



Evaluation of Different Doses of the Aromatase Inhibitor Letrozole for the Treatment of Ectopic Pregnancy and Its Effect on Villous Trophoblastic Tissue

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Received: 4 December 2021 / Accepted: 29 May 2022 / Published online: 14 June 2022
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Abstract

Letrozole, an aromatase inhibitor, has recently been introduced as a favorable medical treatment for ectopic pregnancy. We aimed at evaluating the effects of different doses of letrozole for termination of ectopic pregnancy and study their effects on villous trophoblastic tissue. Sixty patients with undisturbed ectopic pregnancy were classified into three equal groups. Group I: the control group that contained women who underwent laparoscopic salpingectomy, Group II: patients who received letrozole (5 mg day⁻¹) for 10 days, and Group III: patients who received letrozole (10 mg day⁻¹) for 10 days. Subsequently, the β -hCG levels were determined on the first day and after 11 days of treatment. Group IV consisted of patients of GII and GIII; their β -hCG did not drop below 100 mIU/ml within 11 days, and underwent salpingectomy. Placental tissues from patients undergoing salpingectomy either from the control group or GIV were processed for the evaluation of estrogen (ER) and progesterone (PR) receptors, vascular endothelial growth factor (VEGF), and cleaved caspase 3 (CC-3) expression. Cases exposed to high dose letrozole 10 mg day⁻¹ resulted in a higher ectopic pregnancy resolution rate of 85% (17/20), while the resolution rate of the low dose letrozole-treated group (5 mg day⁻¹) was 65% (13/20), and also showed a significant reduction in β -hCG levels on the 11th day, 25.63 ± 4.29 compared to the low dose letrozole group 37.91 ± 7.18 ($P < 0.001$). Meanwhile, the letrozole-treated group GIV showed markedly reduced expression of ER, PR, and VEGF and a significant increase in the apoptotic index cleaved caspase-3 compared to the control group ($P < 0.001$). The utilization of letrozole at a dose of 10 mg day⁻¹ for medical treatment of ectopic pregnancy results in a high-successful rate without any severe side effects. Letrozole depriving the placenta of estrogen that had vascular supporting signals resulted in destroying the vascular network with marked apoptosis.

Keywords Letrozole · Ectopic pregnancy · Induced abortion · Trophoblastic tissue ER · PR · VEGF · Cleaved caspase-3

Introduction

Ectopic pregnancy is the implantation and growth of the embryo outside the uterine cavity, which accounts for maternal morbidity and mortality [1]. Ectopic pregnancy affects around 1–2% of all pregnancies [2]. Although ectopic pregnancy-related mortality was recently reduced, ruptured ectopic pregnancies represent approximately 6% of all maternal deaths [3]. Although some ectopic pregnancies

resolve spontaneously and need only conservative treatment, others continue to grow and lead to tubal rupture that results in severe or persistent abdominal pain and life-threatening intraabdominal hemorrhage that required further interference. Medical and surgical treatment are two methods for the management of growing ectopic pregnancy [4, 5]. Recently, medical treatment has been used as the first choice to treat the early discovered ectopic pregnancy. While using methotrexate as a medical treatment for ectopic pregnancy was associated with terrible adverse effects on different body systems, as well as high proportions of failure in ectopic pregnancy termination [6]. Therefore, it is necessary to find more cost-effective and safe alternatives. Letrozole, a non-steroidal competitive aromatase inhibitor, is approved for the

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management of estrogen-sensitive breast cancer. Letrozole inhibits estrogen production from androgen aromatization via inhibition of the electron transfer chain of cytochrome P450 (CYP19A1) [7]. Letrozole is also used in the treatment of many gynecological disorders, mainly due to its high tolerability, low cost, and low side effects, including the reduction of the size of uterine myomas [8, 9], induction of ovulation in cases of polycystic ovarian syndrome [9], and the reduction of endometriosis recurrence [10–12]. In humans, letrozole inhibits estrogen production during pregnancy and negatively affects placental, estrogen, progesterone, and VEGF expression [6, 13], with subsequent disruption of progesterone physiological function, which was required to support early pregnancy [14], and the interruption of VEGF vascular support signals, which is the angiogenic factor involved in implantation and placentation of ectopic pregnancy [15], which drew attention to how letrozole hinders pregnancy progression [6].

Therefore, our objective was to evaluate different doses of letrozole for the treatment of ectopic pregnancy to reach the optimal and most safe dose with the highest success rate of ectopic pregnancy termination, and also, to investigate the possible underlying mechanisms through determining ER and PR expression, apoptosis signaling molecules, and their consequence on placental angiogenesis.

Patients and Methods

This study included 60 patients with undisturbed ectopic pregnancy admitted to the Department of Obstetrics and Gynecology of the Faculty of Medicine of Zagazig University from October 2020 to July 2021. Inclusion criteria include patients with confirmed ectopic pregnancy on vaginal ultrasound examination, together with β -hCG titers < 3000 mIU/ml, between 19 and 34 years old, with asymptomatic and hemodynamically stable pregnancy. Exclusion criteria included β -hCG levels > 3000 mIU/ml, hemoglobin level < 10 g/dl, platelet count $< 150,000$ /ml, and elevated liver enzymes, blood urea, or serum creatinine. The patients were equally categorized into three groups: patients who were undergoing laparoscopic salpingectomy (the control group GI), patients who were medically treated with 5 mg day^{-1} of letrozole using two tablets (2.5 mg of Femara) every day for 10 days (group II), and patients who medically treated with 10 mg day^{-1} of letrozole using four tablets (2.5 mg of Femara) every day for 10 days (group III). Meanwhile, each woman selected her treatment, and all patients had no contraindications to letrozole treatment. The β -hCG levels, platelet count, liver enzymes, and serum creatinine were assessed on the 1st and 11th days of treatment. Patients who had no successful response to medical treatment (β -hCG levels had not decreased below 100 mIU/

ml on the 11th day after treatment with letrozole) either from group II or III had to undergo salpingectomy and formed group IV. Patients with a β -hCG level below 100 mIU/ml on the 11th day of treatment divided by the total number of patients in the group refer to the success rate of abortion.

Tissue samples from laparoscopic salpingectomy either from control group GI or group IV were collected and fixed with 10% buffered formal saline and impeded in paraffin blocks [16], and processed for estrogen (ER), and progesterone (PR) isoforms, using a real-time PCR (RT-PCR) as previously described [17–19], and vascular endothelial growth factor (VEGF), and cleaved caspase-3 were detected by immunohistochemistry.

The Institutional Review Board of Zagazig University Hospital authorized this study with an approval number of ZU-IRB/19/N/106/13, and all patients gave their informed written consent.

Immunohistochemical Study

Immunohistochemical staining for VEGF in a 1/20 dilution (a mouse monoclonal antibody (JH121) Catalog # MA5-13,182, Invitrogen, Thermo Fisher Scientific, USA) and cleaved caspase-3 1/50 dilution (a recombinant Rabbit Monoclonal Antibody (9H19L2), Catalog # 700,182, Invitrogen, Thermo Fisher Scientific, USA). Briefly, Paraffin blocks were cut into $4 \mu\text{m}$ cuts. Immunostaining was performed using an autostainer from Leica BOND-MAX™ (Leica GmbH, Nussloch, Germany), using the manufacturer's guidelines [20]. The slides were dewaxed in Bond™ dewax solution (Leica Microsystems) and rehydrated in Leica Microsystems (Bond Wash Solution). Antigen retrieval at pH 6 was done via Bond Epitope Retrieval 1 Solution (Leica Microsystems) at 100°C for 30 min. Slides were incubated with monoclonal primary antibodies against (VEGF, and cleaved caspase-3) for 20 min at RT. Biotin off-bond polymer refines recognition (Leica Microsystems) was used to visualize the primary antibody interacting with tissue slices [21]. After post-primary amplification and recognition with the Novolink polymer revealing apparatus, the slides were counterstained with hematoxylin (Leica Microsystems). Lastly, the slides were dried, cleared, and fixed by DPX.

Morphometric Analysis

A Leica light microscope (DM500, Switzerland) was used in conjunction with a Leica digital camera (ICC50, Switzerland) to obtain the images. Software “ImageJ” (version 1.48v National Institute of Health, Bethesda, Maryland, USA) was utilized for image testing [22, 23]. A 10 dissimilar non-overlying randomly chosen fields were observed from each slide to quantitatively estimate the percentage

of positive cells for cleaved caspase-3 with a $\times 40$ objective [24], and for VEGF scoring by multiplying the mean percentages of positive cells and the intensity of the color, color intensity was measured as 1, 2, and 3 for weak, moderate, and strong intensity, respectively. [25]

Quantitative Real-Time PCR

Total RNA was extracted using Trizol reagent (Invitrogen), according to the manufacturer's protocol. The total RNA concentration was measured using a spectrophotometer. First-strand complementary DNA (cDNA) was prepared from total RNA (3 μg) by reverse transcription (RT) using M-MLV reverse transcriptase (Invitrogen) and random primers (9-mers; TaKaRa Bio). Quantitative real-time PCR (Q-PCR) was performed with a cDNA template (2 μL), and 2 \times Power SYBR Green (6 μL ; TOYOBO Co., Osaka, Japan) containing specific primers (β -actin, ER α , Er β , PR) are given as follows:

Gene name	Primer	Sequence (5'–3')	Fragment (bp)
β -actin	Forward	GGACTTCGAGCAAGA GATGG	234
	Reverse	AGCACTGTGTTGGCG TACAG	
ER α	Forward	AGCACCTGAAGTCT CTGGA	153
	Reverse	GATGTGGGAGAGGAT GAGGA	
ER β	Forward	AAGAAGATCCCGGC TTTGT	173
	Reverse	TCTACGCATTTCCCCTCA TC	
PP14	Forward	GACCAACAACATCTCCCT CAT	170
	Reverse	AAACGGCACGGCTCTFC- CATC	
PR	Forward	GGCGGATCCGTCAG TGGTCTAAATCATTG	351
	Reverse	GGCGAATTCCTGGGTTTG ACTTCGTAGCCC	

PCR was carried out for 40 cycles using the following parameters: denaturation at 95 °C for 15 s, followed by annealing and extension at 70 °C for 60 s. The fluorescence intensity was measured at the end of the extension phase of each cycle. The threshold value for the fluorescence intensity of all samples was manually set. The reaction cycle at which PCR products exceeded this fluorescence intensity threshold during the exponential phase of PCR amplification was the threshold cycle (CT). The expression of the target gene was quantified relative to that of β -actin, a ubiquitous housekeeping gene, based on a comparison of CTs with constant fluorescence intensity.

Statistical Analysis

Data were analyzed using SPSS software (25.0, SPSS Inc. Chicago, IL, USA) and were described as means and standard deviations. One-way ANOVA followed by a post hoc test was used to assess the significance among means, with $P < 0.05$ as the significant level.

Results

In this study, a total of 60 patients with undisturbed ectopic pregnancy were divided into three groups. The first group (G1) was subjected to laparoscopic salpingectomy and served as the control group, while the second and third groups (GII and GIII) were subjected to medical treatments with 5 and 10 mg day⁻¹ of letrozole, respectively. The demographic and clinical characteristics of all patients were presented in Table 1. No significant differences were found between the three groups concerning the age, body mass index, parity, β -hCG levels, platelet count, ALT, AST, and creatinine levels on the day of treatment.

The resolution rate of ectopic pregnancy in the control group GI was 20/20 (100%) of patients compared to 13/20 (65%) of patients in low-dose letrozole-treated group GII and 17/20 (85%) in the high-dose treated group GIII. Ten patients who had no successful response to medical treatment within 11 days of either GII or GIII had to undergo salpingectomy and form GIV.

Biochemical Results

There is no statistically significant difference between the three groups concerning β -hCG levels, platelet count, S. creatinine, ALT, and AST, on the treatment day was found; also, there is no statistically significant difference between the three groups concerning platelet count and S. creatinine on the 11th day of treatment.

While high-dose letrozole-treated group GIII exhibited a significant decrease in β -hCG levels on the 11th day of treatment 25.63 ± 4.29 compared to low-dose letrozole group GII 37.91 ± 7.18 ($P < 0.001$), also high-dose letrozole-treated group GIII exhibited a significant increment in the serum ALT and AST levels (54.680 ± 10.86 and 49.09 ± 4.14 U/L) when compared with the control group (29.92 ± 4.33 and 20.63 ± 2.59 U/L), respectively ($P < 0.001$) (Table 1).

Immunohistochemistry Results

The sections of the control group had low expression of cleaved caspase-3 (Figs. 1 and 2), compared to the GIV group treated with letrozole, which demonstrated numerous positive cells (Figs. 3 and 4). The morphometric

Table 1 Demographic and clinical characteristics differences between the three clinical groups

	Group type			ANOVA			Multiple comparisons						
	Group I (n = 20)			Group II (n = 20)			Group III (n = 20)						
	Mean ± SD	Range		Mean ± SD	Range		Mean ± SD	Range		P-value	I and II	I and III	II and III
Age (years)	24.45 ± 3.13	19–30	26.85 ± 4.14	24.85 ± 2.70	24.85 ± 2.70	24.85 ± 2.70	0.064						
BMI, kg/m ²	21.01 ± 2.021	17.9–24.5	20.53 ± 1.93	22.03 ± 2.19	22.03 ± 2.19	22.03 ± 2.19	0.664						
Parity	0.99 ± 0.66	0–2.1	1.31 ± 0.59	1.06 ± 0.52	1.06 ± 0.52	1.06 ± 0.52	0.207						
B-HCG treatment day	1192.60 ± 193.89	797–1601	1186.10 ± 168.71	1173.75 ± 118.42	1173.75 ± 118.42	1173.75 ± 118.42	0.860						
B-HCG 11th day	22.43 ± 7.67	8.9–36.6	37.91 ± 49.8	25.63 ± 31.1	25.63 ± 31.1	25.63 ± 31.1	<0.001				<0.001	0.279	<0.001
Success abortion rate	100%		13/20	17/20	17/20	17/20	0.012				0.008	0.231	0.144
AST level (U/L) Treatment day	19.50 ± 2.69	14.9–24.3	20.67 ± 2.35	19.25 ± 2.46	19.25 ± 2.46	19.25 ± 2.46	0.173						
AST level (U/L) Day 11	20.63 ± 2.59	20.63 ± 2.59	24.29 ± 2.61	49.090 ± 4.146	49.090 ± 4.146	49.090 ± 4.146	<0.001				0.002	<0.001	<0.001
ALT level (U/L) Treatment day	28.80 ± 1.52	15.7–25.4	29.029 ± 28.8	28.68 ± 1.84	28.68 ± 1.84	28.68 ± 1.84	0.6048						
ALT level (U/L) Day 11	29.92 ± 30.98	26.12–29.92	26.23 ± 32.87	26.00 ± 32.76	26.00 ± 32.76	26.00 ± 32.76	<0.001				0.883	<0.001	<0.001
S. creatinine (mg/dl) treatment day	0.70 ± 0.05	0.61–0.79	0.61 ± 0.37	0.69 ± 0.80	0.69 ± 0.80	0.69 ± 0.80	0.08						
S. creatinine (mg/dl) day 11	0.62 ± 0.08	0.50–0.71	0.54 ± 0.79	0.58 ± 0.69	0.58 ± 0.69	0.58 ± 0.69	0.10						
Platelets (× 10 ³ /μL) treatment day	228 ± 62	161–298	256 ± 55	219 ± 67	219 ± 67	219 ± 67	0.13						
Platelets (× 10 ³ /μL) Day 11	207 ± 59	207 ± 59	222 ± 45	217 ± 59	217 ± 59	217 ± 59	0.37						
Range	132–281	132–281	163–281	140–283	140–283	140–283							

Fig. 1 Immunohistochemistry of a few cleaved caspase-3 positive cells in villous trophoblastic cells of the control group (400×)

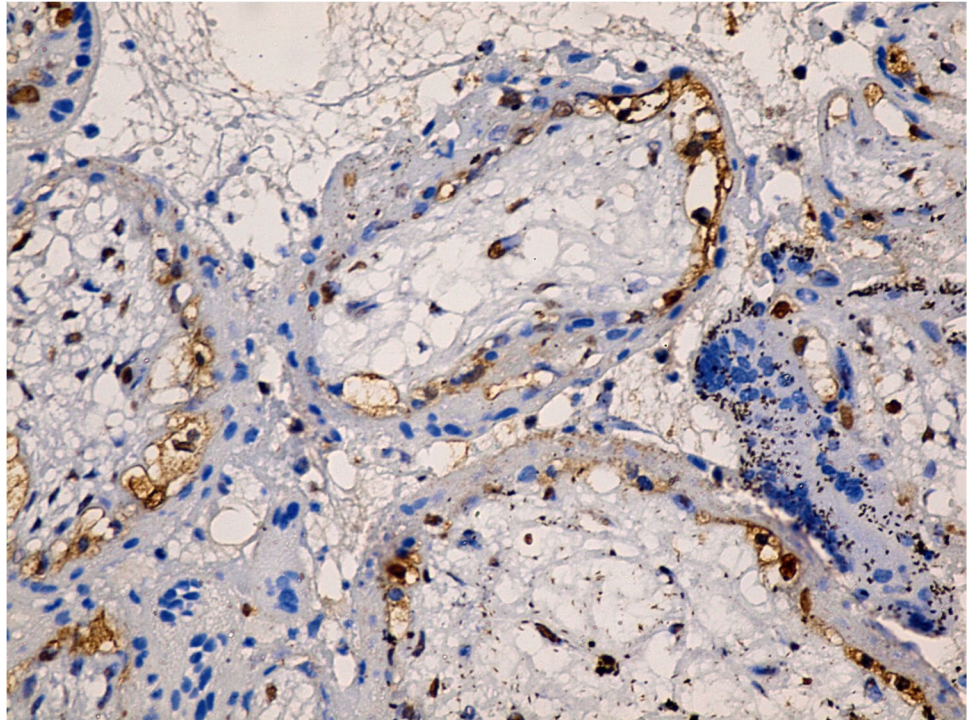
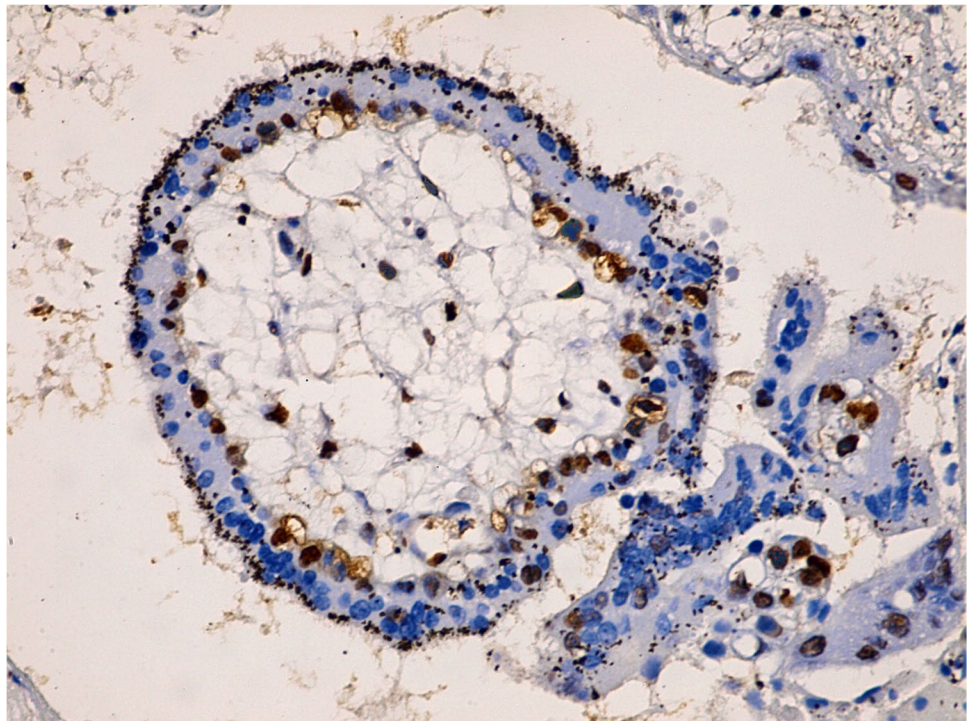


Fig. 2 Immunohistochemistry of few cleaved caspase-3 positive cells in outer trophoblasts and inner villi mesenchymal core cells of the control group (400×)



examination of the mean percentage of cleaved caspase-3 positive cells of the control group GI showed (18 ± 2 , SD), which was significantly decreased than the letrozole-treated group GIV (38 ± 4) ($P < 0.001$) (Table 2).

Placental tissues immunolabelled with VEGF from the control group exhibited strong cytoplasmic expression in villous syncytiotrophoblast, cytotrophoblast, Hofbauer cells of the stroma, and internal fetal capillaries (Figs. 5

Fig. 3 Immunohistochemistry of numerous cleaved caspase-3 positive cells in the outer trophoblastic cells of the letrozole-treated group IV (400×)

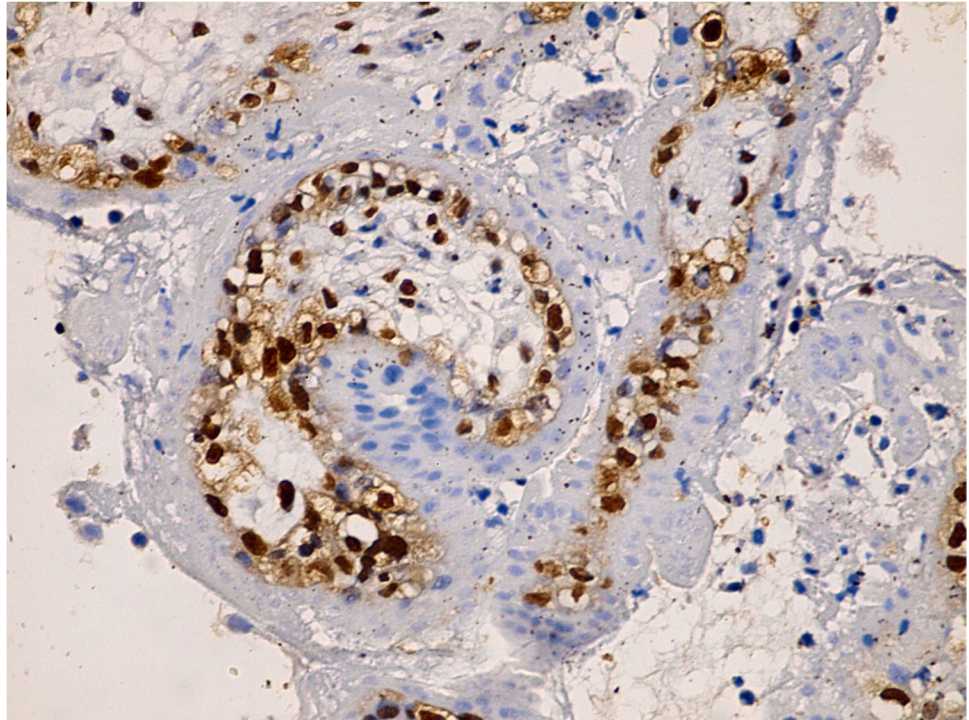
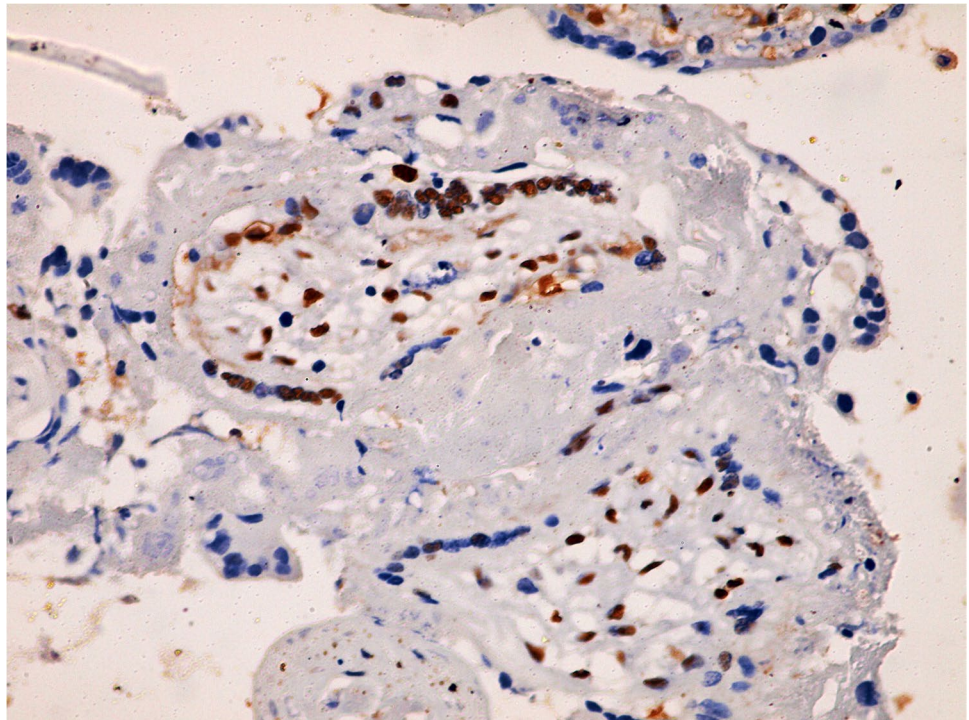


Fig. 4 Immunohistochemistry of numerous cleaved caspase-3 positive cells in the inner villi's mesenchymal core cells of the letrozole-treated group IV (400×)

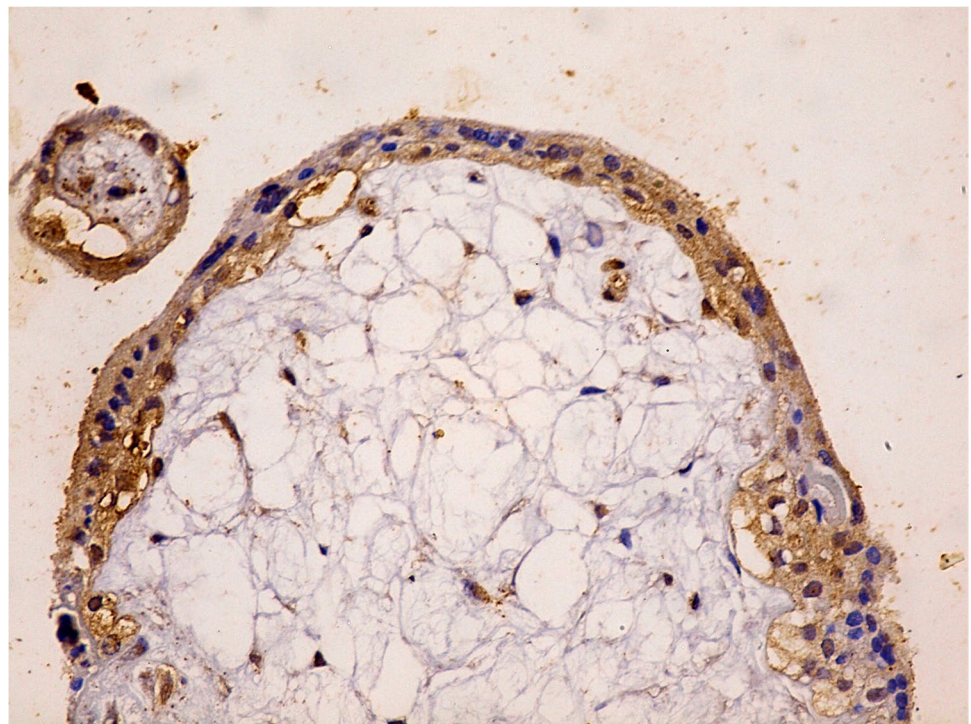


and 6). Meanwhile, the letrozole-treated group showed focal areas with weak expression of cytoplasmic VEGF (Fig. 7). The morphometrical and statistical investigation

of the final score of VEGF expression in the letrozole-treated group GIV showed an exceedingly significant

Table 2 The effects of exposure to letrozole on trophoblastic tissue of control and letrozole-treated groups

		Group type						<i>P</i> -value
		Group I (<i>n</i> = 20)			Group IV (<i>n</i> = 10)			
ER α	Mean \pm SD	0.5	\pm	0.1	0.2	\pm	0.1	< 0.001*
	Range	0.3	–	0.6	0.1	–	0.4	
ER β	Mean \pm SD	0.6	–	0.1	0.3	–	0.1	< 0.001*
	Range	0.4	\pm	0.8	0.1		0.4	
PR	Mean \pm SD	0.9		0.2	0.4	\pm	0.2	< 0.001*
	Range	0.6	–	1.2	0.2	–	0.7	
Caspase-3C	Mean \pm SD	18	\pm	2	38	\pm	4	< 0.001*
	Range	15	–	26	32	–	47	
VEGF	Mean \pm SD	121.27	\pm	9.1	64.31	\pm	4.37	< 0.001*
	Range	102.34	–	145.26	56.64	–	73.23	

Fig. 5 Immunohistochemistry of strong expression of VEGF in outer trophoblastic cells of the control group villi (400 \times)

decrease in the expression (64.31 ± 4.37) compared to the control group GI (121.27 ± 9.1) ($P < 0.001$) (Table 2).

Quantitative Real-Time PCR Results:

In this study, the RT-qPCR results showed a markedly significant decrease in the levels of ER α , ER β , and PR mRNA expression levels of the letrozole-treated group GIV (0.2 ± 0.1 , 0.3 ± 0.1 , and 0.4 ± 0.2), respectively, compared to the control group (0.5 ± 0.1 , 0.6 ± 0.1 , and 0.9 ± 0.2) ($P < 0.001$).

Discussion

Ectopic pregnancy is extrauterine implantation usually in the fallopian tube; some ectopic pregnancies resolve spontaneously, but others continue to grow and lead to rupture of the tube, resulting in life-threatening complications. The medical management of ectopic pregnancy has emerged as an important alternative to the surgical one. Surgical treatment of ectopic pregnancy is an invasive method that carries a significant financial burden on healthcare systems and families. It can also be accompanied by serious

Fig. 6 Immunohistochemistry of strong VEGF expression in inner villi mesenchymal core cells of inner villi control group (400×)

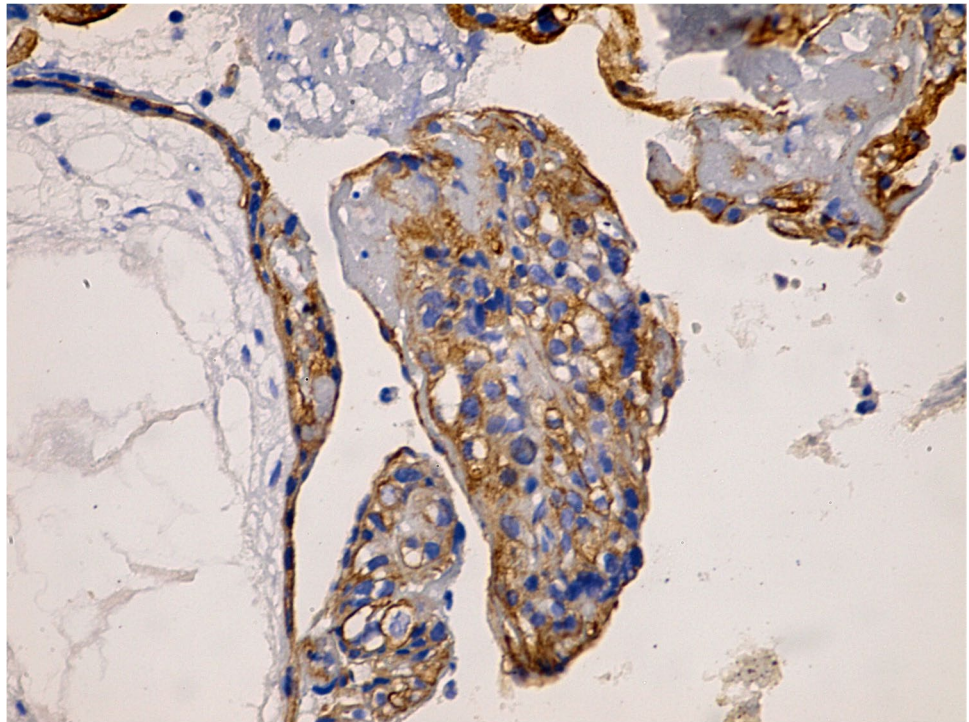
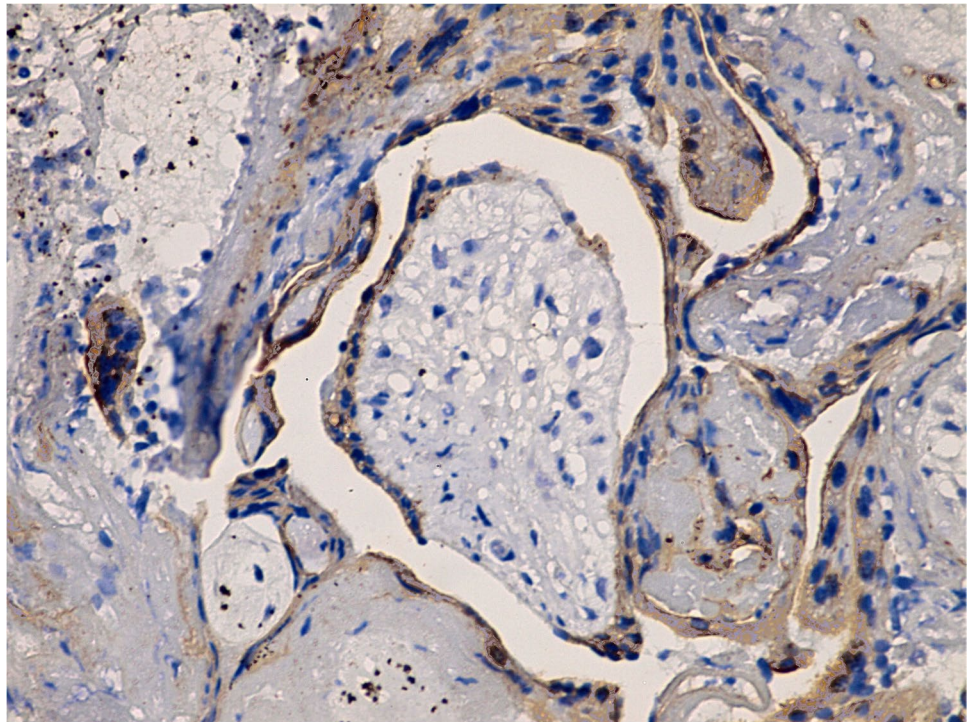


Fig. 7 Immunohistochemistry of weak VEGF expression in outer trophoblasts and inner core cells of the villi of the letrozole-treated group IV (400×)



side effects for pregnant women, such as uterine rupture, sepsis, and death especially when it is performed in unprepared places and not under the supervision of healthcare professionals [26]. The improved safety profile of letrozole usage compared to methotrexate and its successful use to

manage different gynecological conditions encourage further research into its use as a medical treatment for ectopic pregnancy [27].

Our study showed that the resolution rate for ectopic pregnancy using a high dose of letrozole (10 mg day^{-1}) was

85%, while the resolution rate for a low dose of letrozole of 5 mg day⁻¹ was 65%, which was nearly in agreement with a recent study by Mitwally et al. (2020) [27] found that the human resolution rate for ectopic pregnancy using 5 mg day⁻¹ of letrozole was 86%. This difference may be attributed to the relatively small number of participants in both studies. On the other hand, these results were in agreement with the experimental study by Tiboni et al. 2009 [7], who reported similar findings in rats treated with letrozole (0.04 mg kg⁻¹) equivalent to a 2.5-mg daily human dose.

There was a difference in the resolution rate between the high-dose administered letrozole group GIII 85% and the low-dose administered letrozole group 65%, but statistically, these differences did not appear $P=0.144$ due to the small number of participants in this study, but the resolution rate of the high-dose group 85% was very close to the ideal results of the control group 100% to the extent that there was no statistical difference between them $P=0.230$, while the resolution rate of the low-dose group 65% could not get close enough to the optimal results of the control group 100% to dissolve the significant statistical difference between them, where the results between them were statistically significant difference $P=0.008^*$, also, the higher dose letrozole group was able to reduce the β -hCG in a way that is close to the total removal of the ectopic pregnancy by surgery (control group), where there was no statistically significant difference between them $P=0.2779$, while there was a significant statistical difference between the low-dose group and the control group $<0.001^*$ regarding the reduction of β -hCG.

In this study, the Letrozole-treated group IV showed a significant reduction in ER and PR expression, which play a major role in maintaining pregnancy. Low estrogen levels caused by the aromatase inhibitor letrozole can cause inhibition of progesterone receptors that disrupt the physiological functions of progesterone, which are necessary to maintain early pregnancy. [27].

Vascularization occurs in the human placenta by the formation of new blood vessels from pluripotent precursor cells in the mesenchymal core of the villi, rather than starting from fetal blood cells; the placental villi begin to vascularize on day 21 after conception. [25]. Our results showed that the trophoblastic tissues of the letrozole-treated group IV showed a marked reduction in vascular endothelial growth factor (VEGF) compared to the expression of VEGF in the trophoblastic tissue of the control group. VEGF is a powerful mitogenic agent that promotes the formation of blood vessels in the fetoplacental unit that keeps the fetus alive. [28]. Estrogen is a primary stimulatory factor for VEGF, promoting the development of the vascular network and uterine permeability; therefore, antiestrogenic drugs, such as aromatase inhibitors, are angiogenesis inhibitors and can result in complete failure of fetal expansion by interfering with placenta formation and embryonic vascular development. [13,

29–35]. Additionally, in ectopic pregnancy, maternal cells at the implantation site usually show very limited decidual differentiation, if any [30, 31], which markedly attenuates the secretion of placental growth factor PIGF, in addition to the unfavorable environment represented by hypoxia, added further inhibition of PIGF expression [36]; at the same time, as a compensatory mechanism, ectopic trophoblastic tissue increases VEGF expression more than normal intrauterine conception responding to hypoxia, and other stimulators including estrogen [37–41], which enables it to continue to grow, while tubal implantation that cannot overcome these unfavorable conditions of implantation undergoes spontaneous resolution [42–44]

Apoptosis, or cell death, is a process that occurs naturally during the growth and development of tissues (such as the placenta). Preeclampsia and intrauterine growth restriction are two clinical obstetrics pathologies that have enhanced placental apoptosis [7].

In our study, the placental apoptotic index was measured using the cleaved caspase-3 expression which was found to be higher in trophoblast cells of the letrozole-treated group GIV in comparison to the trophoblastic tissue of the control group that showed lower expression. Caspase-3 is one of the proteases involved in the initiation and execution of apoptosis. Therefore, limiting caspase activity is essential for effective cell survival management.

Investigations of the current study found a considerable increase in liver enzymes (ALT and AST) in the high-dose letrozole-treated group compared to the control and low-dose administered group. These results were experimentally observed by Aydin et al. (2011) [45], who reported an increase in liver function indices in female rats given 1 mg kg⁻¹ letrozole. Moreover, up to 1% of women with prolonged letrozole administration have been associated with increased liver enzymes. These increases are generally asymptomatic and self-limited and do not require a dosage modification [27]. Letrozole is an inhibitor of CYP 2C19 and is metabolized by the cytochrome P450 (CYP 3A4 and CYP 2A6) system in the liver; therefore, extreme doses of letrozole can induce liver injury by a toxic or immunogenic metabolite. [46]. The high dose of letrozole 10 mg/day used in our study and 12.5 mg/day were used safely by Pritts et al. (2011) [47] in human patients for induction of ovulation and controlled hyperstimulation of the ovary.

Conclusion

Letrozole deprives the placenta of estrogen signals, which have a vascular supporting effect, destroying the placental vascular system with marked apoptosis. This study showed that using 10 mg day⁻¹ of letrozole resulted in a considerably safe and high success rate of ectopic pregnancy termination

without imposing any serious side effects, but even with the higher dose of letrozole, preparation for potential salpingectomy is needed.

Recommendations and Limitations

The main limitation of this study was the small number of participants; this study draws attention to the necessity of determining the optimal and safe dose of letrozole to achieve the highest success rate in medical termination of ectopic pregnancy, and the results must be confirmed with a large number of participants, and variable studies which facilitate accurate statistical calculations, before being accepted as a standard treatment or a guideline implement.

Funding Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB).

Data Availability Data openly available in a public repository.

Code Availability Not applicable.

Declarations

Ethics Approval The Institutional Review Board of the Zagazig University Hospital authorized this study with an approval number of ZU-IRB/19/N/106/13.

Consent to Participate All patients receive written informed consent.

Conflict of Interest The authors declare no competing interests.

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References

- Marion LL, Meeks GR. Ectopic pregnancy: history, incidence, epidemiology, and risk factors. *Clin Obstet Gynecol*. 2012;55(2):376–86.
- Medicine PCotASfR. Medical treatment of ectopic pregnancy: a committee opinion. *Fertility and sterility*. 2013;100(3):638–44.
- Perkins KM, Boulet SL, Kissin DM, Jamieson DJ, Group NAS. Risk of ectopic pregnancy associated with assisted reproductive technology in the United States, 2001–2011. *Obstet Gynecol*. 2015;125(1):70.
- Varma R, Gupta J. Tubal ectopic pregnancy. *BMJ Clinical Evidence*. 2009;2009.
- Pereira N, Gerber D, Gerber RS, Lekovich JP, Elias RT, Spandorfer SD, et al. Effect of Methotrexate or Salpingectomy for Ectopic Pregnancy on Subsequent In Vitro Fertilization-Embryo Transfer Outcomes. *J Minim Invasive Gynecol*. 2015;22(5):870–6.
- Lee VC-Y, Gao J, Lee K-F, Ng EH-Y, Yeung WS-B, Ho P-C. The effect of letrozole with misoprostol for medical termination of pregnancy on the expression of steroid receptors in the placenta. *Hum Reprod*. 2013;28(11):2912–9.
- Tiboni G, Marotta F, Castigliengo A, Rossi C. Impact of estrogen replacement on letrozole-induced embryopathic effects. *Hum Reprod*. 2009;24(11):2688–92.
- Gurates B, Parmaksiz C, Kilic G, Celik H, Kumru S, Simsek M. Treatment of symptomatic uterine leiomyoma with letrozole. *Reprod Biomed Online*. 2008;17(4):569–74.
- Kashani BN, Centini G, Morelli SS, Weiss G, Petraglia F. Role of medical management for uterine leiomyomas. *Best Pract Res Clin Obstet Gynaecol*. 2016;34:85–103.
- Ferrero S, Venturini PL, Gillott DJ, Remorgida V. Letrozole and norethisterone acetate versus letrozole and triptorelin in the treatment of endometriosis related pain symptoms: a randomized controlled trial. *Reprod Biol Endocrinol*. 2011;9(1):1–7.
- Nash CM, Philp L, Shah P, Murphy KE. Letrozole pretreatment prior to medical termination of pregnancy: a systematic review. *Contraception*. 2018;97(6):504–9.
- Shi L, Shi S-Q, Given RL, Von Hertzen H, Garfield RE. Synergistic effects of antiprogestins and iNOS or aromatase inhibitors on establishment and maintenance of pregnancy. *Steroids*. 2003;68(10–13):1077–84.
- Albrecht ED, Pepe GJ. Estrogen regulation of placental angiogenesis and fetal ovarian development during primate pregnancy. *Int J Dev Biol*. 2010;54(2–3):397.
- Paltieli Y, Eibschitz I, Ziskind G, Ohel G, Silbermann M, Weichselbaum A. High progesterone levels and ciliary dysfunction—a possible cause of ectopic pregnancy. *J Assist Reprod Genet*. 2000;17(2):103–6.
- Lam PM, Briton-Jones C, Cheung CK, Leung SW, Cheung LP, Haines C. Increased messenger RNA expression of vascular endothelial growth factor and its receptors in the implantation site of the human oviduct with ectopic gestation. *Fertil Steril*. 2004;82(3):686–90.
- Bancroft JD, Gamble M. *Theory and practice of histological techniques*: Elsevier health sciences; 2008.
- Kim SC, Park M-N, Lee YJ, Joo JK, An B-S. Interaction of steroid receptor coactivators and estrogen receptors in the human placenta. *J Mol Endocrinol*. 2016;56(3):239–47.
- Shanker YG, Jagannadha RA. Progesterone receptor expression in the human placenta. *Mol Hum Reprod*. 1999;5(5):481–6.
- Shanker YG, Sharma S, Rao AJ. Expression of progesterone receptor mRNA in the first trimester human placenta. *IUBMB Life*. 1997;42(6):1235–40.
- Alabiad MA, Harb OA, Taha HF, El Shafaay BS, Gertallah LM, Salama N. Prognostic and clinic-pathological significances of SCF and COX-2 expression in inflammatory and malignant prostatic lesions. *Pathol Oncol Res*. 2019;25(2):611–24.
- Shalaby AM, Aboregela AM, Alabiad MA, El Shaer DF. Tramadol promotes oxidative stress, fibrosis, apoptosis, ultrastructural and biochemical alterations in the adrenal cortex of adult male rat with possible reversibility after withdrawal. *Microsc Microanal*. 2020;26(3):509–23.
- Ahmed MM, Gebriel MG, Morad EA, Saber IM, Elwan A, Salah M, Fakhr AE, Shalaby AM, Alabiad MA. Expression of immune checkpoint regulators, cytotoxic T-lymphocyte antigen-4, and

- programmed death-ligand 1 in epstein-barr virus associated nasopharyngeal carcinoma. *Appl Immunohistochem Mol Morphol*. 2021;29(6):401–8.
23. Tawfeek SE, Shalaby AM, Alabiad MA, Albackoosh A-AAA, Albakoush KMM, Omira MMA. Metanil yellow promotes oxidative stress, astrogliosis, and apoptosis in the cerebellar cortex of adult male rat with possible protective effect of scutellarin: a histological and immunohistochemical study. *Tissue Cell*. 2021;73:101624.
 24. De Falco M, Fedele V, Cobellis L, Mastrogiacomo A, Leone S, Giraldi D, et al. Immunohistochemical distribution of proteins belonging to the receptor-mediated and the mitochondrial apoptotic pathways in human placenta during gestation. *Cell Tissue Res*. 2004;318(3):599–608.
 25. Demir R, Kayisli U, Seval Y, Celik-Ozenci C, Korgun E, Demir-Weusten A, et al. Sequential expression of VEGF and its receptors in human placental villi during very early pregnancy: differences between placental vasculogenesis and angiogenesis. *Placenta*. 2004;25(6):560–72.
 26. Javanmanesh F, Kashanian M, Mirpang S. Comparison of using misoprostol with or without letrozole in abortion induction: a placebo-controlled clinical trial. *Journal of Obstetrics, Gynecology and Cancer Research (JOGCR)*. 2018;3(2):0-.
 27. Mitwally MF, Hozayen WG, Hassanin KM, Abdalla KA, Abdalla NK. Aromatase inhibitor letrozole: a novel treatment for ectopic pregnancy. *Fertil Steril*. 2020;114(2):361–6.
 28. Watanabe A, Yasumizu T, Hoshi K, Katoh R, Kawaoi A, Shibuya M. Vascular endothelial growth factor expression in the rat uterus and placenta throughout pregnancy. *Acta Histochem Cytochem*. 1998;31(5):419–26.
 29. Hyder SM, Nawaz Z, Chiappetta C, Stancel GM. Identification of functional estrogen response elements in the gene coding for the potent angiogenic factor vascular endothelial growth factor. *Cancer Res*. 2000;60(12):3183–90.
 30. Mueller MD, Vigne J-L, Minchenko A, Lebovic DI, Leitman DC, Taylor RN. Regulation of vascular endothelial growth factor (VEGF) gene transcription by estrogen receptors α and β . *Proc Natl Acad Sci*. 2000;97(20):10972–7.
 31. Ruohola JK, Valve EM, Karkkainen MJ, Joukov V, Alitalo K, Härkönen PL. Vascular endothelial growth factors are differentially regulated by steroid hormones and antiestrogens in breast cancer cells. *Mol Cell Endocrinol*. 1999;149(1–2):29–40.
 32. Cullinan-Bove K, Koos RD. Vascular endothelial growth factor/vascular permeability factor expression in the rat uterus: rapid stimulation by estrogen correlates with estrogen-induced increases in uterine capillary permeability and growth. *Endocrinology*. 1993;133(2):829–37.
 33. Shifren JL, Tseng JF, Zaloudek CJ, Ryan IP, Meng YG, Ferrara N, et al. Ovarian steroid regulation of vascular endothelial growth factor in the human endometrium: implications for angiogenesis during the menstrual cycle and in the pathogenesis of endometriosis. *J Clin Endocrinol Metab*. 1996;81(8):3112–8.
 34. Reynolds LP, Redmer DA. Angiogenesis in the placenta. *Biol Reprod*. 2001;64(4):1033–40.
 35. Cheung CY. Vascular endothelial growth factor: possible role in fetal development and placental function. *J Soc Gynecologic Investigation: JSIGI*. 1997;4(4):169–77.
 36. Ahmed A, Dunk C, Ahmad S, Khaliq A. Regulation of placental vascular endothelial growth factor (VEGF) and placenta growth factor (PlGF) and soluble Flt-1 by oxygen—a review. *Placenta*. 2000;21:S16–24.
 37. Torry DS, Torry RJ. Angiogenesis and the expression of vascular endothelial growth factor in endometrium and placenta. *Am J Reprod Immunol*. 1997;37(1):21–9.
 38. Daniel Y, Geva E, Lerner-Geva L, Eshed-Englender T, Gamzu R, Lessing JB, et al. Levels of vascular endothelial growth factor are elevated in patients with ectopic pregnancy: is this a novel marker? *Fertil Steril*. 1999;72(6):1013–7.
 39. Felemban A, Sammour A, Tulandi T. Serum vascular endothelial growth factor as a possible marker for early ectopic pregnancy. *Hum Reprod*. 2002;17(2):490–2.
 40. Fasouliotis SJ, Spandorfer SD, Witkin SS, Liu H-C, Roberts JE, Rosenwaks Z. Maternal serum vascular endothelial growth factor levels in early ectopic and intrauterine pregnancies after in vitro fertilization treatment. *Fertil Steril*. 2004;82(2):309–13.
 41. Kucera-Sliutz E, Schiebel I, König F, Leodolter S, Sliutz G, Koelbl H. Vascular endothelial growth factor (VEGF) and discrimination between abnormal intrauterine and ectopic pregnancy. *Hum Reprod*. 2002;17(12):3231–4.
 42. Shalev E, Peleg D, Tsabari A, Romano S, Bustan M. Spontaneous resolution of ectopic tubal pregnancy: natural history. *Fertil Steril*. 1995;63(1):15–9.
 43. Craig LB, Khan S. Expectant management of ectopic pregnancy. *Clin Obstet Gynecol*. 2012;55(2):461–70.
 44. Hendriks E, Rosenberg R, Prine L. Ectopic pregnancy: diagnosis and management. *Am Fam Physician*. 2020;101(10):599–606.
 45. Aydin M, Oktar S, Özkan OV, Alçin E, Öztürk OH, Nacar A. Letrozole induces hepatotoxicity without causing oxidative stress: the protective effect of melatonin. *Gynecol Endocrinol*. 2011;27(4):209–15.
 46. Gharia B, Seegobin K, Maharaj S, Marji N, Deutch A, Zuberi L. Letrozole-induced hepatitis with autoimmune features: a rare adverse drug reaction with review of the relevant literature. *Oxford medical case reports*. 2017;2017(11).
 47. Pritts EA, Yuen AK, Sharma S, Genisot R, Olive DL. The use of high dose letrozole in ovulation induction and controlled ovarian hyperstimulation. *International Scholarly Research Notices*. 2011;2011.

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