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Research Article

Do Embryo Morphokinetics Vary According to Female Infertility Indication?

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Abstract

Objective: This study aimed to characterize embryo morphokinetic dynamics in patients with polycystic ovary syndrome (PCOS) and endometriosis, and to investigate their association with basal hormonal parameters

Materials and Methods: Patients treated at the Istanbul Memorial Hospital Assisted Reproductive Technologies and Reproductive Genetics Center between [year] and [year] were retrospectively evaluated. Embryo development in women aged 25–38 years diagnosed with polycystic ovary syndrome (PCOS) (n=50), endometriosis (n=50) or unexplained infertility (UEI) (n=50) was compared with that of a control group (n=1,043) using outcomes obtained from the Time-Lapse Embryo Monitoring System (TLEMS). A total of 150 egg retrieval cycles and the corresponding embryos were retrospectively analyzed. Clinical pregnancy was defined by elevated serum hCG levels and confirmation of a positive gestational sac. Embryo transfer involved a single embryo selected based on embryoscope assessment, while sperm selection was performed using IMSI. Statistical analyses were conducted using SPSS version 26.

Results: The study demonstrated that embryos derived from women with endometriosis exhibited significantly delayed developmental kinetics compared with those from the other study groups, particularly at the tPNf, t2, tSC, tM, tSB, tB, and tEB stages ($p<0.05$). In contrast, embryos from patients with PCOS showed accelerated developmental progression during the tSC stage compared with the EMS group ($p<0.05$). AMH levels were significantly lower in the endometriosis group and significantly higher in the PCOS group relative to the other groups ($p<0.05$). Basal LH concentrations were also significantly elevated in the PCOS group ($p<0.05$), whereas oocyte maturation rates were significantly reduced compared with the remaining groups ($p<0.05$). Despite these differences in morphokinetic and hormonal parameters, pregnancy outcomes were comparable among all groups.

Conclusion: Embryos from women with endometriosis exhibited slower development. Additionally, low AMH levels were noted in patients with endometriosis. The relationship between oocyte maturation, AMH, and LH levels with rapid embryo morphokinetics in patients with PCOS warrants further investigation using molecular markers and PCOS phenotypes.

Keywords: Endometriosis, PCOS, UEI, embryo morphokinetics, AMH, LH

Abbreviations: PCOS: Polycystic Ovarian Syndrome; IVF: *In vitro* fertilization; TLS: Time Lapse Embryo Screening; UEI: Unexplained Infertility; HCG: Human Chorionic Gonadotrophin; IMSI: Intracytoplasmic Morphologically Selected Sperm Injection; AMH: Antimullerian Hormone; LH: Luteinizing Hormone; DOR: Diminished Ovarian Response; FSH: Follicle Stimulating Hormone; ART: Assisted Reproductive Technology; TSH: Thyroid Stimulating Hormone; OPU: Ovum Pick Up; GnRH: Gonadotrophin Releasing Hormone; βHCG: Beta Human Chorionic Gonadotrophin; ICSI Intracytoplasmic Sperm Injection; HTF: Human Tubal Fluid Media; BMI: Body Mass Index

Introduction

Assisted Reproductive Technologies (ART) have undergone substantial advances, and embryo development has increasingly been investigated using time-lapse imaging systems [1]. Women seeking IVF treatment are frequently diagnosed with conditions such as PCOS, endometriosis, or UEI [2-6]. PCOS is a prevalent endocrine disorder that affects at least 10% of women of reproductive age and is associated with infertility. Endometriosis is characterized by the presence of functional endometrial glands and stroma outside the uterine cavity and is recognized as a major cause of female infertility [3,4]. Furthermore, in a subset of infertile couples, despite comprehensive diagnostic evaluations, the etiology of infertility remains unexplained (UEI) [6].

Previous studies have investigated the impact of these conditions on embryo morphokinetics and IVF outcomes [3,7]. *Flores, et al. (2022)* demonstrated prolonged developmental timings in embryos derived from women with endometriosis, particularly at the t2, t5, tC, tM, and tB stages, compared with embryos from unaffected women. In contrast, research evaluating morphokinetic parameters in PCOS has yielded inconsistent findings. *Basheer, et al. (2020)* reported accelerated embryo development in women with PCOS and observed that embryos from PCOS patients reached the morula stage more rapidly than those from non-PCOS patients. Additionally, they noted higher miscarriage rates among hyperandrogenic PCOS patients and suggested that these patients exhibited faster progression to the t5, t8, and morula stages. Conversely, several studies have reported no significant differences in embryo morphokinetics or IVF outcomes between women with and without PCOS.

Furthermore, altered ovarian physiology in PCOS, including disrupted *in vitro* maturation (IVM) and reduced oocyte quality, has been associated with impaired embryo developmental competence [8]. Some investigators have reported delayed blastocyst formation and prolonged timing to the eight-cell stage in embryos derived from PCOS patients compared with embryos from non-PCOS patients. Serum estradiol concentrations have been reported to be elevated in women with endometriosis and reduced in those with PCOS [9]. Several studies have also demonstrated increased anti-Müllerian hormone (AMH) levels in patients with PCOS [10-12]. However, the existing literature investigating embryo morphokinetics in both PCOS and endometriosis remains limited and yields conflicting results.

In the present study, in patients diagnosed with PCOS and endometriosis, embryo morphokinetic parameters, oocyte yield, maturation and fertilization rates, as well as basal hormonal profiles, including FSH, LH, AMH, estradiol, TSH, and PRL, were prospectively evaluated. In addition, demographic and clinical characteristics were analyzed in relation to pregnancy outcomes. The primary objective of this study was to investigate differences in embryo morphokinetics and IVF outcomes in patients with different infertility indication, and to identify potential predictive factors associated with clinical outcomes and pregnancy rates.

Material and Methods

The present study included patients diagnosed with PCOS, endometriosis, and UEI, whose embryos were monitored using a Time-Lapse System (TLS) incubator (EmbryoScope®). Participants underwent ART treatment at the Istanbul Memorial Hospital Assisted Reproductive Technologies and Reproductive Genetics Center between October 2011 and December 2021. A total of 150 patients were enrolled, with 50 individuals allocated to each study group. The study evaluated embryo morphokinetic parameters in addition to basal hormonal profiles, including FSH, LH, E2, PRL, AMH, and TSH levels measured on the third day of the menstrual cycle, as well as serum estradiol (E2) and LH concentrations on the day of trigger administration. Total and mature oocyte yield following oocyte pick-up (OPU), semen parameters, and pregnancy outcomes were also analyzed. Exclusion criteria included severe male factor infertility, maternal age >38 years, recurrent pregnancy loss, and endometrial thickness <7 mm on the day of embryo transfer.

Ethics Approval

This study received ethical approval on March 8, 2022, with approval number 2022/03-822, in accordance with the ethics committee guidelines established under Articles 14 and 42 of the Higher Education Law No. 2547.

Ovarian Stimulation

For ovarian stimulation, 150-225 IU of recombinant follicle-stimulating hormone (rFSH, (Gonal-F) or a combination of rFSH and recombinant LH (rLH) (Pergoveris) Merck Serono, Turkey) or (Human Menopausal Gonadotropin (HMG) (Menopur, Ferring, Switzerland) were administered on the second day of menstruation, depending on the woman's age and Body Mass Index (BMI). When the primary follicle reached a diameter of 12-13 mm, 0.25 mg gonadotropin-releasing hormone (GnRH) antagonist (Cetrotide; Merck Serono, Turkey) was administered daily. When two or more follicles reached at least 18 mm in diameter, final follicular maturation was triggered using 250 µg recombinant human chorionic gonadotropin (r-hCG; Ovitrelle®; Merck Serono, Switzerland) or GnRH analogue (Lucrin; Abbott Laboratories, USA). OPU was performed 36 h after the trigger.

Follicle Aspiration, Denudation and ICSI

Cumulus-oocyte complexes were retrieved from aspirated follicles, washed in human tubal fluid (HTF; Life Global®, Seattle, USA), and incubated at 6% CO2, 5% O2, and 37°C for 3.5 hours. Denudation was then performed using a hyaluronidase enzyme solution prepared at 40 IU/ml in HTF (Life Global, Seattle, USA). Microinjection was performed 4 h after OPU in HEPES-containing HTF medium (Life Global, Seattle, USA) using Olympus IX70 and IX71 inverted microscopes at ×400 magnification, with conditions

of 6% CO2, 5% O2, and 37°C, following a minimum of 4 h of pre-conditioning. The complexes were placed in pre-incubated containers (EmbryoSlide®, Unisense Fertilitech, Aarhus, Denmark).

Embryo Culture

There were 12 wells in an EmbryoSlide® container. Each well was filled with 25 µL of one-step embryo culture fluid (Life Global, Seattle, USA) containing 10% Human Serum Albumin (HSA) protein supplement (Life Global) and 1.5 mL of paraffin oil (Life Global). After ICSI, embryos were left in these wells and loaded into SEIS (EmbryoScope, Unisense Fertilitech, Aarhus, Denmark). Embryos were cultured in a 6% CO2, 5% O2 incubator at 37°C for five days. On the third day of embryo development, culture media and oil were exchanged by loading them onto a new pre-incubated EmbryoSlide as described above.

Luteal Phase Support

In luteal phase support, Progestan vaginal capsule 200 mg (Koçak Farma, Turkey) was used at different doses in all (fresh and frozen) ET cycles in fresh embryo transfer cycles, starting the day after oocyte retrieval Progestan vaginal capsule three times a day with daily progesterone injection (Progestan dex, 25mg, Koçak Farma, Turkey) were administered. In modified natural (mNC) FET cycles Progestan vaginal capsule 200 mg (twice a day) was administered two days after rhCG administration. In artificial (AC) FET cycles, luteal phase support was initiated as early as the 15th day of estrogen administration, once the endometrial thickness reached at least 8 mm. Patients received Progestan vaginal capsules 200 mg (two capsules twice daily) in combination with a daily subcutaneous injection of Progestan Dex 25 mg.

Nine days after blastocyst transfer, serum β-hCG was measured. When pregnancy occurred, the same daily dose of progesterone was continued until the 10th week of gestation. At 7 weeks, a transvaginal ultrasound was performed to monitor early pregnancy. A viable pregnancy was defined as the presence of a fetal heartbeat at 7 weeks. A live birth was defined as a live-born fetus after 24 weeks of pregnancy. Biochemical pregnancy loss was defined as a spontaneous decrease in β-hCG level after an increase without a gestational sac by ultrasound. Clinical pregnancy loss was defined as pregnancy loss after the visualization of an intrauterine gestational sac by ultrasound.

Statistical Analysis

The morphokinetic parameters of the embryos in the automatically saved cases on an EmbryoViewer computer were transferred to Microsoft Excel for analysis. In this study, 365 embryos from the unexplained infertility (1) group, 266 from the endometriosis (2) group, and 412 from the PCOS (3) group were included in statistical analysis. Embryos that could not be visualized in the EmbryoScope® incubator and that were not fertilized were excluded from the study. All statistical analyses were performed using SPSS version 26 (SPSS Inc., Chicago, IL, USA) and Excel 2007 (Microsoft Inc., California, USA). The Shapiro-Wilk test was used to determine whether the cases fit the normal distribution. Those with p-values greater than 0.05 were considered to be within the normal distribution. The ANOVA test was used for cases with a normal distribution, and the Kruskal-Walli's test was used for those without. The post-hoc Tukey test was applied to groups with a significant difference in the normally distributed groups. Bonferroni test was used for groups that did not fit the normal distribution and showed a substantial difference. The Chi-Square test was used to determine the pregnancy rates. When evaluating demographic and clinical parameters according to pregnancy outcomes, Student's t-test was used for normally distributed data and the Mann-Whitney U test for non-normal data. Statistical significance was set at $p<0.05$.

Results

Comparison of Demographic and Clinical Characteristics of Study Groups

Demographic and clinical characteristics of the study groups are shown in Table 1. According to the statistical results, a significant difference was found between the clinical parameters BMI, female age, trigger day E2, and trigger day LH in the groups diagnosed with unexplained infertility and PCOS, as well as between the groups diagnosed with endometriosis and PCOS. AMH levels were found to be significant among all groups. Age was significantly different between the UEI and PCOS groups ($p = 0.001$) and between the endometriosis and PCOS groups ($p <0.001$), with younger patients in the PCOS group. The BMI was significantly higher in the UEI and PCOS groups ($p < 0.001$) and in the endometriosis and PCOS groups ($p < 0.001$), indicating a higher BMI in the PCOS group. Significant differences in AMH, UEI, and endometriosis ($p = 0.050$) were observed between the UEI and PCOS groups ($p < 0.001$), and between the endometriosis and PCOS groups ($p < 0.001$). Trigger day values were significantly different between the E2, UEI, and PCOS groups ($p < 0.001$) and between the endometriosis and PCOS groups ($p < 0.001$), with the highest values observed in the PCOS group. LH levels were significantly different between the UEI and PCOS groups ($p = 0.008$) and between the endometriosis and PCOS groups ($p = 0.001$). The highest LH level was observed in the PCOS group (Table 1).

Comparison of Oocyte Count, Maturation, Fertilization and Sperm Parameters in Study Groups

Metaphase II (MII) oocytes, fertilized oocytes, maturation (%), fertilization (%) rates, and sperm parameters in the study groups are presented in Table 2. In the groups diagnosed with Unexplained Infertility (UEI) and PCOS, there was a significant difference between the endometriosis diagnosed and PCOS groups in the groups diagnosed with UEI and PCOS. The maturation rate was significantly different between the UEI and PCOS groups. The number of MII oocytes was significantly different between the UEI and PCOS groups ($p = 0.014$) and between the endometriosis and PCOS groups ($p < 0.001$), with the highest MII oocytes observed

in the PCOS group. The number of fertilized oocytes was also statistically significant between UEI and PCOS (p = 0.003) and between endometriosis and PCOS (p <0.001), with the highest number in the PCOS group. Similarly, the number of oocytes retrieved was significantly different between the UEI and PCOS groups and between the endometriosis and PCOS groups (both p < 0.001), with the highest number in the PCOS group. The maturation differences between the UEI and PCOS groups were statistically significant (p = 0.035), with lower values in the PCOS group (Table 2).

Table 1: Demographic and Clinical Characteristics of the Study Groups.

		N	Mean ± Standard deviation	P-Value
AGE	UEI	50	32,22±3,04	,001 (UEI vs PCOS) ,000 (EMS vs. PCOS)
	Endometriosis	50	33,00±3,01	
	PCOS	50	29,66±4,26	
BMI (kg/m^2)	UEI	50	23,18±3,42	,000 (UEI vs. PCOS) ,000 (EMS vs. PCOS)
	Endometriosis	50	22,93±3,17	
	PCOS	50	28,39±6,00	
E2 (pg/ml)	UEI	48	43,94±24,18	,168
	Endometriosis	45	47,36±24,31	
	PCOS	48	38,50±19,41	
AMH (ng/dl)	UEI	48	3,11±2,28	,050 (UEI vs. EMS) ,000 (UEI vs. PCOS) ,000 (EMS vs. PCOS)
	Endometriosis	48	1,84±1,00	
	PCOS	41	6,56±4,00	
TSH (uIU/ml)	UEI	50	1,82±0,81	,656
	EMS	49	1,93±1,02	
	PCOS	50	1,98±0,95	
FSH (mlU/ml)	UEI	14	7,64±2,15	,594
	EMS	24	8,10±3,61	
	PCOS	4	9,75±7,17	
PRL (ng/ml)	UEI	50	17,84±7,23	,951
	EMS	50	17,84±8,31	
	PCOS	50	17,39±9,11	
Trigger Day E2 (pg/ml)	UEI	48	2364,21±1586,65	,000 (UEI vs. PCOS) ,000 (EMS vs. PCOS)
	EMS	49	2212,57±1449,06	
	PCOS	49	3989,78±2532,82	
Trigger Day LH (mlU/ml)	UEI	48	2,18±1,76	,010 (UEI vs. PCOS) ,010 (EMS vs. PCOS)
	EMS	42	2,15±1,55	
	PCOS	47	3,63±3,30	
LH (mlU/ml)	UEI	49	6,46±2,39	,008 (UEI vs. PCOS) ,001 (EMS vs. PCOS)
	EMS	43	5,87±2,12	
	PCOS	49	8,33±4,16	

***Note:** UEI, unexplained infertility; EMS, endometriosis; PCOS, polycystic ovary syndrome; BMI, body mass index; E2, estradiol; AMH, anti-Müllerian hormone; TSH, thyroid-stimulating hormone; FSH, follicle-stimulating hormone; PRL, prolactin; LH, luteinizing hormone; N, number of cases. Values are presented as mean±standard deviation. Comparisons between groups were made using Student's t-test. Statistical significance (p<0.05) is indicated in bold.

Table 2: Comparison of Oocyte Count, Maturation, Fertilization and Sperm Parameters in Study Groups.

		N	Mean ± Standard deviation	P-Value
Metaphase-II (MII) Oocyte	UEI	50	10,02±4,92	,014 (UEI vs. PCOS),014 (UEI vs. PCOS) ,000 (EMS vs. PCOS)
	Endometriosis	48	8,04±3,74	
	PCOS	50	13,90±7,27	
Fertilized Oocyte (2PN)	UEI	50	8,08±4,45	,003 (UEI. vs. PCOS) ,000 (EMS vs. PCOS)
	Endometriosis	48	6,38±3,09	
	PCOS	50	11,16±5,92	
The oocyte from the OPU process	UEI	50	11,38±5,11	,000 (UEI vs. PCOS) ,000 (EMS vs. PCOS)
	Endometriosis	48	9,58±4,66	
	PCOS	50	17,60±8,99	
Maturation (%)	UEI	50	87,94±12,67	,035 (UEI vs. PCOS)
	Endometriosis	48	86,60±12,75	
	PCOS	50	81,13±15,17	
Fertilization (%)	UEI	50	79,46±18,00	,975
	Endometriosis	48	79,22±17,42	
	PCOS	50	80,04±20,30	
Sperm Volum (ml)	UEI	50	3,32±1,06	,491
	Endometriosis	42	3,06±1,33	
	PCOS	38	3,39±1,59	
Sperm Concentration (million/ml)	UEI	50	32,28±15,61	,463
	Endometriosis	42	34,14±19,12	
	PCOS	38	29,63±13,38	
Total motility (%)	UEI	50	40,26±6,38	,538
	Endometriosis	42	42,00±8,17	
	PCOS	38	41,50±8,80	
Progressive motility +4 (%)	UEI	50	4,48±2,58	,472
	Endometriosis	42	5,05±3,07	
	PCOS	38	5,16±2,85	
Progressive motility +3 (%)	UEI	50	25,44±6,11	,480
	Endometriosis	42	27,14±6,82	
	PCOS	38	26,16±7,28	
Mobile in place (%)	UEI	50	10,34±2,01	,290
	Endometriosis	42	9,76±2,15	
	PCOS	38	10,18±2,28	

***Note:** UEI: Unexplained Infertility; EMS: Endometriosis; PCOS: Polycystic Ovary Syndrome. N: Number of Cases. Values are presented as mean±standard deviation. Comparisons between groups were made using Student's t-test. Statistical significance ($p<0.05$) is indicated in bold.

Comparison of Morphokinetic Characteristics of Embryos in Study Groups

A comparison of the morphokinetic characteristics of the embryos in the study groups is presented in Table 3. The tPNf stage was statistically significant (p = 0.001) between the endometriosis and PCOS groups, showing that embryos developed more slowly in the endometriosis group. The t2 stage was significantly different between the UEI and endometriosis groups (p = 0.005) and between the endometriosis and PCOS groups (p = 0.011), with embryos in the endometriosis group developing more slowly. The tSC stage (p=0.030) was significantly different between the UEI and endometriosis groups, as well as between the UEI and PCOS (p=0.018) and EMS and PCOS (p<0.001) groups. The results indicated the lowest embryo development rate in the endometriosis group and the highest rate in the PCOS group. The TM stage differed significantly between the UEI and endometriosis groups (p = 0.004) and between the endometriosis and PCOS groups (p < 0.001), with endometriosis showing the slowest progression to the morula. The tSB stage was statistically significant between UEI and endometriosis (p = 0.004) and between EMS and PCOS (p < 0.001), with endometriosis embryos developing more slowly. The tB stage was significantly different between UEI and endometriosis (p < 0.001) and between endometriosis and PCOS (p < 0.001), indicating the slowest development in the endometriosis group. The tEB stage showed significance between UEI and endometriosis (p = 0.001) and between endometriosis and PCOS (p = 0.001), with the endometriosis group developing more slowly. The transition from the compaction stage to the morula stage (TSC-tM) was significantly faster in the UEI group than in the endometriosis (p < 0.001) and PCOS (p < 0.001) groups, indicating that UEI embryos developed more rapidly during this phase.

Table 3: Morphokinetic Characteristics of Embryos in Study Groups.

		N	Mean ± Standard deviation	P-Value
tPNa	UEI	359	8,06±2,14	,587
	Endometriosis	251	8,33±2,39	
	PCOS	351	8,28±3,58	
tPNf	UEI	358	23,81±3,64	,001 (EMS vs PCOS)
	Endometriosis	256	24,50±5,01	
	PCOS	372	23,40±5,76	
t2	UEI	358	26,30±3,72	,005 (UEI vs EMS) ,011 (EMS vs PCOS)
	Endometriosis	259	27,67±5,00	
	PCOS	405	26,77±5,27	
t3	UEI	356	36,49±6,09	,765
	Endometriosis	265	36,59±5,70	
	PCOS	406	36,31±6,34	
t4	UEI	353	38,48±6,10	,110
	Endometriosis	264	39,49±6,00	
	PCOS	403	38,67±6,86	
t5	UEI	348	48,42±8,93	,709
	Endometriosis	262	48,44±8,19	
	PCOS	399	47,89±9,12	
t6	UEI	345	51,61±7,98	,129
	Endometriosis	258	52,92±8,31	
	PCOS	386	51,55±8,66	
t7	UEI	343	54,39±8,80	,173
	Endometriosis	253	55,85±9,08	
	PCOS	380	55,17±9,17	
t8	UEI	339	58,17±10,56	,365
	Endometriosis	239	59,65±11,00	
	PCOS	363	58,55±10,05	

t9	UEI	335	67,71±11,36	,874
	Endometriosis	230	67,84±11,06	
	PCOS	338	67,40±9,83	
tSC	UEI	313	79,93±10,12	,030 (UEI vs. EMS) ,018 (UEI vs. PCOS) ,000
	Endometriosis	162	82,44±10,70	
	PCOS	237	77,53±9,99	
tM	UEI	281	88,06±9,21	,004 (UEI vs. EMS) ,000 (EMS vs. PCOS)
	Endometriosis	191	90,82±10,02	
	PCOS	289	86,81±8,79	
tSB	UEI	258	97,38±7,77	, ,001 (UEI vs EMS) ,001 (EMS vs PCOS)
	Endometriosis	185	100,37±9,23	
	PCOS	234	97,32±8,22	
tB	UEI	230	104,47±7,30	,000 (UEI vs. EMS) ,000
	Endometriosis	156	107,85±8,04	
	PCOS	223	104,28±7,51	
tEB	UEI	180	110,02±6,42	,026 (UEI vs EMS) ,007 (EMS vs PCOS)
	Endometriosis	103	113,37±9,27	
	PCOS	164	109,81±6,24	
t2-t8	UEI	339	32,01±9,14	,666
	Endometriosis	239	32,89±9,17	
	PCOS	363	32,36±8,92	
tSC-tM	UEI	281	8,61±6,36	,000 (UEI. vs EMS) ,000 (UEI vs PCOS)
	Endometriosis	191	28,26±34,91	
	PCOS	289	30,00±34,32	
tSB-tEB	UEI	180	14,79±3,72	,074
	Endometriosis	103	16,03±5,88	
	PCOS	164	22,53±25,24	

***Note:** UEI, Unexplained Infertility; PCOS: Polycystic Ovary Syndrome; N: Embryo Count. Values are presented as mean±standard deviation. Comparisons between groups were made using Student's t-test. Statistical significance ($p<0.05$) is indicated in bold.

Comparison of Pregnancy Outcomes in Study Groups

In the UEI group, 33 women (n=42) achieved pregnancy; in the endometriosis group, 30 women (n=44) reached pregnancy; and in the PCOS group, 32 women (n=41) became pregnant. However, no significant differences were observed between the groups ($p>0.05$). Pregnancy outcomes were similar among the groups (UEI: 78%, PCOS: 68%, endometriosis: 69%).

Comparison of Demographic and Clinical Parameters According to Pregnancy Outcomes

All patient data showed that cases with high fertilization rates ($p=0.001$) and progressive motility (+4 progressive motile sperm) values in sperm parameters were statistically significant ($p = 0.021$) and were directly linked to positive pregnancy outcomes. Fertilization rate and sperm progressive motility were found to be essential factors in achieving pregnancy in all cases.

Comparison of Demographic and Clinical Parameters by Pregnancy in the Unexplained Infertility Group

A comparison of the demographic and clinical parameters according to pregnancy in the UEI group is shown in Table 4.

Comparison of Demographic and Clinical Parameters According to Pregnancy in the Endometriosis Group

A comparison of the demographic and clinical parameters according to pregnancy in the endometriosis group is shown in Table 5. The results indicated that cases with a high fertilization rate were statistically significant ($p = 0.014$) and directly proportional to positive pregnancy outcomes (Table 5).

Table 4: Comparison of demographic and clinical parameters by pregnancy in the unexplained infertility group.

	Pregnancy (Positive 1, Negative 0)	N	Mean ± Standard deviation	P Value
BMI (kg/m²)	0	9	21,64±2,69	,414
	1	33	23,33±3,66	
AGE	0	9	31,56±2,79	,275
	1	33	32,33±3,05	
AMH (ng/dl)	0	9	5,02±4,07	,278
	1	31	2,58±1,41	
E2 (pg/ml)	0	8	36,75±11,34	,086
	1	32	46,06±27,94	
TSH (ulU/ml)	0	9	1,91±1,08	1,000
	1	33	1,76±,75	
FSH (mlU/ml)	0	2	7,05±1,34	,727
	1	9	7,96±2,30	
LH (mlU/ml	0	9	6,69±2,18	,908
	1	32	6,85±2,38	
Trigger Day E2 (pg/ml)	0	8	2913,50±2653,12	,055
	1	32	2349,10±1391,32	
Trigger Day LH (mlU/ml)	0	9	3,39±2,42	,631
	1	31	1,87±1,08	
MII Oocyte	0	9	12,78±9,08	,880
	1	33	9,48±3,55	
Fertilized Oocyte (2PN)	0	9	10,11±8,01	,880
	1	33	7,79±3,34	
Number of oocytes	0	9	13,89±9,35	,857
	1	33	10,88±3,92	
Maturation (%)	0	9	91,43±10,17	,546
	1	33	87,74±12,78	
Fertilization (%)	0	9	77,71±17,97	,567
	1	33	80,74±18,28	
Sperm Concentration (milyon/ml)	0	9	29,00±17,01	,567
	1	33	31,18±12,97	
Total motility (%)	0	9	40,11±6,39	,740
	1	33	40,03±6,03	
Progressive motility (+4) (%)	0	9	3,67±1,00	,487
	1	33	4,55±2,86	

***Note:** N, number of cases; BMI, body mass index; AMH, anti-Müllerian hormone; E2, estradiol; TSH, thyroid-stimulating hormone; FSH, follicle-stimulating hormone; PRL, prolactin; LH, luteinizing hormone. The Mann-Whitney U test was performed, as shown in this table. Statistical significance was set at P<0.05.

Table 5: Comparison of demographic and clinical parameters by pregnancy in the endometriosis group.

	Pregnancy (Positive 1, Negative 0)	N	Mean ± Standard deviation	P Değeri
BMI (kg/m²)	0	14	23,63±3,62	,388
	1	30	23,10±3,07	
AGE	0	14	33,71±3,36	,850
	1	30	32,87±2,80	
AMH (ng/dl)	0	14	2,03±1,17	,510
	1	28	1,92±,89	
E2 (pg/ml)	0	14	47,07±21,27	,683
	1	26	50,08±25,88	
TSH (ulU/ml)	0	14	1,90±1,36	,484
	1	29	2,05±,85	
FSH (mlU/ml)	0	4	7,73±3,28	1,000
	1	16	7,45±2,68	
PRL (ng/ml)	0	14	16,79±7,33	,687
	1	30	18,39±9,05	
LH (mlU/ml	0	14	6,11±2,41	,897
	1	24	5,90±1,85	
Trigger Day E2 (pg/ml)	0	14	2257,57±1789,44	,276
	1	29	2198,00±1376,55	
Trigger Day LH (mlU/ml)	0	11	2,57±1,73	,731
	1	25	1,96±1,31	
MII Oocyte	0	14	8,07±3,95	,969
	1	29	8,41±3,69	
Fertilized Oocyte (2PN)	0	14	5,93±3,22	,610
	1	29	6,97±2,97	
Number of oocytes	0	14	9,57±5,11	,805
	1	29	10,03±4,54	
Maturation (%)	0	14	86,55±14,43	,884
	1	29	86,60±11,95	
Fertilization (%)	0	14	69,34±21,04	,014
	1	29	84,63±11,53	
Sperm Concentration (milyon/ml)	0	11	37,18±15,63	,435
	1	25	34,56±21,29	
Total motility (%)	0	11	40,55±4,74	,520
	1	25	43,72±9,46	
Progressive motility (+4) (%)	0	11	4,36±1,43	,498
	1	25	5,56±3,79	

***Note:** N, number of cases; BMI, body mass index; AMH, anti-Müllerian hormone; E2, estradiol; TSH, thyroid-stimulating hormone; FSH, follicle-stimulating hormone; PRL, prolactin; LH, luteinizing hormone. The Mann-Whitney U test was performed, as shown in this table. Statistical significance was set at $P<0.05$.

Comparison of Demographic and Clinical Parameters According to Pregnancy in the PCOS Group

A comparison of the demographic and clinical parameters according to pregnancy in the PCOS group is shown in Table 6. The results indicated that cases with a high fertilization rate were statistically significant ($p < 0.001$) and this percentage was directly related to positive pregnancy outcomes (Table 6).

Table 6: Table of Comparison of demographic and clinical parameters by pregnancy in the PCOS group.

	Pregnancy (Positive 1, Negative 0)	N	Mean ± Standard deviation	P Değeri
BMI (kg/m²)	0	9	28,84±5,88	,195
	1	32	27,77±6,27	
AGE	0	9	31,00±5,57	,546
	1	32	28,94±3,96	
AMH (ng/dl)	0	7	7,54±3,78	,798
	1	26	7,04±4,37	
E2 (pg/ml)	0	8	34,10±18,37	,651
	1	31	38,29±18,52	
TSH (ulU/ml)	0	9	2,15±1,13	,889
	1	32	2,13±,91	
FSH (mlU/ml)	0	2	12,75±10,25	,667
	1	2	6,75±3,61	
PRL (ng/ml)	0	9	14,54±4,00	,631
	1	32	16,49±9,47	
LH (mlU/ml	0	9	8,63±4,04	,753
	1	31	8,77±4,46	
Trigger Day E2 (pg/ml)	0	8	3619,50±2611,78	,171
	1	32	4072,81±2708,54	
Trigger Day LH (mlU/ml)	0	8	4,40±2,05	,874
	1	30	3,91±3,86	
MII Oocyte	0	9	13,22±9,80	,609
	1	32	13,72±6,71	
Fertilized Oocyte (2PN)	0	9	8,67±7,65	,120
	1	32	11,44±5,05	
The oocyte from the OPU process???	0	9	16,22±12,47	,566
	1	32	17,91±8,57	
Maturation (%)	0	9	86,44±12,01	,195
	1	32	78,49±16,02	
Fertilization (%)	0	9	60,33±19,23	,001
	1	32	84,94±17,80	
Sperm Concentration (milyon/ml)	0	7	28,14±15,03	,566
	1	22	31,05±14,58	
Total, motility (%)	0	7	41,43±6,83	,746
	1	22	43,45±9,31	
Progressive motility (+4) (%)	0	7	4,43±1,90	,328
	1	22	5,86±3,17	

***Note:** N, number of cases; BMI, body mass index; AMH, anti-Müllerian hormone; E2, estradiol; TSH, thyroid-stimulating hormone; FSH, follicle-stimulating hormone; PRL, prolactin; LH, luteinizing hormone. The Mann-Whitney U test was performed, as shown in this table. Statistical significance was set at $P<0.05$.

Discussion

In the present study, embryos derived from women with endometriosis exhibited slower developmental kinetics compared with those from the PCOS and UEI groups. This finding may be attributed to oxidative stress–related impairment of embryo development in endometriosis. In contrast, embryos from women with PCOS demonstrated significantly accelerated developmental progression, potentially associated with elevated LH and AMH levels observed in this group. With respect to clinical parameters, serum AMH concentrations were significantly lower in the endometriosis group compared with both the PCOS and control groups? This observation may reflect oxidative stress–induced ovarian damage in patients with endometriosis, resulting in diminished ovarian reserve and reduced AMH production. Conversely, the highest AMH levels were detected in the PCOS group, likely secondary to increased follicular number and elevated AMH secretion. In addition, serum E2 levels, trigger-day LH concentrations, and day-2 LH levels were significantly higher in women with PCOS than in the other study groups.

Despite these differences, no significant intergroup differences were observed in pregnancy outcomes. Endometrial receptivity in endometriosis did not appear to adversely affect pregnancy achievement in the present cohort. Furthermore, fertilization rates and positive pregnancy outcomes were significantly associated with a higher proportion of progressively motile sperm. We also observed that positive pregnancy rates were significantly associated with higher PRL levels in the UEI group, as well as with increased fertilization rates in the endometriosis and PCOS groups. Although women with PCOS yielded a greater number of oocytes, oocyte maturation rates were significantly lower compared with the UEI group. While maturation rates were also lower relative to the endometriosis group, this difference did not reach statistical significance.

Freis, et al. (2017) conducted a study with patients in a group with Unexplained Infertility (UEI) and women with endometriosis [13]. According to the TLS results in this study, a significant difference was found between the two groups in CS (2-8) and CS (4-8) times. Based on this difference, the embryos of women with endometriosis develop slower than those of patients with unexplained infertility. We found that only the tPNf and t2 stages developed significantly slower in the EMS group until t8 stage. However, the embryos of women with endometriosis generally developed markedly slower than those of other groups. Additionally, Freis et al. reported that patients with endometriosis have lower oocyte and embryo quality. They stated that the results were similar between the groups with unexplained infertility and endometriosis in terms of AMH, age, and BMI. The clinical parameter results obtained from our study showed that the AMH level was significantly lower in the EMS group than in the UEI group. Age and BMI did not differ considerably between the EMS and UEI groups.

Ramezani Tehrani, et al. [14] found that AMH levels were low in women with endometriosis [15]. We also found that the AMH levels were low in patients with endometriosis. This finding could be related to poor oocyte quality and slow embryo morphokinetics. This warrants further investigation. According to the study by *Ramezani Tehrani F, et al.,* in contrast to the age-dependent threshold observed in the control group, AMH levels in women with endometriosis do not decline more sharply before the age of 27. These findings suggest that endometriosis can modify the typical pattern of AMH, and therefore, clinicians should interpret AMH levels carefully in this population [14].

Schenk, et al. [16] found no statistically significant differences in morphokinetic parameters between the control and endometriosis groups in their study, which included 1148 embryos (control: n = 596, endometriosis: n = 552) [16]. Additionally, no significant relationship was observed between fetal heartbeats in groups with and without endometriosis [16]. The morphokinetic parameter results showed that embryos developed more slowly in the EMS group than in the UEI group (tPNf, t2, tSC, tM, tSB, tEB, tB, and tSC-tM were significantly slower in the EMS group).

Irez, et al. [17] showed that oocyte fertilization and pregnancy rates in PCOS cases at different ICSI timings were different compared to those in cases with a normal response, and prolonging the duration caused a decrease in fertilization and pregnancy outcomes [17]. Thus, it was inferred that oocyte aging is shorter and faster in PCOS cases related to oocyte sufficiency. In this study, embryo selection methods used today, especially Preimplantation Genetic Diagnosis (PGD), were not applied. The similarity in pregnancy outcomes between the groups in our study can be attributed to embryo selection via PGD. Similarly, our study showed the rate at which PCOS embryos developed, and we believe that molecular studies support this finding.

Chappel, et al. [18] formed two groups of women of similar age and body mass index (BMI), including women with PCOS (n=64) and women without PCOS (n=64) (control group) [18]. They studied 990 and 628 embryos in the PCOS and control groups, respectively. In this retrospective study, when embryos were examined morphokinetically, the rates of reaching stages t7, t8, and t9 (i.e., reaching the morula) were significantly higher in embryos from women with PCOS than in those from the control group. They showed that the other morphokinetic parameters were similar. In this retrospective study, we demonstrated that the embryos of women with PCOS at the morula stage (tSC-tM) were slower than those in the UEI group. We also showed that, during the compaction stage (tSC), embryos from women with PCOS were faster than those from the UEI group. Additionally, *Chappel et al.* [18] showed that women with PCOS had a significantly higher abortion rate (PCOS group,38.1%; control group, 18.8%). However, we found no significant differences in pregnancy outcomes [18].

Wissing, et al. [19] showed in their study that women with hyperandrogenic PCOS exhibited delayed morphokinetic progression from the tPNf to t8 stages compared to women without PCOS [19]. However, they did not demonstrate a significant difference in morphokinetic parameters between women with

normoandrogenic PCOS and those without PCOS. They also found no significant differences in the implantation and clinical pregnancy rates between the groups. Similarly, *Tabibnejad et al.* [20] showed that the tPNf-t8 interval in embryo development parameters of women with PCOS was slower than that in women without PCOS [20]. They found no significant differences between groups in terms of implantation and clinical pregnancy rates. Based on our findings, there were no significant differences in implantation and pregnancy rates.

Llarena, et al. [21] compared embryo morphokinetics between two groups with and without endometriosis [21]. The continuous embryo monitoring system examined 3,471 embryos from the EMS (n = 1078) and non-EMS (n = 2393) groups until day 6. Morphokinetic results showed that the EMS group was significantly slower to reach the t2 and t8 stages, as well as the compaction (tSC), morula (tM), and blastocyst stages (tB), compared to the non-EMS group [21]. However, there were no significant differences in IVF outcomes between these groups. We found a similar result for t2, although the difference was not evident until the tSC stage: tSC was significantly slower between tM and tB. *Kitajima, et al.* [11] compared serum AMH levels between patients diagnosed with endometriosis (n = 90) and those without endometriosis (n = 30) [11]. They showed that serum AMH levels are inversely proportional to age. However, the authors stated that there was no significant difference between the groups. Our study revealed that AMH levels were substantially lower in women with endometriosis than in those without endometriosis.

Bungum, et al. [12] examined serum AMH, FSH, LH, progesterone, testosterone, and estradiol levels in 18 patients n=10 in the control group and n=8 in the PCOS group [12]. According to their results, the serum AMH, LH, and testosterone levels were significantly higher in the PCOS group. FSH levels were considerably lower in the PCOS group. They did not find a significant difference in progesterone or estradiol levels [12].

Blood glucose, TSH, testosterone, FSH, and LH levels of women diagnosed with PCOS (n=33) and a control group of healthy women (n=32). Their results showed that luteinizing hormone (LH), testosterone, and blood glucose levels were significantly higher in women with PCOS than in the control group. However, they did not find a statistically significant difference in the TSH and FSH levels [12].

Conclusion

In patients with endometriosis, embryo development was delayed, with fewer MII oocytes and slower progression through various fertilization stages (tPN, t2, tSC, tM, tSB, tEB, and tSC-tM). Despite the differences in embryo morphokinetics, clinical pregnancy rates were similar between the endometriosis and control groups, regardless of disease severity. This study highlights the potential usefulness of embryo morphokinetics in selecting high-quality embryos in patients with endometriosis. The association between low AMH levels and embryo quality in endometriosis warrants further investigation. In PCOS patients, higher BMI and trigger-day E2 and LH levels were associated with more M2 oocytes, fertilized oocytes, and a lower rate of mature oocytes. Additionally, tSC was faster, while delays were noted in the tSB-tM processes. Future prospective studies should focus on selecting embryos with the best potential for implantation, pregnancy, and live birth in patients with PCOS and endometriosis, considering established parameters.

Declarations

Disclosure Statement /Competing Interests

The authors declare that they have no affiliations with or involvement in any organization or entity with any financial interest in the subject matter or materials discussed in this manuscript.

Ethics Approval

This study received ethical approval on March 8, 2022, with approval number 2022/03-822, in accordance with the ethics committee guidelines established under Articles 14 and 42 of the Higher Education Law No. 2547.

Consent for Publication

Not applicable.

Data Availability

The data analyzed during the current study are available from the corresponding author on reasonable request.

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Author Contributions

TI and SK: Supervision. TI, GO, AO, T.M.A, YKC: Conceptualization, investigation, methodology, and formal analysis. AO, TI: Writing—original draft. TI, AO: Writing—review and editing.

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