomy need is crucial because of these variable findings. Sentinel lymph node biopsy can be an option to evaluate lymph node status in patients with a preoperative AEH diagnosis. This method may overcome the problem related with frozen section accuracy and avoid re-operations in patients diagnosed as having high-risk endometrial cancer in the final pathology.

All our patients received their diagnoses after endometrial biopsies, rather than with dilatation and curettage, which can enable physician to sample larger portions of the endometrial cavity. This issue may underlie the high rate of concurrent carcinoma. The rates of concurrent carcinoma in patients diagnosed as having AEH via dilatation and curettage or via endometrial biopsy were reported as 17-30% and 43-45%, respectively (2, 3). Although one third of patients with concurrent carcinoma were still missed, dilatation and curettage before hysterectomy may be recommended for patients whose disease is diagnosed via endometrial biopsy.

The retrospective design and lack of comparison of perioperative outcomes according to lymph node dissection are the main limitations of our study. However, this study was focused on the role of lymph node dissection in deciding adjuvant treatment of endometrial carcinoma diagnosed in hysterectomy specimens of patients with AEH, rather than perioperative outcomes.

In conclusion, data to support lymph node dissection in all patients with AEH is lacking. Even in patients with endometrial carcinoma, the necessity for routine lymph node dissection is still under debate. However, the high rate of concomitant endometrial carcinoma with risk factors for extrauterine spread in a considerable number of patients with AEH should be kept in mind. Patients should be informed about this risk, and the value of lymph node dissection in patients with AEH should be demonstrated with large-scale studies in the near future.

Ethics Committee Approval: *This study was designed as a retrospective data assessment; therefore, ethics committee approval was not required.*

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

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Blastocyst transfer does not improve cycle outcome as compared to D3 transfer in antagonist cycles with an elevated progesterone level on the day of hCG

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Abstract

Objective: To evaluate the association between progesterone elevation on the day of human chorionic gonadotropin (hCG) administration and clinical pregnancy rates of gonadotropin-releasing hormone (GnRH) antagonist in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) cycles with the transfer of embryos at different developmental stages (day-3 versus day-5 ETs).

Material and Methods: This is a retrospective analysis of fresh IVF/ICSI; 194 cycles out of 2676 conducted in a single center.

Results: A total of 2676 cycles were analyzed, of which 386 had no progesterone measurements available. Two hundred eighteen cycles had progesterone elevation (p>1.5 ng/mL) giving an overall incidence of 9.5%. Twenty-four cycles were excluded from further analysis. Of the remaining 194 cycles, 151 had day-3 transfers and 43 had blastocyst transfers. There was no statistically significant difference in pregnancy and clinical pregnancy rates per transfer between the D3-ET and D5-ET groups (46% vs. 49%, and 39% vs. 35%, respectively).

Conclusion: The results of this study suggest that blastocyst transfer does not improve cycle outcomes compared with D3 transfer in GnRH antagonist cycles with an elevated progesterone level on the day of hCG. (J Turk Ger Gynecol Assoc 2017; 18: 133-8)

Keywords: Blastocyst transfer, human chorionic gonadotropin administration, progesterone elevation

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Introduction

Attention has been extensively paid during the last 20 years to serum progesterone measurement during ovarian stimulation. Since the early 90s, many studies have documented that a premature and excessive progesterone elevation above a certain threshold, before triggering of ovulation, might negatively affect of in vitro fertilization (IVF) outcomes (1-3).

Elevated progesterone (P_4) levels in the follicular phase has been a matter of debate in terms of IVF cycle outcomes. Apart from the discrepancies in definition of "elevated P_4," some studies claimed no effect at all (4-6), whereas others reported poorer outcomes (7-12). A favorable effect on pregnancy rates was also documented in an earlier study (13).

It was reported for the first time in 1991 that serum progesterone may increase during the last few days of ovarian stimulation (14). This has been widely confirmed during the last two decades, but the incidence of progesterone elevation greatly varies between published studies (2-35%) (15, 16).

This increase does not reflect "premature luteinization". Progesterone elevation occurs because the risk of endogenous leutinizing hormone (LH) surge is usually controlled by simultaneous administration of gonadotropin-releasing hormone (GnRH) analogues or antagonists. Progesterone elevation during ovarian stimulation is primarily related to the intensity of the ovarian response to follicle-stimulating hormone (FSH), but is also dependent on the studied population, which may consist of normal or good repsonders.

It has been supposed that premature elevation of P_4 advances the endometrium and leads to embryo-endometrial asynchrony. High serum P_4 levels on the day of human chorionic gonadotropin (hCG) administration induce both

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advanced endometrial histologic maturation and differential endometrial gene expression, which decrease endometrial receptivity and might be related to implantation failure (17, 18). Progesterone elevation prematurely opens the window of implantation, modifies endometrium receptivity, and is associated with a defective implantation. A management strategy with robust evidence is lacking in such cycles. Postponing embryo transfers (ET) may result in better synchronization between the embryo and the already ahead-of-phase endometrium.

Papanikolaou et al. (11) and Elgindy (9) concluded that elevated P_4 had a detrimental effect on day-3 but not day-5 ETs. The authors suggested that on the fifth luteal day, the endometrium had sufficiently recovered to allow for normal implantation. On the other hand, Hill et al. (19), Huang et al. (20), Corti et al. (21), and Ochsenkühn et al. (10) suggested that progesterone elevation on the day of hCG triggering had a negative impact on IVF outcomes, even with blastocyst transfers. These results were contrary to the findings of previous studies (9, 11).

The aim of our study was to evaluate the association between progesterone elevation on the day of hCG administration and clinical pregnancy rates of GnRH antagonist IVF/ICSI cycles with the transfer of embryos at different developmental stages (day-3 vs. day-5 ETs).

Material and Methods

Subjects

This is a retrospective analysis of fresh IVF/intracytoplasmic sperm injections (IVF/ICSI); 194 cycles out of 2676 conducted from January 2006 to August 2011 in a single center. The study protocol was approved by the instutional review board of the hospital; informed consent was waived due to the retrospective nature of this study.

The study inclusion criteria were: (1) GnRH antagonist cycles with a P_4>1.5 ng/mL on day of hCG, (2) in which ≥8 MII oocytes were retrieved and (3) at least three 8-cell embryos were present on day 3, (4) women were aged <40 years with regular cycles, (5) day-3 FSH level of <10 IU/L, antral follicle count of >5, and (6) an endometrial thickness ≥8 mm on the hCG day. Each patient was included in the study only once in the data set of the present study.

The exclusion criteria were: (1) use of frozen-thaw ETs, (2) previous history of poor ovarian response, (3) pre-implantation genetic diagnosis (PGD) cycles, (4) GnRH agonist- trigerred cycles, (5) the use of testicular sperm, (6) known endocrine disorders, (7) cases where blood was drawn and analyzed in another laboratory.

The following patient characteristics were assessed: cause of infertility, age, duration of gonadotropin stimulation, E2 and P_4 levels on the hCG day, the numbers of oocytes retrieved, MII and 2PN fertilized oocytes, and transfered embryos.

Controlled ovarian hyperstimulation protocol

The GnRH antagonist protocol was initiated on day 2 of the menstrual cycle with either hMG or rFSH (Menogon, Ferring, Switzerland or Gonal F 75 IU ampules; Serono, Geneva, Switzerland; 150-300 IU/d) for ovarian stimulation. The dose was adjusted for each patient according to the follicular growth detected using ultrasonography after the 5th day of drug administration. GnRH antagonist Orgalutran (Organon, Netherlands) 0.25 mg/dL per day was started on stimulation day 5.

Ovarian follicular development and oocyte retrieval

When at least two follicle were ≥18 mm, 10,000 IU hCG (Pregnyl SC freeze-dried ampoule, MSD, Baxter Pharmaceutical Solutions LLC, Bloomington, USA) or 250 μgr of rec-hCG (Ovitrelle, Serono, Germany) was administered to trigger ovulation.

Oocyte retrieval was performed at hour 35 after hCG injection. An oocyte pick-up was completed using a 17-gauge needle for oocyte retrieval under local anesthesia. The oocyte–corona complexes were denuded, intracytoplasmic sperm injection was performed after 2 hours of incubation, and embryos were transferred on days 3 or 5.

Embryo transfer and luteal phase support

The fertilized oocytes were observed for morphology on day 3. One hundred fifty-one participants underwent ET on day 3 (D3-ET), and 43 underwent ET on day 5 (D5-ET) because there was an adequate number of high-quality embryos available. Only high-quality embryos were transferred both on D3-ET and D5-ET. The choice of the ET day was mainly based on the embryo morphology, clinicians' preference for cryopreservation of spare embryos on day 3, and workload of the laboratory. Embryologists graded embryos as good, fair, or poor in line with the simplified Society for Assisted Reproductive Technology scoring system (22).

Both groups were tested for serum βhCG 12 days after ET and transvaginal ultrasound was scheduled 3 weeks afterwards to confirm clinical pregnancy. The luteal phase was supported with intravaginal micronized progesterone (Progestan 200 mg; Koçak, Tekirdağ, Turkey) as 600 mg/day, starting on the day of oocyte retrieval.

Hormonal evaluation

On the day of hCG trigger, serum P_4 and E2 levels were measured on a blood sample drawn at 10:00 AM. We used a microparticle enzyme immunoassay (Axsym System; Advia Centaur, Siemens), which has a sensitivity of 0.21 ng/mL. For the

P_4 assay, the intra- and interassay coefficients of variation are 7.2% and 5.7%, respectively. The E2 assay has a sensitivity of 7.0 pg/mL, with intra- interassay coefficients of variability of 11.3% and 5.0%, respectively. We selected a serum progesteron level of 1.5 ng/mL on the day of hCG administration as a cutoff level for an adverse cycle outcome as evidenced by the literature (9).

Evaluation of in vitro fertilization/intracytoplasmic sperm injection results

Clinical pregnancy and early pregnancy loss rates of D3-ET and D5-ET groups were evaluated. Clinical pregnancy rate was the primary outcome. Clinical pregnancy was defined as the presence of a gestational sac on transvaginal sonography.

Statistical analysis

The statistical analysis of the study was performed using the Statistical Package for the Social Sciences 20.0 (SPSS Inc., Chicago, IL, USA) and G*Power 3 (Düsseldorf, Germany) (23). Categorical variables in the data set are given with frequencies and percentages, but the continuously changing variables are given with mean, standard deviation, median, minimum and maximum values. The compliance of the measurement variables with normal distribution was analyzed using the Shapiro-Wilk test. In the comparison of two groups of variables with normal distribution, the difference between the two means was determined using the significance test (t test), and the comparison of variables that do not show normal distribution was performed using the Mann-Whitney U test. In the comparison of categorical variables between groups, Yates's corrected Chi-square test was used. In all the statistical analyses in the study, comparisons under a p-value of 0.05 were considered statistically significant.

Results

A total of 2676 cycles were analyzed, of which 386 had no progesterone measurements available. Two hundred eighteen cycles were noted to have progesterone elevation (p>1.5 ng/mL) giving an overall incidence of 9.5%. Twenty-one cycles were excluded from further analysis because there were fewer than three 8-cell embryos on day 3 or no blastocysts on day 5. Three additional cycles were excluded because of total embryo freezing and no fresh ET in the given cycle. Of the remaining 194 cycles, 151 had day-3 transfers, and 43 had blastocyst transfers. There was no statistically significant difference in the demographics between the two study groups (Table 1).

The mean age of D3-ET group was 30.65±4.12 years (range, 19-40 years), and that of the D5-ET group was 29.9±3.07 years (range, 22-36 years). Both groups had a similar duration of controlled ovarian hyperstimulation. The mean level of serum progesterone on the day of hCG administration was 1.83±0.49 ng/mL in the D3-ET group and 1.92±0.87 ng/mL in the D5-ET group (p>0.05). The D5-ET group had a significantly higher mean estradiol level on the day of hCG (3940.7±1928.20 vs. 2803.62±1639.73 pg/mL, p=0.001). The mean number of oocytes retrieved was significantly higher (22.23±8.93 vs. 15.63±7.76, p=0.001) in the D5-ET group, along with a higher number of MII (17.1±6.75 vs. 10.86±5.86, p=0.001) and 2PN fertilized oocytes (13.33±5.03 vs. 7.81±4.59, p=0.001), respectively. There was no statistically significant difference in the mean number of embryos transferred in the day 3 and day 5 groups (2.2 vs. 1.8, respectively) (Table 2).

There was no statistically significant difference in pregnancy and clinical pregnancy rates per transfer between the D3-ET and D5-ET groups (46% vs. 49%, and 39% vs. 35%, respectively) (Figure 1).

Table 1. Patient overview

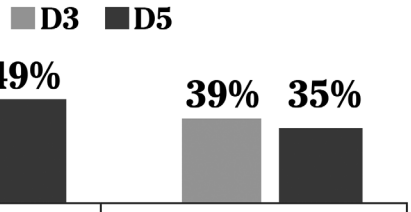
	D3-ET	D5-ET	p
Age, years	30.65±4.12	29.9±3.07	NS
BMI, kg/m^2	27.32±3.16	28.7±3.84	NS
Infertility period, years	4.62±3.30	3.63±2.46	NS
Day 3 FSH, mIU/mL	6.48±2.30	6.28±2.10	NS
Day 3 E2, pg/mL	38.52±10.59	43.59±20.15	NS
Previous IVF trials, n	0.74±1.1	0.76±1.01	NS
Type of infertility Primary infertility, % Secondary infertility, %	 87 13	 74 26	NS
Diagnosis			
Male factor	65 (43%)	20 (46%)	NS
Unexplained infertility	37 (25%)	8 (18%)	NS
Endometriosis	20 (14%)	6 (14%)	NS
Tubal factor	10 (6%)	3 (7%)	NS
PCOS	14 (9%)	4 (9%)	NS
Others	5 (3%)	2 (6%)	NS

ET: embryo transfer; BMI: body mass index; FSH: follicle-stimulating hormone; IVF: in vitro fertilization; PCOS: polycystic ovary syndrome; D3-ET: embryo transfer on day 3; D5-ET: embryo transfer on day 5; E2: estradiol; NS: not significant

Table 2. Cycle parameters in D3–ET and D5–ET groups in patients with P_4 >1 ng/mL on the day of hCG

	D3-ET (n=151)	D5-ET (n=43)	p
Ovarian stimulation, days	8.95±1.6	9.0±1.55	NS
E_2 on hCG day, pg/mL	2803.62±1639.73	3940.7±1928.20	0.001
P_4 on hCG day, ng/mL	1.83±0.49	1.92±0.87	NS
Oocytes retrieved, n	15.63±7.76	22.23±8.93	0.001
MII, n	10.86±5.86	17.1±6.75	0.001
2PN, n	7.81±4.59	13.33±5.03	0.001
Embryos transferred, n	2.2±0.5	1.8±0.8	NS

D3-ET: embryo transfer on day 3; D5-ET: embryo transfer on day 5; ET: embryo transfer; hCG: human chorionic gonadotropin; E2: estradiol; P4: progesterone; MII: metaphase II; PN: pronucleus, NS: not significant



	beta hCG (+)	Clinical pregnancy
D3	46%	39%
D5	49%	35%

Figure 1. Pregnancy and clinical pregnancy rates per transfer between groups

Discussion

In the present study, D5 transfer was not found to be superior to D3 transfer of embryos in patients with elevated P_4 levels, respectively, when the cutoff level for P_4 was set at 1.5 ng/mL on the day of hCG administration.

Elevated P_4 in the late follicular phase of an IVF cycle is claimed to result in worse cycle outcomes. This negative effect is believed to be more prevalent in cycles with a higher oocyte yield; such a negative effect may ensue with a relatively higher P_4 elevation. It is more likely that the elevated P_4 levels reflect the total amount of progesterone secreted by maturing follicles, and these levels have been found to correlate positively with the number of mature follicles and with estradiol levels on hCG day. In the present study, we also documented an increase in E2 levels in correlation with number of mature follicles. Although non-significant, P_4 levels were slightly elevated in the D5-ET group, in which the number of oocytes was significantly higher. The first attempt to critically evaluate the existing literature regarding P_4 elevation on the day of hCG and its role in pregnancy achievement was published in 2007 (6). The results of that review were confounded by the different GnRH analogue protocols administered. Moreover, the majority of the included studies that failed to demonstrate a negative association used an arbitrarily defined threshold value of 0.9 ng/mL. Following that meta-analysis, a prospective study by Elgindy (9) claimed that an increased P_4 level of ≥1.5 ng/mL on hCG day was associated with an adverse effect on clinical outcomes. A meta-analysis of Kolibianakis et al. (24) evaluated the results of five eligible studies of GnRH antagonist cycles. They reported that women with elevated P_4 level on the hCG administration day had decreased probability of clinical pregnancy per cycle. Another meta analysis of Venetis et al. (16) provided convincing data that elevation of serum P_4 secretion was associated with low pregnancy rates whatever the GnRH analogue used. A very recent meta-analysis that reviewed only antagonist cycles documented that women with elevated P_4 levels >1.5 ng/mL on hCG day had more oocytes and higher E2, as well as decreased probability of pregnancy per cycle (25).

The crucial question is how should physicians manage patients with elevated progesterone levels during late follicular phase. Proposed cycle management strategies in the event of high P_4 on the day of hCG may be to freeze all embryos and transfer them back in a natural or hormone-replacement cycle, to favor a D5 ET, to start with a lower FSH dose in the next cycle, to use hp-hMG instead of rFSH, and/or earlier administration of hCG for triggering final oocyte maturation in high-risk patients. None of the aforementioned strategies have been tested so far for their efficacy in this setting.

For women with an elevated P_4 level on the day of hCG, extending culture and transferring embryos on D5 might have been a sound strategy because the most probable mechanism of impairement that high P_4 in the follicular phase causes is the advancement of endometrial maturation and early closure of the endometrial implantation window; day-5 ET may restore this asynchronization in such cycles.

In several studies it is claimed that endometrial advancement due to controlled ovarian hyperstimulation and raised P_4 could be recovered on day 5 (26). Papanikolaou et al. (11) designed a study to determine if there was an effect of elevated P_4 on hCG day on pregnancy outcomes, and whether this effect might be associated with the developmental stage of the embryo transferred. According to this study, even modest rises of P_4 in the follicular phase has a detrimental effect on the implantation potential of good-quality cleavage stage embryos (11). On the contrary, premature luteinization in the blastocyst transfer subgroup had no effect on pregnancy outcomes.

Hill et al. (19) confirmed the recent publications by demonstrating a negative impact of elevated serum P levels on the day of hCG administration on live birth. This negative effect was also demonstrated in both cleavage and blastocyte stage ETs for both poor and good embryos. Huang et al. (20) reported that the negative association of P_4 elevation with clinical pregnancy rates was noted both in D-3 and blastocyte stage ET cycles and confirmed decreased clinical pregnancy rates in GnRH agonist IVF/ICSI cycles regardless of the developmental stage of the transferred embryos. Ochsenkühn et al. (10) documented a similar reduction in pregnancy rates in the study of blastocyte transfers. However, their study had no cohort of cleaveage embryos to use for direct comparision. Corti et al. (21) and Ochsenkühn et al. (10) suggested that progesterone elevation on the day of hCG triggering has a negative impact on IVF outcomes, even with blastocyst transfers. These results were contrary to the findings of Papanikolaou et al. (11). The study of Papanikolaou addressed whether the adverse effect of follicular phase P_4 elevation could be alleviated by a blastocyst

transfer. In contrast to what this retrospective study suggested, in our data set, D5 transfer was not found as superior to D3 transfer of good quality embryos in patients with elevated P_4 levels. Our study was retrospective in design but, the inclusion criteria enabled us to select patients with a cohort of good quality embryos on day 3 that might have a good chance of D5 transfer if they had been allowed to stay in extended culture. Thus, the two transfer groups presented a similar embryo development profile in culture. The reason for the lack of efficiency of D5 transfer strategy may be the fact that the advanced endometrium may still have not recovered from the action of elevated follicular P_4. To our knowledge, there is no solid biologic evidence to confirm this endometrial recovery with concomittant D3 and D5 endometrial biopsies.

The choice of 1.5 ng/mL as a threshold level for P_4 in our study is less than ideal because the threshold for poor cycle outcomes may change with the ovarian response of the patient. In better responders, a higher P_4 threshold is more plausible. As such, the reason for failure of observing improved outcomes with a D5 ET strategy in our study may be that our patient population comprised either normal or good responder patients and thus a higher threshold could have revealed a positive treatment effect in favor of D5 transfer. The strongest effect of progesterone elevation on pregnancy rates was observed between 1.5 and 1.75 ng/mL in the study of Venetis et al. (16). Nevertheless, as the degree of ovarian response is increased, the failure of a beneficial effect for D5 ETs still may persist. In poor responders with an elevated P_4 level, the management strategy could have been to freeze all embryos rather than to try a D5 transfer due to the lack of availability of an adequate number of embryos for extended culture.

The limitation of our study is its retrospective nature; this kind of study has selection bias. Also, the number of D5 transfers was lower than that of D3, which also weakned the study power.

In conclusion, the results of this retrospective study suggest that blastocyst transfer does not improve cycle outcomes as compared with D3 transfer in GnRH antagonist cycles with an elevated progesterone level on the day of hCG. Therefore, a prospective randomized control trial on an intention-to-treat basis is needed to compare single D3 and single blastocyst transfers in this setting to reach a more definitive answer to the problem.

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Ethics Committee Approval: *Ethics committee approval was received for this study from the ethics committee of IVF Center, Memorial Atasehir Hospital, Istanbul, Turkey*

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Microcystic, elongated, and fragmented pattern of invasion in relation to histopathologic and clinical prognostic factors in endometrioid endometrial adenocarcinoma

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Abstract

Objective: To investigate the association of microcystic, elongated, and fragmented (MELF) pattern of invasion with prognostic factors in endometrioid endometrial adenocarcinoma (EEA).

Material and Methods: Stained tissue sections from 83 cases of EEA operated by the same gynecologic oncologist were reviewed to identify cases showing MELF-type invasion in this retrospective study. The association of MELF pattern with age, tumor grade, depth of myometrial invasion, and presence of lymphovascular space invasion (LVSI) was analyzed.

Results: FIGO grade 2 and grade 1 tumors were evident in 53.0% and 38.6% of patients, respectively. Depth of myometrial invasion was <50% in 72.0% of patients, and LVSI was absent in 77.1%. MELF pattern was confirmed in 35 (42.2%) patients. Presence of MELF pattern was associated with significantly higher mean ± standard deviation age (62.9±6.9) years vs. 58.9±9.1 years, p=0.033), and found to be more likely in patients with high-grade tumor (FIGO grade III; 85.7% vs. 14.3%, p<0.001), deep (≥50%) myometrial invasion (78.3% vs. 21.7%, p<0.001), and presence of LVSI (94.7% vs. 5.3%, p<0.001) as compared with absence of MELF pattern.

Conclusion: In conclusion, our findings revealed a high rate of MELF pattern among patients with EEA alongside the association of MELF pattern with poor prognostic factors such as high grade tumor, deep myometrial invasion, and LVSI. (J Turk Ger Gynecol Assoc 2017; 18: 139-42)

Keywords: Endometrioid endometrial adenocarcinoma, microcystic, elongated, fragmented pattern, tumor grade, myometrial invasion, lymphovascular space invasion

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Introduction

Endometrioid endometrial adenocarcinoma (EEA) has a favorable prognosis with overall 5-year survival rates of 90%, reaching 93-94% for International Federation of Gynecology and Obstetrics (FIGO) grade 1 and 2 tumors (1, 2).

Besides the increased risk of extra-uterine spread of tumor with the depth of myometrial invasion, certain patterns of myometrial invasion have also been proposed as a potential prognostic factors in endometrial carcinomas, which may play a role in predicting the pattern of lymph node involvement as well as metastasis (3-7).

The microcystic, elongated, and fragmented (MELF) pattern is a distinctive presentation of myometrial invasion with unusual changes to neoplastic glands, which was first described by Murray et al. (8) in 2003. Although it was initially thought to represent a simple degenerative process, with identification of the restriction of MELF pattern invasion

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to myoinvasive low-grade carcinomas of endometrioid type, these changes have been suggested to be an active cellular process indicating a specific tumor-stromal interaction (4, 5).

Tumors with MELF pattern have been associated with deposits of carcinoma in lymph nodes that resemble hystiocytes, which forms the basis of application of keratin stains to identify single tumor cells in lymph node sinuses, as well as experimentally in the setting of sentinel lymph node evaluation (9). Nonetheless, despite the documented association of MELF pattern with lymphovascular space invasion (LVSI) and lymph node metastasis, it remains uncertain as to whether presence of MELF pattern invasion has clinical significance in EEA (5-8, 10). Our study was therefore designed to study and examine the frequency of MELF pattern invasion in patients with EEA and to determine its association with clinical and histopathologic prognostic factors.

Material and Methods

Stained tissue samples from 83 cases of operated EEA were reviewed to identify cases showing MELF-type invasion in this retrospective study. The inclusion criterion was endometrioid-type endometrial adenocarcinoma. Non-endometrioid-type adenocarcinoma was excluded. The study was conducted in full accordance with local GCP guidelines and current legislation, and our institutional ethics committee gave permission to use patient data for publication (19.10.2016/487).

Data on age, tumor grade, depth of myometrial invasion and LVSI were recorded for each patient. Tumor samples were re-evaluated by the same pathologist to investigate MELF pattern. The association of MELF pattern with age, tumor grade, depth of myometrial invasion, and presence of LVSI was analyzed. Tissue samples were graded using the grading system identified by FIGO: a solid non-squamous content up to 5% was considered grade 1, 6 to 50% as grade 2, and >50% as grade 3 (11). Hysterectomy specimens stained with hematoxylin and eosin (H&E) were evaluated to identify MELF pattern exhibition and MELF-positive cases as previously described by Murray et al. (8). LVSI tumor fragments present in the endothelium-lined vascular/lymphatic spaces, either in the tumor or distant from it, were also evaluated in H&E stained specimens with or without MELF-expression.

Depth of myometrial invasion was determined based on the tumor invading the myometrium down to deepest margin and lying over the endomyometrial junction and classified as invasion through <50% or ≥50% of the myometrial thickness.

Statistical analysis

MedCalc Statistical Software version 12.7.7 (MedCalc Software BVBA, Ostend, Belgium; http://www.medcalc.org; 2013) was used for statistical analysis. The Chi-square (χ^2) test and Fisher's exact test were used to compare categorical data, and numeric data were analyzed using Student's t-test for independent variables with normal distribution. Data are expressed as mean ± standard deviation (SD), minimum-maximum and percentage (%) where appropriate. $P<0.05$ was considered statistically significant.

Results

FIGO grades 2, 1, and 3 tumors were evident in 53.0%, 38.6%, and 8.4% of patients, respectively. Depth of myometrial invasion was <50% in 72.0% of cases (stage IA), and LVSI was absent in 77.1%. MELF pattern invasion was confirmed in 35 (42.2%) patients (Table 1).

Presence of MELF pattern was associated with significantly higher mean age, 62.9±6.9 years vs. 58.9±9.1 years, p=0.033, and found to be more likely in cases of high-grade tumor (FIGO grade III; 85.7% vs. 14.3%, p<0.001), deep (≥50%) myometrial invasion (78.3% vs. 21.7%, p<0.001), and lymphovascular invasion (94.7% vs. 5.3%, p<0.001) as compared with absence of MELF pattern invasion (Table 1).

Table 1. Prognostic factors in the overall study population with respect to MELF pattern invasion

	MELF Pattern		
	Absent (n=48)	**Present (n=35)**	**p value**
Age (years), mean±SD	58.9±9.1	62.9±6.9	**0.033[a]**
Tumor grade	**n (%)**		
Grade I	28±96.6	1±3.4	**<0.001[b]**
Grade II	19±43.2	25±56.8	
Grade III	1±14.3	6±85.7	
Depth of myometrial invasion <50%	42±71.2	17±28.8	**<0.001[c]**
Lymphovascular space invasion	1±5.3	18±94.7	**<0.001[c]**

[a]: Student's t-test; [b]: Fisher's exact test; [c]: Chi-square test; MELF: microcystic, elongated, and fragmented

Discussion

Our findings revealed the presence of MELF pattern invasion in 42.2% of patients with EEA and greater likelihood of MELF positivity in older patients; the presence of factors indicates poor prognosis including deep myometrial invasion, high grade tumor, and LVSI.

The incidence of MELF in our cohort (42.2%) was higher than the incidence (23.1%) reported previously in Turkish patients (n=121) (7), but consistent with the upper level of the range (7 to 48%) for the overall incidence of MELF reported in different studies among patients with EEA (5, 7, 10, 12, 13). Our findings support the more frequent observation of deep myometrial invasion and LVSI in MELF-positive cases (7), unlike previous reports indicating the more common observation of MELF invasion in low-grade (FIGO grade 1 or 2) EEAs (5-7). The percentage of patients with and without MELF pattern was significantly higher in high-grade (FIGO grade 3) tumors in our cohort. Indeed, cervical stromal involvement, lymph nodal metastasis, and clinically advanced stage were reported to be more common in the presence of MELF pattern invasion, and MELF pattern positivity, as well as involvement of cervical stroma were shown to be independent determinants of lymph node metastasis (7).

In total, 18 of 19 patients with LVSI and 28 with >50% myometrial invasion (94.7% and 78.3%, respectively) had MELF pattern invasion in our study. This supports data from a past study, which indicated that MELF-positive cases were more likely to exhibit LVSI and ≥50% MI than MELF-negative cases (78.5% vs. 13.9% and 78.5% vs. 32.2%, respectively) (7). MELF pattern, when accompanied with fibromyxoid stromal response, was shown be related to LVSI and a worse long- term prognosis (8), and an invasive glandular morphology was suggested to be a stronger marker of LVSI risk than the depth of invasion (7).

High-grade histologic subtype, older age, myometrial invasion depth, and positivity of LVSI are well-established interacting adverse prognostic factors that are predictive of disease outcome in endometrial neoplasia (1, 2, 6, 14). Presence of LVI and MELF-type invasion were shown to be significantly associated with an increased likelihood of nodal metastasis (5). Thus, the increased likelihood of poor prognostic factors among MELF-positive cases in our cohort emphasizes the implications of accurate histologic assessment of myometrial invasion and recognition of MELF pattern invasion in clinical practice in terms of grading of EEAs, and assessing long-term prognosis given its association with LVSI, lymph node metastasis, and extra-uterine disease (4, 5, 7, 8, 10, 14). In addition, a past study that analyzed risk factors for recurrence in low-grade EEA showed that MELF myoinvasion pattern, deep myoinvasion, LVSI, and high-grade tumor in metastatic foci were factors that predicted increased recurrence risk at sites other than the vagina (5).

The likelihood of increased tumor progression and adverse prognosis in the presence of MELF pattern invasion in EEAs has been associated with enhanced dissemination of neoplastic cells due to a fibromyxoid stromal reaction creating a low resistance milieu, as well as a likelihood of transition of epithelial mesenchyme that allows surrounding stromal infiltration (4, 5, 8, 14, 15). Nevertheless, the clinical relevance of MELF pattern positivity in endometrial adenocarcinoma has not yet been elucidated given that the relation of MELF invasion to lymph node metastasis or poor prognosis was not confirmed in some studies (5, 6-8, 10).

In conclusion, our findings revealed a high rate of MELF pattern invasion among patients with EEC alongside the association of MELF pattern invasion with poor prognostic factors such as high-grade tumor, deep myometrial invasion, and lymphovascular invasion. Our findings emphasize the importance of accurate histologic assessment of myometrial invasion, and the likely role of identifying MELF pattern invasion with frozen section or probe curettage in configuring surgical treatment among patients with EEA. Nonetheless, larger scale studies are necessary to justify the consideration of MELF pattern invasion as a risk factor for higher-stage disease, lymph node metastasis, and thus poor outcomes in EEA.

Ethics Committee Approval: *Ethics committee approval was received for this study from the ethics committee of Medipol University School of Medicine (No: 19.10.2016/487).*

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Exploring the umbilical and vaginal port during minimally invasive surgery

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Abstract

This article focuses on the anatomy, literature, and our own experiences in an effort to assist in the decision-making process of choosing between an umbilical or vaginal port. Umbilical access is more familiar to general surgeons; it is thicker than the transvaginal entry, and has more nerve endings and sensory innervations. This combination increases tissue damage and pain in the umbilical port site. The vaginal route requires prophylactic antibiotics, a Foley catheter, and a period of postoperative sexual abstinence. Removal of large specimens is a challenge in traditional laparoscopy. Recently, there has been increased interest in going beyond traditional laparoscopy by using the navel in single-incision and port-reduction techniques. The benefits for removal of surgical specimens by colpotomy are not new. There is increasing interest in techniques that use vaginotomy in multifunctional ways, as described under the names of culdolaparoscopy, minilaparoscopy-assisted natural orifice surgery, and natural orifice transluminal endoscopic surgery. Both the navel and the transvaginal accesses are safe and convenient to use in the hands of experienced laparoscopic surgeons. The umbilical site has been successfully used in laparoscopy as an entry and extraction port. Vaginal entry and extraction is associated with a lower risk of incisional hernias, less postoperative pain, and excellent cosmetic results. (J Turk Ger Gynecol Assoc 2017; 18: 143-7)

Keywords: Single port laparoscopy, culdolaparoscopy, natural endoscopic surgery, colpotomy, postoperative pain

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Introduction

Laparotomy was replaced by laparoscopy for many procedures. With advances in techniques, tools and materials, new methods of minilaparoscopy and single-access surgery (SAS) for umbilical, vaginal or other natural orifices evolved. Abdominal extraction of surgical specimens is not possible when 3.5 mm or smaller ports are the only ones placed in the abdomen, whereas some surgical specimens could be removed via traditional 10-mm or 12-mm laparoscopic umbilical ports. For large specimens, the solutions include extension of the umbilical port or a secondary port, minilaparotomy, morcellation, and colpotomy. The greatest advantage of extraction through an enlarged umbilical incision is the possibility of performing laparoscopic procedures with secondary ports of 5 mm, minilaparoscopy instruments or percutaneous needles. Another problem faced when large specimens have to be extracted through secondary ancillary ports is damage to the inferior epigastric artery during port enlarging (1). The inferior epigastric artery is a collateral branch of the external iliac artery (EIA), which usually arises from the EIA, 6.2 cm above the inguinal ligament. Nevertheless, the EIA can arise from the femoral artery below the inguinal ligament, from the profunda femoral artery, and even as a common trunk with the circumflex iliac artery. Lesions incurred during enlargement of lateral ancillary ports can lead to a severe hemorrhage with potentially serious complications.

SAS is usually performed via a unique navel incision and multichannel ports. Operative laparoscopes with working channels were also used with the aid of percutaneous needle assistance. Port size extension increases tissue damage and can increase the chances of injury to inferior epigastric vessels, port-site hernia (PSH), postoperative pain, and poor cosmetic results (2).

Colpotomy has been published under different names such as vaginotomy, vaginal celiotomy, or culdotomy and circular colpotomy during vaginal hysterectomy. It has been used during surgery and for extraction. Some laparoscopists used colpotomy as an extraction option rather than increasing or making an additional abdominal incision.

Culdolaparoscopy (3), minilaparoscopy-assisted natural orifice surgery (MANOS) (4), and natural orifice transluminal endoscopic surgery (NOTES) use multifunctional vaginal access for insufflation, viewing, surgery, and extraction (5-7). In this paper, we attempt to compare umbilical and vaginal extraction, specifically when large specimen are retrieved, focusing on anatomic differences based on the literature and our own experience.

Vaginal access anatomy

Most general surgeons rarely use a vaginal port for access or extraction of samples. Therefore, this section serves as a refresher for non-gynecologic surgeons to consider vaginal extraction or use of the transvaginal approach in culdolaparoscopy, MANOS or NOTES.

The vagina is a fibro-muscular structure, S-shaped, between 6 and 12 cm long, and 3 to 4 mm thick, being longer in the rear wall (8). The cephalic or upper section of the vagina widens to form a pouch around the cervix, which is known as the fornix. The fornix can be separated in the anterior, posterior and, lateral fornixes. The lateral fornixes are topographic in relation with the endo pelvic fascia and the base of the broad ligaments. The vaginal wall of the posterior fornix is in contact with the peritoneum of the pouch of Douglas (posterior cul-de-sac). The posterior vaginal wall is divided into 3 sections; the upper is the fornix, as previously described. The middle portion is attached to the rectum vaginal septum. The lower portion is attached to the rectovaginal septum and the fibromuscular nucleus of the perineal body. We have not used the anterior fornix colpotomy in these surgeries even though we use the circular colpotomy during vaginal hysterectomies (8, 9). Colpotomy has been used for specimen extraction by laparoscopists in general surgery, gynecology, and urology (10).

Harlaar et al. (11) measured the cavity of the posterior cul-de-sac in ten cadavers. They used latex to make a mold of the posterior cul-de-sac. The mean diameter was 2.6 cm (±0.5 cm) with a range of 2.0-3.4 cm (11). However, these measurements are limited to those of a cadaver. In a live female, the measurements are significantly different when performing laparoscopy (12). We use a uterine manipulator to bring the uterus in an anterior and cephalic direction. This maneuver opens the pouch of Douglas under an endoscopic view. It broadens the posterior "cul-de sac" by several centimeters and allows the placement of a vaginal port, as well as removal of the appendix, gallbladder, ovarian cysts, uterine fibroids, and other types of specimens. When the removed sample is large, suction, drainage, and collapse of a cyst or hollow viscous is performed. For a large solid specimen, hemisecting or morcellating is needed to reduce the size in order to avoid damage by overstretching the anatomically related structures (9).

Umbilical access anatomy

The introduction of SAS has enabled the navel to be reconsidered as an embryonic natural orifice that can be used as the main access for general laparoscopy, offering the advantage of surgery without scars. Therefore, we review these structures in order to prevent complications (13).

Typically, the average navel is 8.2 mm in thickness when measured in the shortest length between the skin and peritoneum. It is slight thicker in women than in men (14). The navel is located at the level of the highest point of the iliac crest, the third to fourth lumbar disc, and equidistant between the tip

of the xiphoid process and the top of the pubic symphysis. The navel is located slightly depressed and down in the middle of the depth of the abdominal wall, facing the body at L4. The skin bottom of the umbilical depression has a protrusion, which is in a bun shape form, the umbilical tuber, which is separated from the peripheral skin by a circular groove. In the center of the tuber is the scar of the umbilical cord. The position of the navel changes during pregnancy or obesity (15). The subcutaneous layer consists of fatty tissue and small arteries, veins and nerves, and is thicker in the obese. The deeper layer of the navel is composed of fibrous tissue covered by sub peritoneal tissue were the umbilical artery and veins run. The umbilical ring is embedded in the middle linea Alba. The navel ring includes the insertion of the urachus, remnants of the umbilical arteries caudally, and the Teres ligament cephalically, all of which are covered by the parietal peritoneum (16).

Differences in umbilical and vaginal innervations

To better understand many of the differences between the umbilicus and vagina as an inlet for surgery, we review the innervations of these organs (17). The umbilical area is very sensitive when compared with others areas in the abdomen. In a study of innervations of the umbilical skin, authors are quoted to have found epidermal and dermal numerous tactile cells and corpuscles, and abundant end-bulblike genital structures. Nerve bundles were observed together with bulbous and lamellar corpuscles with abundant nerve endings both in the papillary and reticular dermis (18).

The vagina receives visceral and somatic nerves. The innervations arrive via the lower hypogastric plexus and the internal pudendal nerve, forming a plexus around the vagina (16, 19). The posterior vaginal fornix area has substantially less sensory innervation than the navel area.

Griebling et al. (20) described a neural-rich submucosal plexus within the region of the vagina, with a small quantity of intraepithelial axons. The sympathetic and parasympathetic nerves are smaller and have even less presence of sensory nerves (20). Their study showed that innervations changed under the influences of estrogen (20, 21).

Postmenopausal women often experience some level of atrophy due to lower estrogen levels, which produce changes in innervations. Treatments with estrogen replacement should improve this condition (22-27).

Investigations on vaginal port and sexual function

Regarding sexual function after posterior colpotomy, Butticè et al. (28) evaluated 71 patients after hybrid transvaginal (NOTES) nephrectomy. Sexual function was evaluated using the Female Sexual Function Index (FSFI) questionnaire the day prior to the operation and 3 months after. The authors showed that the complication risk increased significantly with increasing tumor size. Among the whole cohort, even the FSFI score differences were small; there was a statistically significant decrease in the postoperative period in all domains, except sexual satisfaction. In fact, the patients reported unaltered sexual function after surgery and satisfaction with the result when asked directly. In subgroup analyses, in nulliparous patients (n=60), arousal, sexual desire, orgasm, and satisfaction domains had no significant differences in pre- and postoperative periods. Accordingly, the authors strongly supported the use of hybrid transvaginal NOTES nephrectomy for large renal tumors, especially in nulliparous patients, for unaltered sexual satisfaction (28).

Nevertheless, Vitale et al. (29) also studied quality of life (QoL) and sexual function changes of 20 women with third- and fourth-degree cystocele (according to the Baden-Walker classification), treated using biocompatible porcine dermis graft. The Short Form-36 questionnaire to assess QoL was administrated at baseline and 12 months after surgical treatment. The Pelvic Organ Prolapse/Urinary Incontinence Sexual Questionnaire (PISQ-12), which measures changes of sexual behavior, was used at baseline and 12 months after surgical treatment. Each woman underwent translabial color Doppler ultrasonography to measure clitoral blood flow before and 12 months after surgical treatment. In the results, the authors showed that use of biocompatible porcine dermis grafts to treat severe cystocele considerably improved QoL and sexual function, and did not influence clitoral blood flow. Thus, based on the authors' experience, vaginal prolapse and urinary incontinence treatment (30) should also provide sexual improvement.

The problem has not yet been completely addressed by surgeons, especially in cases of mass extraction or large specimens. Rolli et al. (31) investigated the feasibility and safety of vaginal myomectomy via posterior colpotomy in a series of 46 consecutive procedures. Thirty-two patients underwent successful vaginal myomectomy under general anesthesia and 12 under loco-regional anesthesia, and the median size of the myomas was 50 mm (range, 16-81 mm). The authors showed the feasibility of the technique, but they did not address sexual function after surgery, or whether any patients achieved pregnancy after myomectomy (31).

Studies on surgical comparison of methods

Gynecologists have been using transvaginal access to the peritoneal cavity via a posterior colpotomy for more than a century for diagnostic and operative procedures, and specimen extraction (4). However, since the early 1970s, vaginal access was replaced by laparoscopy (32, 33). The reasons why transvaginal access fell out of favor are

multifactorial, including advances in laparoscopy, perceived technical difficulty, concerns about patient acceptance, infections, taboos, and misconceptions (34). Recently, with the emergence of MANOS and NOTES peritoneoscopy surgery, the vagina has become the most suitable entry and extraction site to operate on and retrieve several organs in different specialties, such as general surgery, urology and, of course, gynecology (34, 35). Ghezzi et al. (2) reported their experience with large specimen extraction using the navel in 1116 gynecologic laparoscopy procedures. There were no problems with direct surgical extraction of specimens or with endobags. The only complication was a laceration of the epigastric artery. Neither PSH nor metastases occurred. Another study of Ghezzi et al. (33) randomly compared the transumbilical and transvaginal routes for retrieval of adnexal masses in laparoscopy. The results of this study suggest that retrieval of adnexal masses following laparoscopic excision via a posterior vaginotomy causes less postoperative pain than transabdominal specimen extraction through the navel port (35).

Bulian et al. (36) successfully performed hybrid transvaginal cholecystectomies (TVC) using rigid instruments and compared this technique with the traditional laparoscopic technique. In their comparison, there were no postoperative differences in terms of pain and sex in the TVC group, whereas there were two PSHs found in the laparoscopy group. Patients in the TVC group were more satisfied with the results compared with those who underwent traditional laparoscopy. The transvaginal access procedures were mostly performed using prophylactic antibiotics, and required Foley catheter insertion and sexual abstinence. The authors suggested that the postoperative abstinence period varies among different groups, from 14 to 42 days (34).

A recent review (37) reported 11 years' experience of removing specimens via posterior colpotomy in 230 patients and included 899 cases collected over 17 publications. The data suggested that the removal of transvaginal specimens represented a safe, feasible, and applicable procedure in the field of laparoscopic gynecology. A 13-year experience report of 2431 single-port laparoscopic cholecystectomies performed using a laparoscope with a 6-mm working channel and percutaneous needle assistance documented excellent cosmetic results with a low incidence of PSH (38).

When considering the differences in the two oncologic staging accesses, because laparoscopy should be preferred over laparotomy as stadiation procedure in early-stage and advanced ovarian cancer, it is indisputable to think about the advantages of the umbilical than the vaginal port.

In fact, Rossetti et al. (39) evaluated the feasibility, safety, and effectiveness of laparoendoscopic single-site surgery (LESS) for the assessment of peritoneal carcinomatosis resectability in 55 patients with advanced-stage ovarian cancer (AOC). The authors reviewed the medical records of patients with AOC who underwent LESS for operative examination. Standard cytoreductive laparotomy surgery was performed. The peritoneal cancer score was completed in 49 (94%) patients using LESS; 34/37 (92%) patients who were considered to have resectable disease were effectively optimally debulked. The authors concluded that LESS was a feasible and safe alternative minimally invasive procedure to assess the resectability of patients with AOC (39).

Conclusion

In the era of reduced-port surgery and NOTES, the ideal method to access and perform surgery in the abdominal cavity and extract specimens is under scrutiny. Both navel and vaginal sites are safe and feasible for use by trained laparoscopist surgeons. A review of the literature and our own experience show that the transvaginal route is associated with reduced or no risk of PSH, reduced postoperative pain, faster recovery of common activity, and excellent cosmetic results.

When the vaginal option is not available, the lack of benefits is obvious, specifically if large specimens are to be extracted. In such cases, enlargement of an abdominal port inlet or an additional incision is needed, which increases tissue damage and pain. These maneuvers greatly limit many of the advantages of the minimally invasive approach.

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How to personalize ovarian stimulation in clinical practice

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Abstract

Controlled ovarian stimulation (COS) in *in vitro* fertilization (IVF) cycles is the starting point from which couple's prognosis depends. Individualization in follicle-stimulating hormone (FSH) starting dose and protocol used is based on ovarian response prediction, which depends on ovarian reserve. Anti-Müllerian hormone levels and the antral follicle count are considered the most accurate and reliable markers of ovarian reserve. A literature search was performed for studies that addressed the ability of ovarian reserve markers to predict poor and high ovarian response in assisted reproductive technology cycles. According to the predicted response to ovarian stimulation (poor- normal- or high- response), it is possible to counsel couples before treatment about the prognosis, and also to individualize ovarian stimulation protocols, choosing among GnRH-agonists or antagonists for endogenous FSH suppression, and the FSH starting dose in order to decrease the risk of cycle cancellation and ovarian hyperstimulation syndrome. In this review we discuss how to choose the best COS therapy, based on ovarian reserve markers, in order to enhance chances in IVF. (J Turk Ger Gynecol Assoc 2017; 18: 148-53)

Keywords: In *in vitro* fertilization, controlled ovarian stimulation, individualization, anti-Müllerian hormone, antral follicle count

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Introduction

Controlled ovarian stimulation (COS) in *in vitro* fertilization (IVF) cycles is the crucial point from which good oocyte retrieval and couple's prognosis depend. Several protocols have been studied in order to find the therapy that ensures the best outcomes in terms of pregnancy and live birth, minimizing iatrogenic risks, and the risk of cycle cancellation due to poor response or ovarian hyperstimulation syndrome (OHSS). In recent years, the concept of "one size fits all" has evolved into a concept of "individualization" in IVF. This should also reduce costs and the dropout rate of patients, mainly caused by the physical and psychological burden (1). Treatment individualization is based on ovarian reserve. The ovarian response to COS largely depends on a woman's ovarian reserve, the stimulation regimen itself is a secondary factor. Serum anti-Müllerian hormone (AMH) and ultrasound antral follicle count (AFC) in particular have been shown to be the most sensitive markers. Another strategy for the individualization of treatment is based on the response in previous IVF cycles (if a previous cycle had a good performance, the same protocol can be used).

In the Italian scenario, a strong consensus exists among physicians on the importance of the prediction of ovarian response to treatment. Ovarian reserve markers are assessed in as many as 80% of women who enter IVF programs, and the majority of physicians agree that AMH and AFC are the most reliable factors for predicting ovarian response (2). The choice of therapy is a very important clinical point because of the possibility of using various kinds of drugs [gonadotrophin-releasing hormone (GnRH)-analogues or antagonists, different gonadotrophin preparations, adjuvant therapies]. Moreover, the selection of the follicle-stimulating hormone (FSH) starting dose is fundamental for IVF outcomes (3-5). In this review, we discuss how to choose the best therapy in order to improve IVF outcomes on the basis of marker-guided ovarian response predictions.

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Evidence acquisition

A literature search was performed for studies that addressed the ability of ovarian reserve markers to predict ovarian response in IVF cycles. A systematic search of Medline, EMBASE, Cochrane library, and Web of Science databases was conducted using the keywords, anti-Müllerian hormone, AMH, antral follicles, AFC, poor/high response, and IVF. Criteria were identified in the title and/or abstract of the publications. Additional journal articles were identified from the bibliographies of included studies as well as textbooks. Literature available up to January 2017 was included.

Evidence synthesis

Ovarian reserve markers

We found that many ovarian reserve markers have been proposed in recent years. Serum FSH, measured on day 3-5 of the menstrual cycle, and estradiol are the most employed markers in reproductive medicine. The problem is that FSH is an indirect marker of ovarian reserve and its serum levels are out of range only when ovarian reserve is severely compromised. As a consequence, the literature reports suboptimal sensitivity and specificity for this marker in predicting ovarian response to gonadotrophins. Various cut-off values (from 10 to 15 IU/L) have been proposed for predicting poor ovarian response, but the large percentage of patients with normal values limits the usefulness of the marker.

In the last 10 years, serum AMH and ultrasound AFC have shown to measure the real ovarian follicle pool very accurately. The pool of 2 to 9 mm antral follicles measured using ultrasound when performing AFC is the same that produces AMH, so AFC and AMH are highly correlated and have the same performance in evaluating follicle quantity (6). AFC and serum AMH have shown similar predictive value for ovarian response and number of retrieved oocytes, and a better performance than other ovarian reserve markers in predicting ovarian response in IVF (7-9). A few studies found that AMH was the strongest predictor of ovarian response, whereas other studies demonstrated a stronger predictive value for AFC (10).

AMH has very little intra- and inter-cycle variability. With new recent automated assays we have repeatable and comparable dosages among laboratories. AFC is characterized by a certain intra-cycle variability and intra-and inter-observer variability due to different methodology for counting antral follicles; which class of antral follicles better correlates with the number of retrieved oocytes has yet to be demonstrated (2-5 mm, 4-6 mm or 5-10 mm). In clinical practice, 2-10 mm follicles are counted in order to obtain the AFC (11, 12). Three-dimensional (3D) automated follicular tracking decreases both intra- and inter-observer variability (13), but it requires advanced ultrasound equipment, which is not yet available everywhere. AFC and AMH are useful instruments for the individualization of ovarian stimulation regimens and for the choice of FSH starting dose in particular (2, 6). Several studies reported a linear correlation between AMH and live birth rates (14); however, the predictive value of AFC is less clear. Thus, AMH appears more useful when counselling couples about the chances of live birth after IVF.

Predictive models

Age is one of the most reliable indicators of ovarian response, but women of similar age may have wide variations in ovarian response due to different dimensions in the pool of recruitable antral follicles (15). Despite the usefulness of markers of ovarian reserve in order to individualize ovarian stimulation regimens, the literature is still lacking practical algorithms that may help physicians in choosing the right therapy and few studies proposed to individualize the treatment on a single marker, AFC or AMH.

A large randomized control trial (RCT) is ongoing with the aim of evaluating live birth rates and the cost-effectiveness of individualizing gonadotrophin starting doses on the basis of AFCs. In this study, women are categorized into groups based on AFCs and randomized to receive either individualized or standard gonadotrophin doses (16). Two studies have been published reporting the efficacy of serum AMH levels in tailoring gonadotrophin dose selection (5, 17). Nelson et al. (5) published a prospective non-randomized study that included more than 500 women undergoing IVF for whom the therapeutic protocol (standard long agonist or antagonist protocol) and FSH starting dose were chosen on the basis of basal AMH levels. The result of the personalized approach was a reduction of both the extremes of ovarian reserve with a reduction in excessive responses and cancelled cycles due to poor response (5). A retrospective study by Yates on 769 women at first IVF cycle demonstrated that an individualized, AMH-guided, controlled ovarian hyperstimulation protocol significantly improved positive clinical outcomes, reduced the incidence of complications, and reduced the financial burden associated with assisted reproduction (17). A recent pilot study compared the efficacy and safety of two algorithms, one based on AMH and the other on AFC, to determine the starting dose of recombinant FSH (rFSH) for ovarian stimulation in 348 women. Patients were assigned to receive an FSH starting dose of 150, 225 or 375 IU on the basis of pre-treatment AMH or AFC. The study reported no difference in terms of clinical pregnancy, multiple pregnancies and miscarriage rates between the two groups, but there was a statistically significant difference in ovarian response, with a major proportion of hyper responses in the AFC-tailored group (18).

Complex predictive models

Different variables are implicated in ovarian response (19-21). This concept brought about the elaboration of complex algorithms in order to better predict ovarian response and to define the right FSH starting dose. A prospective study tested a model including age, AFC, ovarian volume, Doppler ovarian score, and smoking status (19), but the complexity in measured variables did not permit wide clinical application. Another study proposed a model based on age, body mass index (BMI), day 3 serum FSH and AFC (22), which was later tested in the CONsistency in r-FSH Starting dOses for individualized tReatmenT (CONSORT) study (23). This model was not applied in clinical practice because the coefficients for computing the algorithm were not published. Moreover, the FSH starting dose calculated by the model was often lower than those proposed by clinical practice and led to iatrogenic poor responses. This was confirmed in a successive prospective study, where the CONSORT calculator was used to calculate the FSH starting dose for 197 women undergoing IVF cycles: the calculated dose was too different to the dose recommended by physicians to be applied (24).

A more recent study created a model through a retrospective analysis based on age, AFC, and day 3 serum FSH, with AFC being the most significant predictor of ovarian response (25). According to the model, in a woman aged 30 years with a normal day 3 FSH of 4 IU/L and an AFC of 16, the most appropriate gonadotrophin dose is 150 IU daily. A similar nomogram based on AMH had previously been developed by the same group and included AMH, age, and day 3 serum FSH (26). Based on the nomogram, a woman aged 30 years with FSH of 4 IU/L and AMH 4 ng/mL would require a gonadotrophin dose of 150 IU/daily. A model incorporating AMH has recently been validated retrospectively in two independent IVF centers in Italy. In both centers, the application of the nomogram resulted in more appropriate FSH starting doses compared with empirically chosen treatment. This easy-to-use algorithm could be useful in daily clinical practice for increasing the number of patients reaching optimal ovarian response (27). The AMH-based approach for COS individualization has recently been further confirmed by the results of a multicenter randomized phase-3 trial, the Evidence-based Stimulation Trial With Human rFSH in Europe and Rest of World 1 study (ESTHER-1). The study compared the efficacy and safety of a new recombinant FSH (follitropin delta) with an AMH and BMI-tailored dose with conventional recombinant FSH (follitropin alfa). The use of the new gonadotropin resulted in similar ongoing pregnancy and live birth rates, with fewer excessive and poor responses compared with the control group (28).

The prediction of ovarian response based on ovarian reserve markers may be useful for the choice of stimulation protocols and of any supplementary therapies, as discussed below.

Ovarian response prediction and management

After the ovarian response has been predicted, the physician has to choose the most adequate COS protocol and FSH starting dose in order to obtain an optimal oocyte retrieval. An egg collection of between 8 and 15 oocytes should guarantee the highest chances of pregnancy. Egg retrievals of less than 8 oocytes reduce pregnancy rates because of the lack of adequate numbers of good embryos to transfer. Retrievals of more than 15 oocytes may expose patients to the risk of OHSS. This means that physicians should try to obtain a moderate follicular recruitment in high responders. All protocols have demonstrated similar performance in IVF outcomes for predicted poor responders, as such the best protocol is the least stressful for the patient.

Predicted poor response

The prognosis in IVF cycles depends on age and ovarian reserve. Assisted reproductive technology (ART) can only partially provide against the decay of fertility induced by age and reduction of ovarian reserve: pregnancy rates in women aged over 40 are less than 10%, and only slightly higher in younger women with severely reduced ovarian reserve. A low ovarian reserve translates into an inadequate response to controlled ovarian stimulation, insufficient egg retrieval, and maybe to poor oocytes and embryo quality. Poor ovarian response is defined as the retrieval of <4 oocytes following a standard IVF protocol (29). The incidence of poor ovarian response in IVF cycles ranges from 10 to 20% and the prevalence increases with advancing age.

The criteria used to identify poor ovarian responders are both anamnestic (age, shortening of the menstrual cycle, previous ovarian surgery) and clinical, based on the study of ovarian reserve. The problem of using ovarian reserve markers is in defining acceptable cut-off levels for predicted poor response. The literature reports several values for AMH and AFC in the prediction of the poor response. The variability could be explained by factors such as the small sample size of some studies and variability in the measurement of markers. According to published data, a cut-off value of AMH ranging between 0.7-1.3 ng/mL may be considered acceptable for the prediction of poor response in IVF, with sensitivity and specificity (20). AFC can be used to reliably predict ovarian response in IVF, but there is high variability in cut-off levels in the literature (10). Recent studies reported AFC cut-off values for the prediction of poor response ranging between <5 and <7 (30).

The assessment of ovarian reserve in these patients is useful during pre-treatment counseling in order to advise couples about the possibility of cycle cancellation and poor prognosis,

and reduces drop-out rates. Although poor ovarian reserve is associated with poor IVF outcomes, the diagnosis of poor ovarian reserve is not acceptable as the only factor leading to direct exclusion of couples from undergoing ART treatment programs. In fact, AFC and AMH, which are the best predictive markers, have a false positive rate of 10-20%. Moreover, the accuracy of these markers is not high in the prediction of pregnancy (31), and the possibility of achieving pregnancy, especially in young women, is quite acceptable (32).

Unfortunately, there is currently insufficient evidence to recommend a particular treatment for women defined as poor responders. Treatment with a GnRH antagonist protocol instead of a GnRH agonist protocol was initially proposed for such women because it avoids the profound suppression of endogenous FSH and luteinizing hormone (LH) concentrations in the early follicular phase at the stage of follicular recruitment, giving hope of a better egg retrieval. Several trials and meta-analyses showed that the long GnRH agonist and GnRH antagonist regimens were comparable in their efficacy in terms of IVF outcomes for poor responders (33).

The few studies published on women with predicted poor response undergoing their first IVF cycle reported similar outcomes using both protocols. As shown by Nelson et al. (5), the GnRH antagonist protocol was associated with fewer days of gonadotrophin stimulation but the prognosis for these women remained poor, with clinical pregnancy rates ranging between 16% and 11% (5). We think that the choice of therapeutic protocol should aim to gain patient compliance and cost reduction in poor responder patients (17). A recent multicenter randomized trial demonstrated the non-inferiority of a mild ovarian stimulation strategy with a GnRH antagonist compared with a standard approach with a GnRH agonist. The ongoing pregnancy rate was 12.8% (25/195) for mild ovarian stimulation versus 13.6% (27/199) for conventional ovarian stimulation [95% confidence interval: (0.57-1.57)], and the duration of ovarian stimulation and amount of gonadotrophins used were significantly lower in the mild stimulation strategy (34).

Different studies performed on predicted poor responders showed that increasing FSH dose did not correlate with the number of retrieved oocytes (35). The maximum number of oocytes that could be retrieved in women was strongly limited by the number of recruitable antral follicles in the ovaries and a higher gonadotrophin dose was not able to compensate for the lack of substrate.

In conclusion, prediction of poor response can have positive results in terms of patient compliance and reduction of costs, but it does not seem to produce a significant improvement in IVF outcomes (36).

Predicted high response

The term 'hyper response' refers to the retrieval of >15 oocytes (37) following a standard COS protocol. The prevalence rate in IVF cycles is estimated to be around 7% and decreases with the woman's age. Young age, long menstrual cycles, polycystic ovary syndrome (PCOS), and hyper response in a previous cycle (38) are suggestive of high ovarian reserve, but the stronger predictors of hyper response are AMH and AFC. AMH cut-off levels proposed in literature for the prediction of hyper response vary according to the assay used (DSL, IBC or AMH gen II), but AMH serum levels >3.5 ng/mL have good sensitivity and specificity (2). An AFC value of >16 has been shown to be the most appropriate cut-off for hyper response (8, 39).

The measurement of ovarian reserve markers has a relevant value in patients with high ovarian reserve. First, it allows counselling couples about the potential risks associated with treatment, such as OHSS. Secondly, it permits choosing the treatment according to the predicted ovarian response. In patients with high ovarian reserve, COS individualization is crucial because it improves IVF outcomes and avoids the iatrogenic risk of OHSS. Recent studies demonstrated that the use of GnRH antagonists in predicted high responders was associated with a reduction in the incidence of OHSS. As a consequence, a reduction in cycle cancellation and patient hospitalization was achieved with a significant reduction in costs (17, 40). A large RCT including 1050 first IVF cycles recently demonstrated that the incidence of severe OHSS (5.1% vs. 8.9%; p=0.02) and moderate OHSS (10.2% vs. 15.6%; p=0.01) was significantly lower in the GnRH antagonist group compared with the agonist group, respectively, and pregnancy rates were similar in the two groups (40).

In GnRH antagonist protocols, initial follicular recruitment and selection is undertaken using endogenous endocrine factors prior to starting exogenous gonadotrophin administration. This leads to a lower number of growing follicles when compared with the standard long GnRH agonist protocol, which is why GnRH antagonist protocols are the first-choice treatment in women with high ovarian reserves at risk of OHSS. Secondly, GnRH antagonist protocols allow the possibility of inducing final oocyte maturation with an GnRH analogue instead of human chorionic gonadotrophin (hCG). This seems to significantly reduce the risk of OHSS, but it is associated with lower pregnancy rates in fresh IVF cycles because of an adverse effect on endometrium receptivity due to the absence of hCG (41). Implantation rates, clinical pregnancy rates, ongoing pregnancy rates, and survival rates of frozen-thawed embryos are similar in hCG with GnRH agonist trigger protocols, which demonstrates that GnRH agonist protocols do not impact on oocyte quality (42).

A strategy to improve outcomes in GnRH agonist-triggered cycles is the addition of a low dose (1500 IU) of hCG, administered 35 h or 5 days after the triggering bolus of GnRH agonist; however, this approach does not eliminate severe OHSS (23). Owing to the enhanced effectiveness of vitrification, segmentation in GnRH agonist-triggered cycles through the freezing of all embryos for transfer in subsequent cycles may be the optimal strategy to eliminate the risk of OHSS while maintaining elevated pregnancy rates (41, 43).

The FSH starting dose is another crucial determinant of ovarian response to stimulation. In women with high ovarian reserve, the choice of an unduly low gonadotrophin dose could lead to mono or pauci-follicular development. On the other hand, the choice of an excessive dose could lead to excessive ovarian response with subsequent OHSS risk. We believe that predictive algorithms based on reliable markers of ovarian reserve, such as those described above (25, 26), may guide physicians in this choice.

GnRH antagonists are better than GnRH-agonist in high responder patients at reducing the occurrence of OHSS, while maintaining comparable clinical pregnancy rates. Moreover, the FSH starting dose must be chosen on the basis of ovarian reserve markers.

Conclusions

Ovarian reserve establishes prognosis in terms of oocyte retrieval and chances of live birth in IVF at any age of the woman. Medical history and a good assessment of ovarian reserve markers guarantee optimal oocyte retrieval, thereby formulating the most appropriate COS protocol for individual patients. The literature indicates how to guide correct management of patients, from predicted poor- to hyper-responders, but much remains to be done to reduce iatrogenic risks and improve IVF outcomes.

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What is your diagnosis?

A P2L2 woman aged 35 years presented to our outpatient department with amenorrhea of 8 months with episodes of on-and-off heavy bleeding per vaginum. She had a history of laparoscopic right salpingectomy for right tubal ectopic pregnancy 8 months previously, which was confirmed on histopathology (HPE). When she presented to us, she was carrying ultrasonography (USG) of her pelvis, computed tomography (CT) of the abdomen and pelvis, and several human chorionic gonadotropin (beta-hCG) reports of last 8 months. Imaging was absolutely normal and beta-hCG values were showing a waxing and waning course (Figure 1), but never came below a discriminatory zone despite two courses of methotrexate (MTX) in doses of 50 mg/m^2 in single dose regimens. A provisional diagnosis of pregnancy of unknown location (PUL) was made at that time because a pregnancy test was positive, serum beta-hCG was 10,304 IU/L, and no intra or extra-uterine gestation could be cited on ultrasound. She had no spells of abdominal pain or fainting attacks. On being referred to our unit, we evaluated her on the lines of gestational trophoblastic neoplasia (GTN) in view of persistently high beta-hCG for the last 8 months (graphical presentation of beta-hCG shown in Figure 1). Routine blood investigations, chest X-ray, USG pelvis, as well as CT chest and brain were found as normal. We did not repeat CT of the abdomen and pelvis. Considering persistently high beta-hCG values without any evidence of pregnancy on imaging, two courses of single agent chemotherapy (MTX-FA) in a multiple dosage regimen (Inj. MTX 1 mg/kg/day on days 1, 3, 5, and 7, and Inj. Folinic acid 15 mg on day 2, 4, 6, and 8) was given intravenously, 2 weeks apart; however, beta-hCG value remained high.

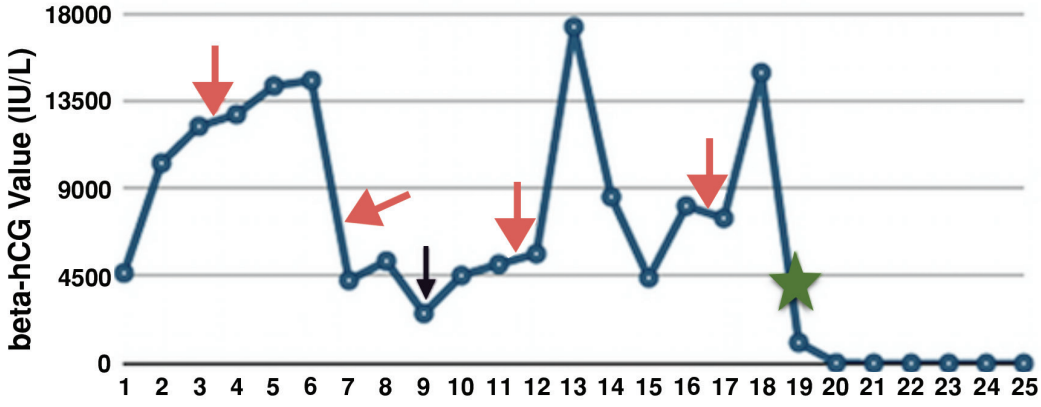

Figure 1. Graphical presentation of beta-human chorionic gonadotropin values (red arrow indicates the timing of single-agent chemotherapy, the purple arrow shows the time of referral to us, and the green star is the time of surgery)

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DOI: 10.4274/jtgga.2017.0023
Presented as a poster at RCOG World Congress, 12-15th April 2015, Brisbane, Australia.

Answer

After a thorough discussion and failure to find the source of beta-hCG, the decision was taken to perform a whole-body positron emission tomography (PET)-CT scan. On PET-CT, an active 5x4-cm malignant lesion was found in the bowel mesentery. No metastasis was found in any other site. The patient underwent exploratory laparotomy. Intra-operatively, a 5x5-cm, firm, well-circumscribed and encapsulated mass was present over the greater omentum, as shown in Figure 2a. The whole mass, with a 2-cm healthy omental margin, was excised. The cut section of the mass showed extensive areas of necrosis and hemorrhage (Figure 2b). The uterus and bilateral adnexa were healthy. The right fallopian tube was found absent intra-operatively. The mass was confirmed as an omental choriocarcinoma on HPE (Figure 3-4). Postoperatively, her beta-hCG value decreased dramatically to 1073 IU/L, and 112 IU/L after one week. She required no adjuvant chemotherapy because there was no evidence of local or distant metastasis. The patient was under regular follow-up with us as well as the medical oncology department. Beta-hCG follow-up was performed weekly until three negative values were obtained, and monthly thereafter for 24 months. During follow-up, her beta-hCG value always remained negative (Figure 1). She never had any signs and symptoms of recurrence. The patient is now disease free.

PUL is defined by the presence of a positive pregnancy test with an absence of either intra or extrauterine pregnancy on transvaginal ultrasound (1). The reported incidence of PUL is 8-10% (1). MTX 50 mg/m^2 has proven to be effective in 90% of cases of ectopic pregnancy and also results in resolution of beta-hCG levels in women with asymptomatic persisting PUL (1). An algorithm for the management of PUL is described in Figure 5 (1).

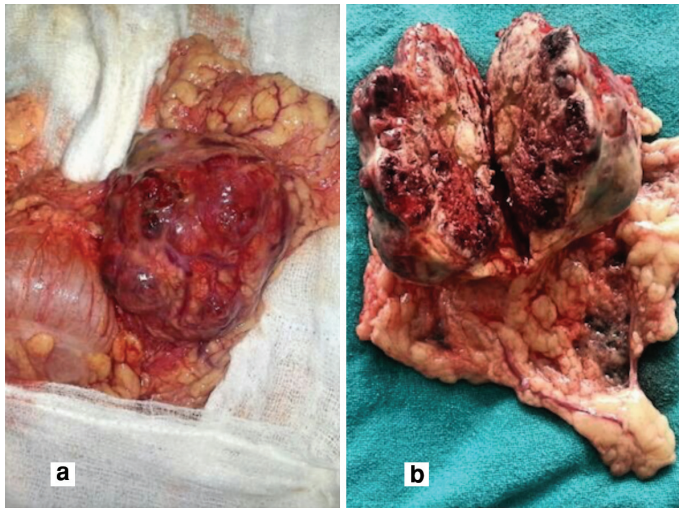

Figure 2. (a). A 5x5-cm firm, well circumscribed and encapsulated mass over the greater omentum
(b). Cut section of the mass showing extensive areas of necrosis and hemorrhage

GTN usually occurs after molar pregnancy (50%), but may also be present after abortions (25%), normal intrauterine pregnancy (22.5%), and ectopic pregnancy (2.5%) (2). Choriocarcinoma per se is a rare tumor. The incidence of choriocarcinoma is 1 in 45,000 pregnancies in Western countries, but a higher incidence is reported from southeast Asia (3). Choriocarcinoma is a malignant and aggressive tumor, associated with high beta-hCG. Most cases of choriocarcinoma are intrauterine and of gestational origin. Extrauterine choriocarcinoma is quite rare, few case reports are present in the literature (4-12). Moreover, choriocarcinoma of the greater omentum is extremely rare, with only two cited cases (8, 11). Most cases

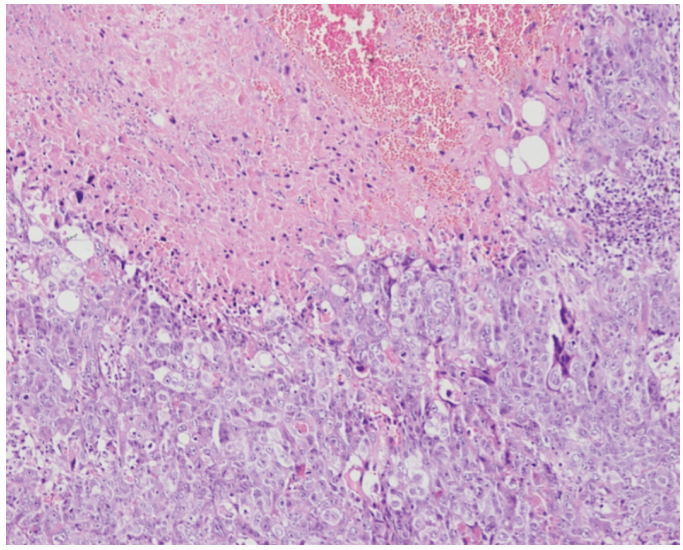

Figure 3. H&E (x100) stained section showing necrotic area along with the tumor

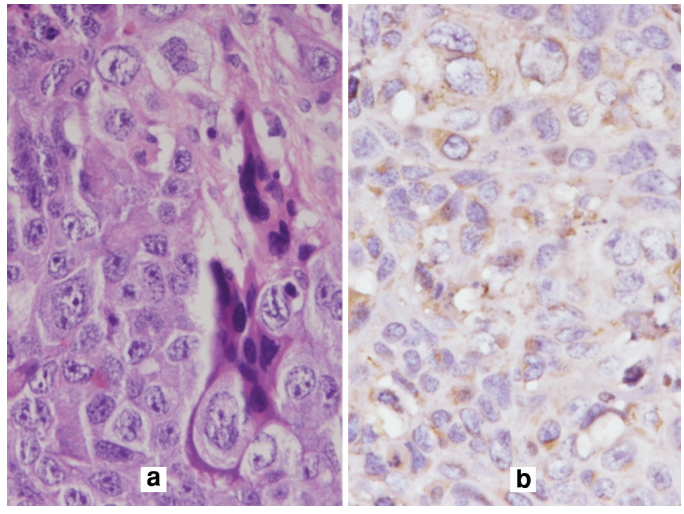

Figure 4. (a). H&E (x400) showing biphasic tumor comprising of giant cells and mononuclear tumor cells, (b). IHC-Positive for beta-human chorionic gonadotropin

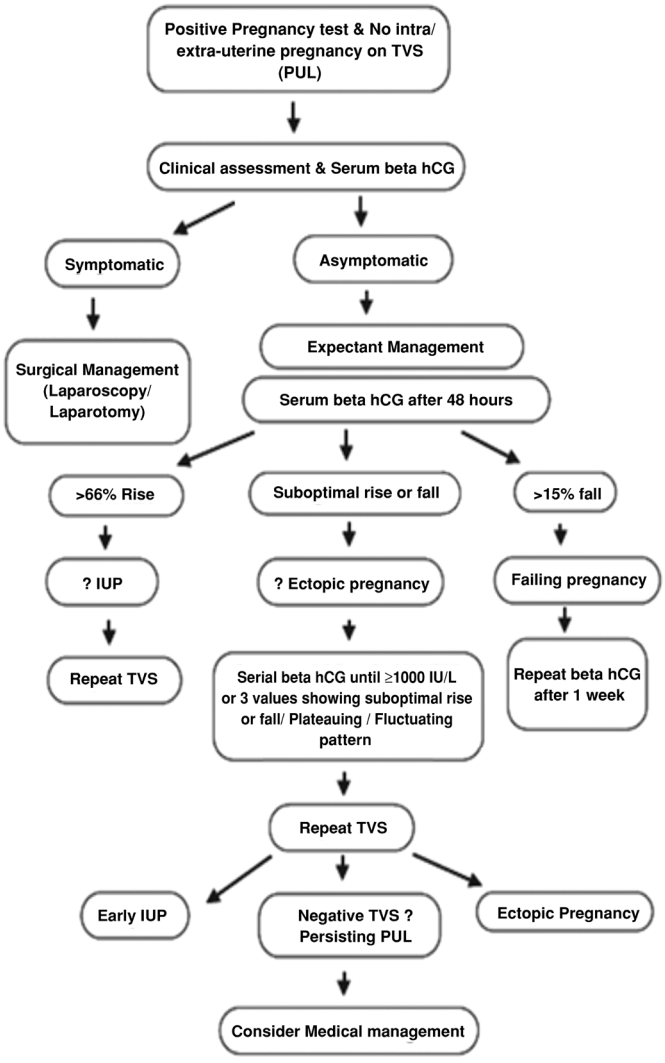

Figure 5. An algorithm for the management of pregnancy of unknown location
IUP: intra-uterine pregnancy; TVS: trans vaginal scan

developed secondary to implantation of trophoblastic tissue following an ectopic gestation (4-8) and sited in the genital tract (tube, ovary, cervix, and vagina) (4-7). A case of omental choriocarcinoma mimicking ectopic pregnancy was reported by Wan et al. (8). Their patient required multiple courses of combined chemotherapy to achieve remission. The plausible etiology of this lesion in our case was the persistence of trophoblastic tissue left during laparoscopic salpingectomy that may have got implanted over the omentum. In the case of primary extrauterine choriocarcinoma, the uterus, tubes, and ovaries show no evidence of pregnancy. On reviewing the literature, four cases of primary choriocarcinoma could be elucidated occurring on the left anterior abdominal wall of the pelvis (9), on the surface of a subserosal uterine leiomyoma (10), in the omentum (11), and the right lower lobe of the lung (12). All these cases were managed by complete resection of tumor ± adjuvant chemotherapy. The prognosis of such cases is not well studied due to its rarity, but according to the above case reports, most patients achieved complete remission.

We report this case of choriocarcinoma of the greater omentum not for its rarity, but for the fact that it was missed despite an intensive examination. Our patient was initially managed along the lines of PUL considering that imaging did not support any ectopic site for the source of beta hCG. That an initial baseline CT showed no tumor goes with the understanding that perhaps the lesion was small enough to be missed on CT, yet it produced enough beta hCG, and was uncompromising to chemotherapy. The lesion may have increased in size because no imaging was repeated during MTX cycles. We decided to perform a PET-CT for the whole body in the absence of an adequate response to standard chemotherapy, which was the turning point in our case because we established the source of beta-hCG. The efficacy of PET-CT in defining such lesions is not known, perhaps due to the paucity of such cases, which typically undergo surgery in good time with a provisional diagnosis of ectopic pregnancy.

Conclusion

Rare sites of choriocarcinoma can be missed despite intensive examinations. A suspicion of disease should always be kept when serial beta hCG levels show a plateau or rising trends despite chemotherapy. PET-CT may be a useful adjunct to map such lesions. Surgical excision of these tumors remains the best modality in resectable cases. Close follow-up is mandatory to achieve complete remission.

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Obesity and insulin resistance are significant predictors of serum leptin levels

To the Editor;

In the December 2016 issue of your journal, Fakor et al. (1) presented an original article entitled "The association between levels of maternal serum leptin in the third trimester and the occurrence of moderate preterm labor" in which the authors elucidated the possible role of leptin in the development of preterm labor in 30 moderate preterm delivering women. The authors demonstrated that low serum leptin levels may have a substantial predictive value for preterm birth before 34 weeks' gestation by altering cytokine balance, cytotrophoblast and angiogenic activity, in the feto-placento-maternal unit. Although the authors presented and discussed their study results effectively in the context of previous studies, we believe that like any other study, there are some methodologic issues related to the present paper. In this context, from a methodologic point of view, we have several concerns including unmatched body mass index (BMI) values of study participants, and the presence of a probable insulin resistance (IR), which is associated with preterm birth and serum leptin levels.

First, as also stated by the authors, numerous factors are strictly related with serum leptin levels including obesity, insulin, glucocorticoids, and thyroid hormones via multiple signaling pathways (2). From these variables, obesity presents a unique importance while evaluating serum leptin levels because of the fact that obese subjects have higher serum leptin values, which correlates body weight percentage, than normal weight subjects (3). The importance of BMI on serum leptin levels was demonstrated in a study by Paul et al. (4). Serum leptin levels and differences between sexes were found to be significant in all body mass indices. In this context, when comparing two groups to evaluate serum leptin levels, it is crucial to match study groups in respect to BMI values. The significantly higher BMI levels of control subjects ($p=0.017$) could be the reason of elevated leptin levels. In order to rule out this bias, an additional statistical analysis is needed to understand if serum leptin levels are affected by the BMI values of study participants.

Second, although the authors stated that they excluded patients with diabetes mellitus, they gave no information about the possible presence of IR in their study group. IR is especially important for a study investigating a connection between leptin and preterm labor because IR contributes to the relatively wide variation in leptin levels, which is seen even at similar levels of body mass (5, 6). Moreover, IR below the thresholds of gestational diabetes mellitus (GDM) can cause adverse maternal and perinatal outcomes. In a recent large-scale retrospective study by Temming et al. (7), it was demonstrated that even in the absence of GDM, IR was associated with increased risks of preterm birth. Based on these data, it is reasonable to suggest that the alterations on serum leptin levels could be associated with IR and resulting preterm labor.

In conclusion, as we see an increasing number of obstetric complications including preterm labor, this study covers an important and interesting topic. We believe that the further understanding of the roles of adipocyte-derived hormones in human pregnancy will provide an insight into metabolic risks associated with preterm labor.

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Author's Response

Dear Editor;

In a letter entitled "Obesity and insulin resistance are significant predictors of serum leptin levels," two main criticisms were mentioned. The first was the effect of body mass index (BMI) in leptin levels, which has been established in many previous studies (1, 2). We mentioned it in the introduction of the paper and also considered BMI as an important confounding variable in our study (3). As a basic study design concept, we tried to match BMI variables in the case and control groups. However, we could not select two BMI-matched groups (p=0.017) because of the lack of patients with preterm labor. Therefore, we decided to decrease the effect of the BMI variable by statistical methods. The other criticism was the effect of insulin resistance (IR) on leptin level. As it was written in methods section of our article (3), we tried not to include conditions related with IR such as diabetes and polycystic ovarian syndrome. When patients are hospitalized in Iran, their blood sugar is checked as a routine lab test. In addition, a glucose tolerance test is performed between 24-28 weeks of pregnancy as a screening program in Iran.

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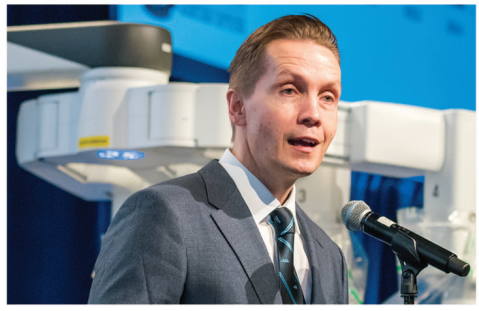

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AAGL
ELEVATING GYNECOLOGIC SURGERY

AAGL POSTGRADUATE COURSES

PG DAY ONE (NOVEMBER 12, 2017)

Morning courses 7:00am – 11:00am	
ROBO-600	**Didactic:** Building a World Class Robotic Program: Simulation, Integration, Application and Evaluation *Chair: Gaby N. Moawad*
ANAT-602	**Didactic:** A Treasury of Pelvic Anatomy: Sacred Knowledge for Surgical Expertise *Chair: David M. Boruta*
URO-604	**Didactic:** Practical Anatomy for Complex Pelvic Surgeries: Things Every Gynecologist and Urogynecologist Should Know *Chair: Anthony G. Visco*
SUTR-606	**Didactic/Simulation Lab:** Laparoscopic Suturing: Practical Applications for Tissue Reapproximation, Intracorporeal and Extracorporeal Knot Tying, Barbed Suture, and Suturing Technologies *Chair: Lydia E. Garcia*
FELO-608	**Didactic:** Career Tools for Life: How to Navigate a Successful MIGS Career of Your Dreams *Co-Chairs: Hye-Chun Hur, Warren Volker*
SAFE-610	**Didactic:** Optimizing Quality and Patient Safety *Chair: Amanda Nickles Fader*
Afternoon courses 12:30pm – 4:30pm	
ROBO-601	**Cadaveric Lab:** Creating Systematic Proficiency *Chair: Devin M. Garza*
ANAT-603	**Cadaveric Lab:** Navigating the Retroperitoneum: The Road to Performing Complex Laparoscopic Gynecologic Surgery *Chair: Yukio Sonoda*
URO-605	**Cadaveric Lab:** Complex Surgical Spaces Demystified with Hands-on Experience: Anatomy Every Gynecologist and Urogynecologist Should Know *Chair: Marlene Corton*
SUTR-607	**Didactic/Simulation Lab:** Laparoscopic Suturing: Practical Applications for Tissue Reapproximation, Intracorporeal and Extracorporeal Knot Tying, Barbed Suture, and Suturing Technologies *Chair: Jamie Kroft*
ENDO-609	**Didactic:** Minimally Invasive Management of Complex Endometriosis: From Imaging Pearls to Fertility-Sparing Surgery to Hysterectomy *Chair: Ken R. Sinervo*
PELV-611	**Didactic:** Pelvic Pain – Making It Right: Effectively Fixing Painful Complications *Chair: Mark W. Dassel*

PG DAY TWO (NOVEMBER 13, 2017)

Morning courses 7:00am – 11:00am	
COMPLX-700	**Didactic:** Oncology: Complex Surgical Anatomy and Procedures *Chair: Pamela T. Soliman*
HYST-702	**Didactic:** Laparoscopic Hysterectomy from Basic to Complex *Chair: Nash S. Moawad*
NEURO-704	**6-HOUR COURSE:** 7:00am – 2:30pm **Didactic w/Live Cadaveric Demo:** Neuropelveology: A Systematic Approach to the Diagnosis and Management of Complex Pelvic Pain and Pelvic Neuropathies *Co Chairs: Michael Hibner, Nucelio Lemos*
VHYS-705	**Didactic w/Live Cadaveric Demo:** Vaginal Hysterectomy: Mastering the Most Minimally Invasive Approach to Hysterectomy and Taking It to the Next Level *Co-Chairs: Johnny Yi, Veronica Lerner*
SUTR-706	**Didactic/Simulation Lab:** Laparoscopic Suturing: Practical Applications for Tissue Reapproximation, Intracorporeal and Extracorporeal Knot Tying, Barbed Suture, and Suturing Technologies *Chair: Grace Y. Liu*
TEACH-708	**Didactic:** Become the Master Shifu You Always Wanted to Be *Chair: Sangeeta Senapati*
HSC-710	**FULL-DAY COURSE:** 7:00am – 3:30pm **Didactic/Simulation Lab:** Advanced Operative Hysteroscopy: Expect the Unexpected *Co-Chairs: Linda D. Bradley, Aarathi Cholkeri-Singh*
Afternoon courses 12:30pm – 4:30pm	
COMPLX-701	**Cadaveric Lab:** Complex Surgical Anatomy/Complications *Chair: Edward J. Tanner*
HYST-703	**Cadaveric Lab:** Laparoscopic Hysterectomy: Navigating the Basic and Complex Disease with Ease *Chair: Karen C. Wang*
SUTR-707	**Didactic/Simulation Lab:** Laboratorio de Simulación en ESPAÑOL: Sutura Laparoscópica: Aplicación práctica para Reaproximación de tejidos, Nudo Intracorpóreo y Extracorpóreo, Sutura Barbada y Tecnologías de Sutura *Chair: Jaime A. Albornoz*
TEACH-709	**Simulation Lab:** Teach the Teacher *Chair: Nicole M. Donnellan*
PUSH-711	**Didactic:** Shoot for the Moon: Surgical Strategy from the Stars *Chair: Audrey Tsunoda*
FIBR-712	**Didactic:** Contemporary Fibroid Therapies and Musical Hits from the 80s: Might There Be an Association? *Chair: M. Jonathon Solnik*

Registration now open!

For more information go to *www.aagl.org*

AAGL BLOCK PROGRAM

Tuesday, November 14 - Congress (Registration Hours 6:30 am — 5:30 pm)				Exhibit Hall Hours 9:30 am — 3:30 pm		
6:00am — 7:45am	Industry Sponsored Breakfasts (6:00 am — 7:45 am)		Surgeon's Breakfast: "*Highlighting Women in Medicine*" (6:00 am — 7: 45am, Additional charge)			
7:30am — 9:30am	CME General Session II — Live Interactive Cadaveric Demonstration: Anatomy/Jordan M. Phillips, M.D. Keynote Address					
9:35am — 11:00am	Exhibit Hall Open/Refreshment Break					
Room Number						
11:00am — 12:00pm	CME Plenary 1 Hysteroscopy	CME Panel 1 The Chronic Pain Patient	CME Surgical Tutorial 1 Vaginal Hysterectomy	Open Comm. 1 Endometriosis	Open Comm. 2 Robotics	Video Session 1 Robotics
12:10pm — 1:10pm	CME Plenary 2 Oncology	CME Panel 2 Maximizing Efficiency in Low Resource Settings	CME Surgical Tutorial 2 Cuff Closure	Open Comm. 3 Emerging Technology & Techniques	Open Comm. 4 Endometriosis	Video Session 2 Endometriosis
1:10pm — 3:30pm	Exhibit Hall Open/Box Luncheon					
2:15pm — 3:15pm	CME Plenary 3 Robotics	Open Comm. 5 Research & Science	Open Comm. 6 New Instruments	Open Comm. 7 Hysteroscopy	Open Comm. 8 Hysteroscopy	Video Session 3 Urogynecology
3:25pm — 5:05pm	CME Plenary 4 Laparoscopy	CME Panel 3 Same Day Discharge +/- ERAS	CME Surgical Tutorial 3 Robotic Surgery: Port Placement and Docking	Open Comm. 9 Laparoscopy	Video Session 4 Hysteroscopy	Video Session 5 Laparoscopy
5:10pm — 6:10pm	CME General Session III — Stump the Professor					
6:10pm — 8:10pm	Industry Sponsored Symposia					
7:30pm — 12:30am	Urban Pub Crawl (Additional charge)					

Wednesday, November 15 - Congress (Registration Hours 6:30 am — 5:30 pm)				Exhibit Hall Hours 9:30 am — 3:00 pm		
6:00am — 7:45am	Barre3 Fitness Class (6:00 am — 7:00 am, Additional Charge)		Industry Sponsored Breakfasts (6:00 am — 7:45 am)			
7:45am — 9:30am	CME General Session IV - Chopped/Business Meeting					
9:30am — 11:00am	Exhibit Hall Open/Refreshment Break					
Room Number						
11:00am — 12:00pm	CME Plenary 5 Urogynecology	CME Panel 4 Special Populations	CME Surgical Tutorial 4 Anterior & Posterior Obliterated Cul-De-Sac	Open Comm. 10 Urogynecology	Open Comm. 11 Robotics	Video Session 6 Single Port Laparoscopy
12:10pm — 1:10pm	CME Plenary 6 Endometriosis	CME Panel 5 Endometriosis: Medical vs. Surgical	CME Surgical Tutorial 5 Uh Oh! Managing Surgical Complications	Open Comm. 12 Oncology	Open Comm. 13 Reproductive Medicine	Video Session 7 Laparoscopy
1:10pm — 3:00pm	Exhibit Hall Open/Box Luncheon					
2:15pm — 3:15pm	CME Plenary 7 Reproductive Issues	Open Comm. 14 Pelvic Pain	Open Comm. 15 Surgical Education	Open Comm. 16 Research & Science	Video Session 8 Endometriosis	Video Session 9 Robotics
3:25pm — 5:05pm	CME Plenary 8 Education, Research & Science	CME Panel 6 Defining Quality Metrics	CME Surgical Tutorial 6 Tips and Tricks for Managing Fibroids	Open Comm. 17 Laparoscopy	Video Session 10 Basic Science, Research & Education	Video Session 11 Laparoscopy
5:10pm — 7:10pm	Industry Sponsored Symposia					
7:00pm — 12:00am	Silent Auction (7:00 pm - 9:00 pm)		Presidential Gala (9:00 pm - 12:00 am, Additional charge)			

Thursday, November 16 - Congress	
8:30am — 12:30pm	CME General Session V - Telesurgery Session

LET'S PUT THE "FUN" IN FUNDRAISING

What's new for 2017?

This year we're excited to offer several unique, fun, and fulfilling opportunities to support the efforts of The Foundation through multiple fundraising events and opportunities. All proceeds from each of these events go toward The Foundation's mission of continued progress in the field of minimally invasive gynecology.

We hope you'll join us for one or more of these events and enjoy the networking and social atmosphere - all for a greater cause.

Silent Auction

Wednesday, November 15, 2017
(7:00 pm - 9:00 pm)
Preceding the Presidential Gala

Imagine yourself on a trip of a lifetime. Or advancing your surgical skills during an observership with a high-profile surgeon. Are championship sports events more your thing? Perhaps you've always wanted to buy an original piece of art. All this and more will be available for bid with all proceeds benefiting the Foundation. Come see if your dreams can be fulfilled.

Congressional Crawl

Monday, November 13, 2017
(8:30 pm - 12:30 am)

No trip to the greater Washington, D.C. Area is complete without touring our national monuments. This evening tour lets you visit them without the usual daytime crowds, allowing you to take in the history and beauty as they're bathed in bright lights against the dark sky - a truly unique and memorable experience.

Urban Pub Crawl

Tuesday, November 14, 2017
(7:30 pm - 12:30 am)

Hip and trendy bars, extraordinary restaurants - these are the cornerstones of what night life in DC has come to be known for. Join us as we visit some of DC's trendiest locales for delicious hors d'oeuvres and creative craft cocktails. Your local hosts will ensure that you have a great time, and a safe time with dedicated shuttle drivers. Networking and fundraising like you've never done before!

Barre3 Fitness Class

Wednesday, November 15, 2017
(6:00 am - 7:00 am)

If fitness is more up your alley, then a sunrise Barre3 class will certainly get your day started right. Barre3 delivers a full body workout using only low-impact movements from 3 different disciplines - ballet barre, pilates, and yoga. No experience is required. Do your body some good while you support the Foundation's efforts. What could be more fulfilling than that?

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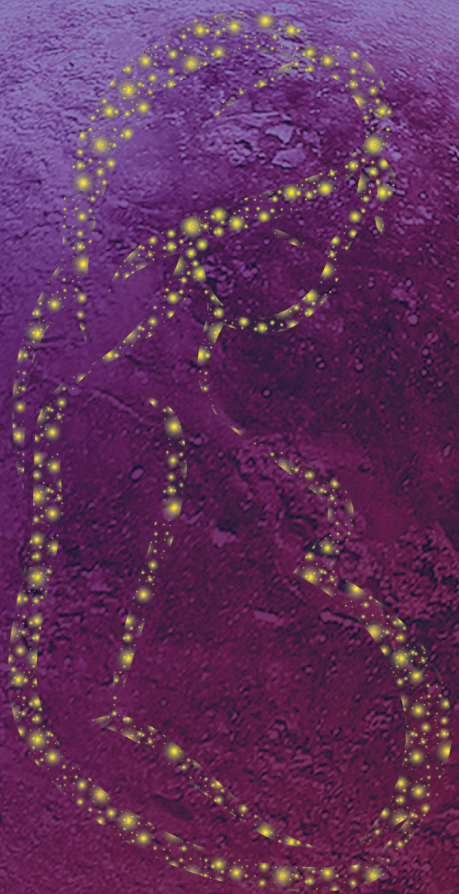

Drosetil

Etinilestradiol 30 mcg
Drospirenon 3 mg

Drospera

Etinilestradiol 20 mcg
Drospirenon 3 mg

Dienille

Etinilestradiol 30 mcg
Dienogest 2 mg

DİENİLLE KÜB ÖZETİ: ÜRÜN ADI: DİENİLLE 2 mg/0,03 mg Film Kaplı Tablet. **FORMÜL:** 2 mg dienogest/0,03 mg etinilestradiol (Her bir film kaplı tablet). **FARMAKOLOJİ:** ATC kodu: G03FA15. DİENİLLE dienogest (progestojen) ve etinilestradiol (östrojen) içeren, antiandrojenik etkili bir kombine oral kontraseptif (KOK)'tir. **ENDİKASYONLAR:** Hormonal kontrasepsiyon. **KULLANIM ŞEKLİ VE DOZU:** Tabletler her gün aynı zamanda ve birbirini izleyen 21 gün boyunca alınır. Bir önceki ay hormonal kontraseptif kullanımı yoksa, tabletler kanamanın ilk günü alınmaya başlanmalıdır. Bir sonraki pakete 7 günlük tabletsiz aradan sonra devam edilir, genellikle ara kanama bu dönemde meydana gelir. Tipik olarak kanama, son hapı aldıktan 2-3 gün sonra başlar ve bir sonraki paketten ilk tablet alınana kadar devam edebilir. **UYGULAMA ŞEKLİ:** Oral. **KONTRENDİKASYONLAR:** İçeriğindeki maddelerden herhangi birine karşı aşırı duyarlılık; venöz tromboz veya pozitif hasta öyküsü; arteriyel tromboz veya pozitif hasta öyküsü veya prodromal olayların varlığı; arteriyel tromboz için ciddi ya da birçok risk faktörünün varlığı, venöz veya arteriyel trombozun kalıtsal veya edinilen yatkınlığının göstergesi olabilecek biyokimyasal faktörler, ağır karaciğer hastalığı veya pozitif hasta öyküsü (karaciğer fonksiyon değerleri normale dönmemişse), karaciğer tümörü veya pozitif hasta öyküsü; steroid bağımlı tümörler; tanı konulmamış vajinal kanama; fokal nörolojik belirtili migren öyküsü; geçmişte yaşanan pankreatit veya ağır hipertrigliseridemi; ağır veya akut böbrek yetmezliği. **ÖZEL KULLANİM UYARILARI VE ÖNLEMLERİ:** DİENİLLE kullanımından önce olası dolaşım bozukluğu risklerine karşı KOK tedavisi dikkatlice tartışılmalıdır. Herhangi bir KOK kullanımı, kullanılmadığı durum ile karşılaştırıldığında venöz tromboemboli riski artar. Bazı epidemiyolojik çalışmalarda uzun süre KOK kullanımının servikal kanser riskinde artışa neden olabileceği bildirilmiştir, ancak bu bulguların KOK kullanımının hangi etkilerine bağlı olabileceği halen tartışılmaktadır. Kendisinde ya da aile öyküsünde hipertrigliseridemi olan kadınlarda, KOK kullanımı sonucu pankreatit riski artabilir. **İLAÇ ETKİLEŞİMLERİ VE GEÇİMSİZLİKLER:** Herhangi bir geçimsizlik yoktur. Diğer ilaçlar ile arasındaki etkileşmeler ara kanamaya ve/veya kontraseptif potansiyel kaybına yol açabilirler. Bu etkiler karaciğer enzim indüksiyonunu arttıran hidantoin, barbitüratlar, primidon, karbamazepin ve rifampisin durumunda gösterilmiş olup; rifabutin, efavirenz, nevirapin, okskarbazepin, topiramat, felbamat, ritonavir, nelvinafir, griseofulvin ve St. John's wort içeren bitkisel ilaçlar için geçerlidir. **GEBELİK VE EMZİRME:** Gebelik döneminde kontrendikedir. Kullanımı sırasında gebelik meydana gelirse kullanım durdurulmalıdır. Emzirme döneminde kullanılmaması tavsiye edilir. **ARAÇ VE MAKİNE KULLANIMI:** Herhangi bir etkisi bulunmamaktadır. **İSTENMEYEN ETKİLER:** KOK kullanan kadınlarda venöz ve arteriyel tromboemboli riski vardır. Sigara, hipertansiyon, kan pıhtılaşması ve lipid metabolizması bozuklukları, ağır obezite, varis, gelişmiş flebit ile tromboz gibi faktörler venöz ve arteriyel tromboemboli riskini artırabilir. **RAF ÖMRÜ VE SAKLAMA KOŞULLARI:** 36 ay-25°C'nin altındaki oda sıcaklığında saklayınız. **TİCARİ TAKDİM ŞEKLİ:** PVC/PVDC-Alu blisterde, 21 film kaplı tablet. **FİYATI:** 34,28 TL (Şubat 2017 itibariyle). **ÜRETİM YERİ:** Laboratorios Leon Farma S.A.La Vallina s/n. Poligono Industrial Navatejera 24008, Leon ISPANYA. **RUHSAT SAHİBİ:** Exeltis İlaç San. ve Tic. A.Ş. Kültür Mah. Nisbetiye Cad. No:56 Akmerkez B Blok Kat: 6 D: 574 Etiler, Beşiktaş/İstanbul. **RUHSAT TARİH/NO:** 16.02.2015-2015/136.

DROSPERA KÜB ÖZETİ: ÜRÜN ADI: DROSPERA 3 mg/0,02 mg film kaplı tablet. **FORMÜL:** 3 mg drospirenon ve 0,02 mg etinilestradiol içeren 24 adet aktif ve 4 adet plasebo beyaz film kaplı tablet. **FARMAKOLOJİ:** ATC kodu: G03AA12. DROSPERA drospirenon (progestojen) ve etinilestradiol (östrojen) içeren, antimineralokortikoid ve antiandrojenik etkili bir kombine oral kontraseptif (KOK)'tir. **ENDİKASYONLAR:** Kontrasepsiyon, hormona bağlı su tutulması ve buna bağlı belirtilerin gözlendiği durumlar, orta derecede akne vulgaris ve premenstruel disforik bozukluk semptomlarının tedavisi. **KULLANIM ŞEKLİ VE DOZU:** Tabletler gösterilen sırayla, her gün yaklaşık aynı zamanda, bir miktar su ile ve birbirini izleyen 28 gün boyunca alınır. Bir önceki ay hormonal kontraseptif kullanımı yoksa, tabletler kanamanın ilk günü alınmaya başlanmalıdır. Tablete 2. ile 5. günler arasında başlanması da kabul edilebilir, bu durumda tablet alımına başlanması itibariyle yedi gün boyunca ilave bir koruma yöntemi kullanılmalıdır.Bir sonraki pakete önceki kutudaki son tablet alımının ertesi günü başlanır. Çekilme kanaması genellikle plasebo tablet alımına başlanmasından 2-3 gün sonra başlar ve bir sonraki pakete başlandığında devam edebilir. Doğru şekilde kullanıldıklarında başarısızlık oranı yılda yaklaşık % 1 kadar olup, bu oran hap alımı unutulduğunda veya doğru alınmadığında artabilir. **UYGULAMA ŞEKLİ:** Oral. **KONTRENDİKASYONLAR:** Venöz veya arteriyel trombotik/tromboembolik olayların veya serebrovasküler bir olayın varlığı ya da öyküsü, bir tromboz prodromunun varlığı veya öyküsü, venöz veya arteriyel tromboz için ciddi ya da birçok risk faktörünün varlığı, fokal nörolojik belirtili migren öyküsü, ağır karaciğer hastalığı (karaciğer fonksiyon değerleri normale dönmemişse) varlığı, ağır veya akut böbrek yetmezliği, karaciğer tümörü varlığı veya öyküsü, steroid bağımlı tümörler, tanı konulmamış vajinal kanama, bilinen gebelik veya gebelik şüphesi, içeriğindeki maddelerden herhangi birine karşı aşırı duyarlılık, pankreatit ya da ağır hipertrigliseridemi öyküsü. **ÖZEL KULLANIM UYARILARI VE ÖNLEMLERİ:** KOK kullanımı ile ender olarak ortaya çıkan arteriyel ve venöz trombotik/tromboembolik hastalıkların risk artışı arasında ilişki bildirilmiştir. Venöz tromboembolizm riski kullanımın ilk yılında en yüksek olup, bir KOK'a başlarken ya da daha uzun aradan sonra) mevcuttur. Servikal kanser için en önemli risk faktörü süregelen human papilloma virus (HPV) enfeksiyonudur. Bazı epidemiyolojik çalışmalarda, uzun süre KOK kullanımının servikal kanser riskinde artışa neden olabileceği bildirilmiştir; ancak bu bulguların KOK kullanımının hangi etkilerine bağlı olabileceği halen tartışılmaktadır. Risk faktörlerinin veya bu durumlardan herhangi birinin ilk kez ortaya çıkması ya da artması halinde hemen doktora başvurulmalıdır. Tedavinin devamlılığı hakkındaki kararı doktor vermelidir. **İLAÇ ETKİLEŞİMLERİ VE GEÇİMSİZLİKLER:** Herhangi bir geçimsizlik yoktur. Oral kontraseptifler ve diğer ilaçlar (HİV proteaz inhibitörleri ve antibiyotikler gibi) arasındaki etkileşmeler ara kanamaya ve/veya kontraseptif başarısızlığa yol açabilirler. Antibiyotik tedavisi (rifampisin ve griseofulvin dışında) alan kadınlar, ilacın sonlanmasını takiben 7 gün boyunca bariyer yöntemi kullanmalıdırlar. **GEBELİK VE EMZİRME:** Gebelik döneminde kontrendikedir. Kullanımı sırasında gebelik meydana gelirse kullanım durdurulmalıdır. Emzirme döneminde kullanılmaması tavsiye edilir. **ARAÇ VE MAKİNE KULLANIMI:** Herhangi bir etkisi bulunmamaktadır. **İSTENMEYEN ETKİLER:** KOK kullanan kadınlarda venöz ve arteriyel tromboemboli riski vardır. Sigara, hipertansiyon, kan pıhtılaşması ve lipid metabolizması bozuklukları, ağır obezite, varis, gelişmiş flebit ile tromboz gibi faktörler venöz ve arteriyel tromboemboli riskini artırabilir. **RAF ÖMRÜ VE SAKLAMA KOŞULLARI:** 36 ay-25°C'nin altındaki oda sıcaklığında saklayınız. **TİCARİ TAKDİM ŞEKLİ:** PVC/PVDC-Alu blisterde, 21 film kaplı tablet. **FİYATI:** 24,92 TL (Şubat 2017 itibariyle). **ÜRETİM YERİ:** Laboratorios Leon Farma S.A.La Vallina s/n. Poligono Industrial Navatejera 24008, Leon İSPANYA. **RUHSAT SAHİBİ:** Exeltis İlaç San. ve Tic. A.Ş. Kültür Mah. Nisbetiye Cad. No:56 Akmerkez B Blok Kat: 6 D: 574 Etiler, Beşiktaş/İstanbul. **RUHSAT TARİH/NO:** 24.02.2015-2015/208.

DROSETİL KÜB ÖZETİ: ÜRÜN ADI: DROSETİL 3 mg/0,03 mg Film Kaplı Tablet. **FORMÜL:** 3 mg drospirenon/0,03 mg etinilestradiol (Her bir film kaplı tablet) **FARMAKOLOJİ:** ATC kodu: G03AA12. DROSETİL drospirenon (progestojen) ve etinilestradiol (östrojen) içeren, antimineralokortikoid ve antiandrojenik etkili bir kombine oral kontraseptif (KOK)'tir. **ENDİKASYONLAR:** Kontrasepsiyon, hormona bağlı su tutulması ile buna bağlı belirtiler, akne ve sebore tedavisi. **KULLANIM ŞEKLİ VE DOZU:** Tabletler gösterilen sırayla, her gün yaklaşık aynı zamanda, bir miktar suyla ve birbirini izleyen 21 gün boyunca alınır. Bir önceki ay hormonal kontraseptif kullanımı yoksa, tablet alımına kanamanın ilk günü başlanmalıdır. Tabletlere 2. ile 5. günler arasında başlanması da kabul edilebilir, bu durumda tablet alımına başlanması itibariyle yedi gün boyunca ilave bir koruma yöntemi kullanılmalıdır. Bir sonraki pakete 7 günlük, sıklıkla çekilme kanamasının izlendiği ve tablet alınmayan dönemi takiben geçilir. Bu kanama, genellikle son tabletin alınmasını takiben 2.-3. gün başlar ve bir sonraki pakete başlandığında devam edebilir. KOK'lar doğru şekilde kullanıldıklarında başarısızlık oranı yılda yaklaşık % 1 olup, ilaç unutulduğunda ya da yanlış kullanıldığında oran artabilir. **UYGULAMA ŞEKLİ:** Oral. **KONTRENDİKASYONLAR:** Venöz veya arteriyel trombotik/tromboembolik olayların veya serebrovasküler bir olayın varlığı ya da öyküsü, bir tromboz prodromunun varlığı veya öyküsü, venöz veya arteriyel tromboz için ciddi ya da birçok risk faktörünün varlığı, fokal nörolojik belirtili migren öyküsü, ağır karaciğer hastalığı (karaciğer fonksiyon değerleri normale dönmemişse) varlığı, ağır veya akut böbrek yetmezliği, karaciğer tümörü varlığı veya öyküsü, steroid bağımlı tümörler, tanı konulmamış vajinal kanama, bilinen gebelik veya gebelik şüphesi, içeriğindeki maddelerden herhangi birine karşı aşırı duyarlılık. **ÖZEL KULLANIM UYARILARI VE ÖNLEMLERİ:** KOK kullanımı ile ender olarak ortaya çıkan arteriyel ve venöz trombotik/tromboembolik hastalıkların risk artışı arasında ilişki bildirilmiştir. Venöz tromboembolizm riski kullanımın ilk yılında en yüksek olup, bir KOK'a başlarken ya da aynı veya farklı bir KOK'a tekrar başlarken (4 hafta ya da daha uzun aradan sonra) mevcuttur. Servikal kanser için en önemli risk faktörü süregelen human papilloma virus (HPV) enfeksiyonudur. Bazı epidemiyolojik çalışmalarda, uzun süre KOK kullanımının servikal kanser riskinde artışa neden olabileceği bildirilmiştir; ancak bu bulguların KOK kullanımının hangi etkilerine bağlı olabileceği halen tartışılmaktadır. Risk faktörlerinin veya bu durumlardan herhangi birinin ilk kez ortaya çıkması ya da artması halinde hemen doktora başvurulmalıdır. Tedavinin devamlılığı hakkındaki karar doktor vermelidir. **İLAÇ ETKİLEŞİMLERİ VE GEÇİMSİZLİKLER:** Herhangi bir geçimsizlik yoktur. Oral kontraseptifler ve diğer ilaçlar (enzim indükleyiciler ve antibiyotikler gibi) arasındaki etkileşmeler, ara kanamaya ve/veya kontraseptif başarısızlığa yol açabilirler. Antibiyotik tedavisi (rifampisin ve griseofulvin dışında) alan kadınlar, ilacın sonlanmasını takiben 7 gün boyunca bariyer yöntemi kullanmalıdırlar. **GEBELİK VE EMZİRME:** Gebelik döneminde kontrendikedir. Kullanımı sırasında gebelik meydana gelirse kullanım durdurulmalıdır. Emzirme döneminde genellikle önerilmemelidir. **ARAÇ VE MAKİNE KULLANIMI:** Herhangi bir etkisi bulunmamaktadır. **İSTENMEYEN ETKİLER:** KOK kullanan kadınlarda venöz ve arteriyel tromboemboli riski vardır. Sigara, hipertansiyon, kan pıhtılaşması ve lipid metabolizması bozuklukları, ağır obezite, varis, gelişmiş flebit ile tromboz gibi bazı faktörler venöz ve arteriyel tromboemboli riskini artırabilir. **RAF ÖMRÜ VE SAKLAMA KOŞULLARI:** 36 ay-25°C'nin altındaki oda sıcaklığında saklayınız. **TİCARİ TAKDİM ŞEKLİ:** PVC/PVDC-Alu blisterde, 21 film kaplı tablet. **FİYATI:** 24.92 TL (Şubat 2017 itibariyle). **ÜRETİM YERİ:** Laboratorios Leon Farma S.A.La Vallina s/n. Poligono Industrial Navatejera 24008, Leon İSPANYA. **RUHSAT SAHİBİ:** Exeltis İlaç San. ve Tic. A.Ş. Kültür Mah. Nisbetiye Cad. No:56 Akmerkez B Blok Kat: 6 D: 574 Etiler, Beşiktaş/İstanbul. **RUHSAT TARİH/NO:** 24.02.2015-2015/205.
REÇETE İLE SATILIR.

Daha geniş bilgi için firmamıza başvurunuz. Tel: 0 212 365 93 30, infoTR@exeltis.com . Herhangi bir şüpheli advers reaksiyon ile karşılaşılması halinde TÜFAM'a bildiriniz. (www.titck.gov.tr; e-posta:tufam@titck.gov.tr) Tel:0 800 314 00 08; Faks: 0 312 218 35 99.

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