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RESEARCH

Correlation between Estradiol and Progesterone Hormone Levels and Endometrial Thickness, Pattern and Vascularization in Patients Undergoing In Vitro Fertilization

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Abstract

Introduction: Currently there is a shift in trends in in vitro fertilization programs related to embryo transfer. Embryo transfer is largely determined by the window of implantation related to endometrial receptivity. Endometrial pattern, thickness and vascularity are biomarkers of endometrial receptivity. Estrogen and progesterone are known to have a role in the development of this biomarker.
Methods: This study used a cross-sectional design. The study was conducted from January to December 2022 at the Morula IVF Fertility Clinic Padang, RSU Bunda Medical Center (BMC) Padang.
Results: At the end of the study, there were 100 respondents with an average age of 25-29 years, most of them were overweight, and almost all of them had primary infertility, and the average duration of infertility was 6.75 ± 3.98 years. At the time of ovulation trigger, the mean estradiol levels were 3070.32 ± 1186.985 pg/ml, progesterone 1.10 ± 0.57 ng/ml, and E2/P4 ratio 3.64 ± 3.10. Most of the respondents had type A endometrial pattern, type I vascularity, and the mean endometrial thickness was 11.58 ± 2.94 mm. There is a statistically significant relationship between estradiol and endometrial thickness and vascularity. In addition, a relationship was also found between progesterone and E2/P4 with endometrial vascularization. However, there is no significant relationship between progesterone and the E2/P4 ratio with the endometrial pattern.
Conclusion: Estrogen, progesterone and E2/P4 are related to endometrial vascularization and estrogen is related to thickness. None of these hormones are associated with endometrial pattern.
Keywords: *estrogen, progesterone, endometrial pattern, endometrial thickness, endometrial vascularization.*

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INTRODUCTION

In vitro fertilization (IVF) conventionally consists of a fresh embryo transfer which may be followed by one or more cryopreserved embryo transfers in a subsequent cycle. Frozen embryo transfer (FET) has been used increasingly in clinical practice in women undergoing IVF over the last few decades, to avoid the adverse effects of ovarian stimulation on endometrial receptivity during early IVF cycles such as ovarian hyperstimulation syndrome (OHSS) by delaying embryo transfer to the next cycle without ovarian stimulation. [1]

The shift in trend from ET to FET is reflected in several studies conducted in Iran in the past 5 years that more FETs have been performed than ETs. The Morula IVF Clinic in Padang is a fertility clinic that provides IVF services with a total of 2574 infertility patients in 2020 and 41 people participating in the IVF program with 11 participants undergoing ET and most of the rest undergoing FET.[2,3,4]

In some literature, the advantages of elective FET are equal or superior (in certain patient subgroups) to ET because it reduces the incidence of ovarian hyperstimulation syndrome (OHSS). Although currently it is reported that FET is better, this technique still has some drawbacks, namely the possibility that the embryo will not survive during the freezing process and that it takes time to cool down before implantation. [5-8]

Some studies still carry out ET, possibly caused by some of the advantages of ET, among others, the embryos are selected in advance so that the time needed to go to implantation is not long because the embryos are directly transferred, and it avoids damage to the embryos during freezing. However, obstetric and perinatal outcomes are generally worse after ET than FET, because of supraphysiological and endometrial estrogen levels that are irregular.[5,7]

Research by Hou et al in 2019 found that the echogenicity pattern grew faster in the high progesterone group which had an effect on lower pregnancy and implantation rates at levels > 1.5 ng/ml at the time of ovulation trigger. Zhang et al in 2018 concluded that estradiol can help the development of the endometrium in women with thin endometrium in a fresh cycle which results in high pregnancy rates in thicker endometrium. Meanwhile, a study by Chen et al in 2015 found higher expression of VEGF-A, VEGF-C, and PLGF in women with high progesterone levels so that vascularization increased with better pregnancy outcomes.[9-11]

However, several studies also found that in patients with different endometrial patterns and thicknesses, progesterone levels did not show a significant difference even though progesterone was found to directly affect pregnancy outcomes, and there was no significant correlation between estradiol levels and endometrial thickness and pregnancy rates in these IVF's patients.[12,13]

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Because of the various hypotheses above, the authors are interested in conducting research on the relationship between levels of the hormones estradiol and progesterone and endometrial thickness, pattern, and vascularity in patients undergoing in vitro fertilization..

METHODS

This is an observational analytic study with a cross-sectional design to assess the relationship between levels of the hormones estradiol and progesterone and endometrial thickness, pattern and vascularity in patients undergoing in vitro fertilization. The research was conducted at the Padang Morula IVF Fertility Clinic, RSU Bunda Medical Center (BMC) Padang in January 2022–January 2023.

The population of this study were all infertility patients undergoing in vitro fertilization at the Padang Morula IVF Fertility Clinic, RSU Bunda Medical Center (BMC) Padang. with inclusion criteria are willing to be research subjects and have signed an informed consent form. Exclusion criteria included patients discontinued before ovulation triggers and patients with uterine abnormalities that could alter the appearance of the endometrium, such as endometrial polyps, endometrial adhesions, adenomyosis, submucous uterine myomas, etc.

Patients who met the inclusion and exclusion criteria and agreed as research samples were subjected to controlled ovarian stimulation and ovulation triggers, then blood samples were taken for the study variables, namely examination of serum levels of the hormones estradiol and progesterone. After that, an examination of the thickness, pattern, and vascularity of the endometrium was carried out by ultrasonography on the same day when the ovulation trigger was given.

In this patient, maternal outcome data were also collected such as age, BMI, type of infertility, duration of infertility, basal hormone profile, AMH, AFC before carrying out IVF. Univariate analysis was used to determine the distribution of the variables studied. Bivariate analysis was used to determine the relationship between levels of the hormones estradiol, progesterone, and the E2/P4 ratio with endometrial patterns and vascularization using a one-way ANOVA test. The statistical test used to determine the relationship between levels of the hormone estradiol and endometrial thickness uses a correlation test

RESULTS

RESULTS

In this study, a total of 24 patients met the inclusion and exclusion criteria.

1. **Univariat analysis**

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Univariate analysis is presented in Table 1-3.

Variable	Group	f	%	Mean	SD	Min	Max
Age (years)	15-19	0	0				
	20-24	0	0				
	25-29	11	44				
	30-34	5	20				
	35-39	6	24				
	40-44	3	12				
	45-49	0	0				
BMI	Underweight	0	0				
	Normoweight	9	36				
	Overweight	7	28				
	Obesity I	8	32				
	Obesity II	1	4				
Infertility type	Primary	24	96				
	Secondary	1	4				
Infertility period				6.72	3,98	1	16
FSH basal hormone profile (mIU/ml)				6.69	1.56	4,8	11,84
LHbasal hormone profile (mIU/ml)				5.60	2.17	0,14	11,03
E2 basal hormone profile (pg/ml)				52.78	71.84	7,90	383
P4 basal hormone profile (ng/ml)				0.58	1.61	0,05	8,25
AMH basal hormone profile (ng/ml)				2.73	2.52	0,64	11,92
AFC				11,36	3.54	6	22
Total		25	100				

Table 2. Estradiol, Progesterone, and E2/P4 Ratio Levels When Giving Ovulation Triggers to Patients Undergoing In Vitro Fertilization

Variable	Mean	SD	Min	Max
Estradiol levels (pg/ml)	3070.32	1186.98	1410	5760
Progesterone levels (ng/ml)	1.10	0.57	0.18	2.56

E2/P4 Rasio	3.64	3.10	0.87	15.00

Table 3. Endometrial Thickness, Pattern and Vascularization When Giving Ovulation Triggers to Patients Undergoing In Vitro Fertilization

Variable	Group	f	%	Mean	SD	Min	Max
Endometrial thickness(mm)				11,58	2,94	8,20	15,90
Endometrial pattern	A	18	72.0				
	B	5	20.0				
	C	2	8.0				
Endometrial vascularization	Type 0	6	24.0				
	Type I	14	56.0				
	Type II	2	8.0				
	Type III	3	12.0				

2. Bivariate Analysis

Bivariate analysis was used to determine the relationship between levels of the hormones estradiol, progesterone, and the E2/P4 ratio with endometrial patterns and vascularization using a one-way ANOVA test. The statistical test used to determine the relationship between levels of the hormone estradiol and endometrial thickness uses a correlation test. (Table 4-9)

Table 4. Correlation between Estradiol Hormone Levels and Endometrial Thickness When Giving Ovulation Triggers to Patients Undergoing In Vitro Fertilization

Variable	r	R^2	P Value
Endometrial thickness(mm)	0.459	0,211	0.021

Table 5. Correlation between Estradiol Hormone Levels and Endometrial Vascularization When Giving Ovulation Triggers in Patients Undergoing In Vitro Fertilization

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Table 6. Relationship Between Progesterone Hormone Levels and Endometrial Patterns When Giving Ovulation Triggers to Patients Undergoing In Vitro Fertilization

Table 7. Relationship Between Progesterone Hormone Levels and Endometrial Vascularization When Giving Ovulation Triggers to Patients Undergoing In Vitro Fertilization

Endometrial vascularization	Mean	SD	CI 95%		P Value
Type 0	0.86	0.30	0.54	1.17	0.039
Type I	0.98	0.51	0.68	1.28	
Type II	1.80	0.02	1.61	1.99	
Type III	1.68	0.81	-0.33	3.69	

Endometrial vascularization	Mean	SD	CI 95%		P Value
Type 0	2188.50	864.97	1280.77	3096.23	0.000
Type I	3103.93	807.31	2637.8	3570.06	
Type II	2328.00	1083.28	-7404.95	12060.95	
Type III	5172.00	834.40	3099.23	7244.77	

Table 8. Relationship Between E2/P4 Ratio With Endometrial Pattern When Giving Ovulation Triggers In Patients Undergoing In Vitro Fertilization

Endometrial pattern	Mean	SD	CI 95%		P Value
A	0.97	0.41	0.77	1.18	0.188
B	1.39	0.82	0.36	2.42	
C	1.54	1.00	-7.48	10.56	

Endometrial pattern	Mean	SD	CI 95%		P Value
A	4.83	4.77	2.45	7.20	0.643
B	5.97	5.41	-0.74	12.68	

C	2.16	0.68	-3.99	8.32	

Tabel 9. Relationship Between E2/P4 Ratio and Endometrial Vascularization When Giving Ovulation Triggers to Patients Undergoing In Vitro Fertilization

Endometrial vascularization	Mean	SD	CI 95%		P Value
Type 0	2.18	0.81	1.32	3.03	0.046
Type I	5.30	4.79	2.53	8.07	
Type II	1.28	0.58	-3.98	6.55	
Type III	10.40	5.44	-3.12	23.93	

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DISCUSSION

In this study, it was found that the most age group was 25-29 years, which included 11 respondents (44%). Most of the respondents had excessive BMI (64%) varying from overweight to obesity and primary infertility (24 respondents or 96%) and the average duration of infertility was 6.72 ± 3.98 years. In this study, the average basal FSH level was 6.69 ± 1.56 mIU/ml, basal LH level was 5.60 ± 2.17 mIU/ml, basal E2 hormone level was 52.78 ± 71.84 pg/ml, progesterone level basal 0.58 ± 1.61 ng/ml, AMH basal hormone levels 2.73 ± 2.52 ng/ml and AFC 11.36 ± 3.54.

In this study, a relationship was found between estradiol hormone levels and endometrial thickness. The same thing was found by Martins et al in 2021, where serum estradiol values on ovulation trigger days below 3000 pg/mL are associated with optimal endometrial thickness which leads to good pregnancy outcomes.[14] This is also in line with a study by Quass et al in 2021, obtaining a high live birth rate observed in the group with the highest endometrial thickness measurement exposed to estradiol levels in the supraphysiological range. This study also mentioned that gonadotropins are the most aggressive ovarian stimulation strategy and are associated with the highest estradiol levels, believed to result in more pronounced endometrial thickening.[15] The mean endometrial thickness at the time of ovulation triggering in this study was 11.58 ± 2.94 mm. This thickness is included in the ideal category. This is in line with a study by Chan et al in 2018, which found that most patients had endometrial thickness when given hCG which ranged from ≥8 to <14 mm with an average endometrial thickness in the <8 mm group was 6.9 ± 0.8 mm and the average in the ≥8 group mm is 10.8 ± 2.1 mm. Research by Polim et al in 2021 at the Morula IVF Clinic in Jakarta shows that estradiol levels <3000 pg/mL are the optimal range for embryo implantation and pregnancy.[17]

Estrogen affects vascularity directly and indirectly. Increased estrogen concentrations are known to increase the average blood flow to many vascular beds. The accumulating evidence suggests that increased uterine arterial blood flow is an ER-mediated phenomenon, leading to increased nitric oxide production and relaxation of VSM cells.[18] In this study, a relationship was found between estradiol levels and endometrial vascularization. This is supported by a 2020 study by Lv et al., which found age-related levels of estrogen, AMH and PROK1 in follicular fluid.[19]

The pattern of the endometrium is influenced by the secretion of endometrial cells which provide a variety of echo images during transvaginal ultrasound.[20] In this study, there was no significant relationship between the hormone progesterone and the endometrial pattern. These results are similar to a study by Mamedalieva in 2021, which found that after administration of progesterone, there were variable endometrial pattern results where in 53.3% of patients, the endometrium had an echo-heterogenic pattern, but in 36.7% had an endometrial pattern that did not match menstrual cycle day.[21] Different results were found by Hou et al in 2019, who found a pattern of echogenicity growing faster in the high progesterone group which had an effect on lower pregnancy and implantation rates at levels > 1.5 ng/ml during ovulation trigger, because it opens and closes more quickly window of implantation that interferes with the embryo transfer process.[9] Although there was no statistically significant relationship, it was found that pattern C had a higher average

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progesterone level than pattern B and pattern B had a higher average progesterone level than pattern A, which is in line with the theory. The statistically insignificant difference may be influenced by the sample size between endometrial patterns A, B and C which are not comparable (18 vs 5 vs 2 respectively), because on the day of the ovulation trigger it is expected that the endometrial pattern A (triple line) will be more numerous for avoid earlier opening of the window of implantation.[22]

During the secretory phase, the most elevated ovarian hormone is progesterone. Vascular endothelial growth factor (VEGF)-A is one of the most important factors in the regulation of angiogenesis in the secretory phase when compared to the proliferative phase. This is based on the relationship between progesterone and endometrial vascularization on the day of ovulation trigger.[23] In this study, a relationship was found between progesterone and endometrial vascularization. These results are supported by Salmasi et al in 2021, also getting progesterone is related to endometrial vascularization. In this study, ovarian stimulation and progesterone administration increased endometrial angiogenesis through upregulation of VEGF protein.[24] Most of the endometrial vascularization of the study participants was in type I (56%) where most of the blood vessels had reached the outer layer of the endometrium. It has been suggested that better endometrial and subendometrial vascularization may lead to better placental development during pregnancy which is associated with a lower risk of miscarriage and a higher chance of live birth after IVF.[25,26] Several recent studies have shown that the implantation rate of frozen transfer embryos is significantly higher than that of fresh transfer embryos because endometrial function is under the influence of hyperstimulation of estradiol or progesterone, especially if there is an increase in progesterone on the day of hCG administration.[27,28]

In this study, there was no relationship between the E2/P4 ratio and the endometrial pattern. This is similar to Hasibuan's findings in 2017 which found that there was no significant correlation between estrogen, progesterone and P/E2 in endometrial pattern and thickness.[29] Different results were found in a study by Klonos et al in 2020 found that the expression of estrogen receptors and progesterone receptors is related to the remodeling of endometrial structure and function. Estrogen in the follicular phase prepares the endometrium for the action of progesterone in the next secretory phase of the cycle. Progesterone is another determining factor in creating the window of implantation and maintaining pregnancy. In the Klonos study, it was found that there was a relationship between the E2/P4 ratio and the endometrial pattern assessing the role of hormones at the receptor level, while in this study the assessment of hormones was based on direct levels.[30] The reason for the non-significance of the relationship between the E2/P4 ratio and the endometrial pattern in this study probably came from the distribution of the sample, the proportion of the sample and the mean levels of progesterone as well as the variations in the endometrial pattern. in the disconnect of this study. In addition, the role of the receptor was not tested which could contribute to results that are different from the theory because it could be that the problem is in the hormone receptor which often occurs in infertility patients.[31]

Estrogen and progesterone work together in influencing endometrial vascularization. Progesterone affects the action of VEGF so that it has an effect on the level of endometrial angiogenesis. This is consistent with increased vascularity in the luteal phase before

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ovulation. Meanwhile, estrogen is known for its vasodilatory effect through its role in nitric oxide, so it also influences endometrial bleeding to prepare for implantation.[18,23] In this study, there was a relationship between the E2/P4 ratio and endometrial vascularization. Research by Care et al in 2014 stated that the role of steroid hormones (E2 and P4) in formation supports the interpretation that macrophages can be used for regulation of endometrial epithelial, stromal and vascular architecture. This study supports the role of macrophages in regulating steroid hormone-induced proliferative events in the menstrual cycle and pregnancy in the rat uterus to establish the epithelial, stromal, and vascular architecture that is essential for normal reproductive competence.[32] In this study, the E2/P4 ratio was related to endometrial vascularity. This is caused by the role of the hormone estrogen on VEGF and progesterone receptors. On the day of ovulation triggering, estrogen levels are high enough that these high levels can synthesize more progesterone receptors. It has been mentioned before that progesterone has a stronger effect on VEGF than estrogen but estrogen also plays a role in the synthesis of the progesterone receptor.[23,33] The ratio of estrogen/progesterone has been proven in several studies related to endometrial vascularization which is responsible for receptivity but the success rate of fresh embryo transfer is still low because the endometrial condition is not yet receptive.[27] Singh et al in 2015 found the overall limit value of the serum E2/P4 ratio at the time of triggering an adverse ovulation, which was below 2.8 in the fresh embryo transfer program while in this study the average E2/P4 was above 2.8 so that it could be predicted through the E2/P4 ratio, success Fresh transfer embryos in this research sample will increase.[34]

CONCLUSION

In this study, it was found that there was a statistically significant relationship between estradiol and endometrial thickness, there was a statistically significant relationship between estradiol and endometrial vascularization, there was no statistically significant relationship between progesterone and endometrial pattern, there was a statistically significant relationship between progesterone and endometrial vascularity, there was no statistically significant relationship between the E2/P4 ratio and endometrial pattern, and there was a statistically significant relationship between the E2/P4 ratio and endometrial vascularity.

SUGGESTIONS

Further research needs to be carried out using a cohort study design to assess the relationship between endometrial pattern, thickness, and vascularity and factors that can affect embryo transfer decisions and pregnancy rates

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