Experimental & Clinical Article

Reproductive Medicine; *Endocrinology and Infertility*

Pregnancy Outcomes of Infertile Patients Presenting to Our Assisted Reproductive Technology Center

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ABSTRACT

OBJECTIVE: In this study, we aimed to evaluate whether the demographic and stimulation characteristics, treatment results, and pregnancy outcomes of infertile couples who applied to our assisted reproductive technology center vary according to the calendar year.

STUDY DESIGN: The original files of 949 patients who underwent assisted reproductive technology treatment were analyzed between January 2012 and December 2017. Assisted reproductive technology cycles were classified according to year and also basal parameters and assisted reproductive technology outcomes were compared by year.

RESULTS: Female age, infertility period, baseline follicle-stimulating hormone, luteinizing hormone, estradiol levels, duration of stimulation, total gonadotropin dose required, peak E2 level, and endometrial thickness on human chorionic gonadotropin administration day were statistically significant between the groups ($p<0.05$). While a statistically significant difference was observed in fertilization rate ($p<0.05$), the following were comparable between the groups ($p>0.05$): number of retrieved and MII oocytes, two pronuclei, day of transfer, embryo transfer technique, single vs. multiple embryo transfer, rates of the Grade-I embryo, biochemical and clinical pregnancy, live birth, ectopic pregnancy, and miscarriage.

CONCLUSION: Infertile women of reproductive age benefit from assisted reproductive technology when trying to become pregnant. Factors that could adversely affect assisted reproductive technology, such as advanced age, have a negative impact on the treatment's success. These repercussions should be taken into account by infertile couples who consider delaying pregnancy.

Keywords: Assisted reproductive technology, Infertility, Pregnancy outcomes

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Introduction

Assisted reproductive technology (ART) employs in vitro fertilization (IVF) techniques, including intracytoplasmic sperm injection (ICSI), to transfer a developing embryo to the uterus following fertilization outside the human body; 2-4% of all pregnancies are attained by IVF-ICSI (1-3). Despite exceptional technological advances and progress in ART in the last few decades, infertility rates are still around 15% worldwide, and unfortunately, take-home baby rates have not reached desired levels (4-6). ART is also used for patients of reproductive age to preserve fertility before gonadotoxic treatment (4,7). As a result, the number of ART centers is increasing.

Ethical and philosophical issues in ART vary widely from country to country. Surrogacy, oocyte or sperm donation, human cloning, and the commercial purchase and sale of human reproductive products are legally prohibited and restricted in Türkiye (8). Many factors can affect the success of ART, such as the age of the infertile couple, embryo quality, endometrial receptivity, and embryo transfer technique (9-12). In this study, we aimed to evaluate the demographic and stimulation characteristics, treatment results, and pregnancy outcomes of infertile couples who applied to our assisted reproductive technology (ART) center between January 2012 and December 2017.

Materials and Method

Study participants and data collection: The ART outcomes of 949 patients who had presented to the ART center of Ali Kemal Belviranli Women's Health and Children's Hospital be-

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tween January 2012 and December 2017 were retrospectively evaluated. ART cycles were classified according to the years (Group 1, 2012; n=67), (Group 2, 2013; n=135), (Group 3, 2014; n=192), (Group 4, 2015; n=285), (Group 5, 2016; n=175), and (Group 6, 2017; n=95). Exclusion criteria were age >45 years, BMI >35 kg/m2, any significant illness or metabolic disorders, and chemotherapy or radiotherapy history. The ethical board approval was given by the institutional review board of Necmettin Erbakan University Meram Medical Faculty (2017/1082) and the study was performed in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. Written and oral informed agreement was given by the participants before the IVF-ICSI procedure for future use.

Data were obtained for female age, body mass index (BMI) (kg/m2), smoking status, duration of infertility, etiology of infertility, menstruation day 3 follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E2) levels, antral follicle count (AFC), male age, total progressive motile sperm count (TPMSC), stimulation parameters, IVF-ICSI outcomes, and perinatal outcomes (clinical pregnancy, live birth, biochemical pregnancy, ectopic pregnancy, and miscarriage rates).

Controlled ovarian stimulation and ovulation triggering: Pituitary down-regulation was achieved using leuprolide acetate in the gonadotropin-releasing hormone agonist (GnRHa) cycles. Recombinant FSH and leuprolide acetate daily together were used for microdose flare-up cycles. Cetrotide or Orgalutran was used in the gonadotropin-releasing hormone antagonist (GnRHant) cycles. For all protocols, recombinant FSH (100–225 IU/day) was used for controlled ovarian stimulation, and the initial gonadotropin dose used was individualized according to the woman's age, serum FSH concentration on day 3, BMI, and previous reflection to ovarian stimulation.

Ovulation triggering was performed by the administration of 250 IU recombinant human chorionic gonadotropin (hCG) (Ovitrelle, Serono, Istanbul, Türkiye) when at least two follicles reached 18 mm in diameter. The oocyte pick-up procedure was performed 36 h after the ovulation triggering. The denuded oocytes were evaluated under light microscopy for the determination of their developmental stage and quality.

Sperm retrieval technique: Semen was obtained by masturbation after 2-3 days of abstinence, and then the samples were left to liquefy for at least 20-30 min at 37 °C before analysis. The concentration, motility, and morphology of the sperm were evaluated, followed by centrifugation (13).

intracytoplasmic sperm injection, fertilization check, and embryo grading: The oocytes obtained from each cycle were cultured at 37°C in a humidified triple gas incubator (Model 3131; Thermo Fisher Scientific, USA) with 6% CO2 and 5% oxygen, in G-IVF PLUS™ (10136; Vitrolife, Goteborg, Sweden) medium for at least 2 h before they were denuded of cumulus cells. Thirty minutes after ICSI, the oocytes were transferred into G-1 PLUS™ (10128; Vitrolife) medium, labeled accordingly, and cultured at 37°C in a humidified 6% CO2 incubator until the fertilization control. ICSI procedure was routinely performed by two senior embryologists in all cycles in our clinic. The decision of which embryo will be transferred is performed by the council attended by two senior embryologists.

Fertilization controls were performed 18-19 h after the ICSI procedure. Only embryos that showed two pronuclei (2PN) were accepted normally fertilized. Day-2 and Day-3 embryos with less than 30% fragmentation, were taken into account to be transferable embryos. After the transfer of the Day-2 and Day-3 embryos, the remaining embryos were cultured further to Day-5 to form blastocysts.

Embryos were assessed according to the European Society for Human Reproduction and Embryology guidelines and categorized into four quality classifications (14). The highest quality embryos were selected for embryo transfer on days 2, 3, and 5 after fertilization. The number of embryos transferred (two or fewer per patient) complied with national regulations in Türkiye.

ET Procedure: Two senior physicians performed the ETs accompanied by ultrasonographic appearance (Logiq 200 Pro, General Electric, Seoul, South Korea) using an embryo transfer catheter system. A sterile speculum was introduced to the vagina in the lithotomy position and the vagina and the cervix were cleared using sterile cotton swabs.

The embryos were loaded by an embryologist into a transfer catheter and the physician deposited the embryos approximately 10 mm from the uterine fundus under USG. All catheters were immediately checked for retained embryos, and blood and the patient remained in the Trendelenburg position for about 15 minutes. Crinone 8% vaginal gel (progesterone, Serono, Istanbul, Türkiye) at a daily dose of 90 mg was used for luteal phase support. Baseline parameters and IVF-ICSI outcomes were compared between the groups. Biochemical pregnancy was detected by hCG levels in venous blood tests performed 12-14 days after embryo transfer, and clinical pregnancy was accepted as those with a gestational sac accompanying fetal heart-beat on ultrasound examination at 4-5 weeks after embryo transfer. Live birth was defined as the birth of a live fetus after 22 weeks of gestational age.

Statistical analysis

The SPSS 15.0 for Windows (SPSS, Chicago, IL, USA) was used for statistical analyses. The continuous variables were checked to be the Kolmogorov-Smirnov test for normal and non-normal distributions. The normally distributed variables were tested by the one-way analysis of variance (ANOVA) test and not-normally distributed variables were tested by the Kruskal-Wallis test. Pearson’s chi-square test or Fisher’s exact test was applied for categorical data. The continuous variables were given as the mean ± standard deviation and the categorical variables were demonstrated as the number of cases and percentages. The Bonferroni adjustment was used to control the type I errors for all possible multiple comparisons. A $p<0.05$ was accepted as statistically significant.

Results

Table I lists the sociodemographic and stimulation characteristics of the participants. The number of admissions to our ART center was highest in 2015 (n=285, 30.0%). While the

Table I: *Demographic and stimulation characteristics of the patients.*

		2012 (Group 1) (n=67)	2013 (Group 2) (n=134)	2014 (Group 3) (n=182)	2015 (Group 4) (n=261)	2016 (Group 5) (n=147)	2017 (Group 6) (n=89)	*p*
Age (years)		31.28+5.57	30.90+5.14[8]	30.43+4.54	30.57+4.42[13]	29.08+4.54[8,13]	29.90+4.32	0.005*
BMI (kg/m2)		26.18+4.23	26.51+4.70	25.85+4.62	24.39+4.65	25.18+4.63	23.32+3.92	0.061
Smoking rate (%)		4.5%	2.2%	7.8%	8.8%	5.2%	10.6%	0.081
Duration of infertility (years)		7.69+4.40[2,4,5]	6.38+3.93	6.11+3.62[2]	6.46+3.71[13]	5.15+3.14[4,13]	5.23+3.12[5]	<0.001*
Etiology of infertility (%)	Male factor	53.1%	42.1	37.6	33.7	28.4	40.9	0.098
	Tubal factor	0.0%	3.8	2.8	1.2	1.4	1.1	
	Unexplained	25.0	33.1	34.3	35.3	44.6	34.1	
	Poor responder	21.9	14.0	25.4	29.8	25.7	23.9	
Baseline-FSH (IU/mL)		6.20+2.12[3,4,5]	6.69+2.05[7,8]	6.97+2.04	7.51+2.82[3,7]	7.66+2.39[4,8]	7.50+2.21[5]	<0.001*
Baseline-LH (IU/mL)		4.47+2.16[2,3,4]	5.04+2.74	5.70+2.56[2]	5.76+3.18[3]	5.80+2.93[4]	5.18+2.91	0.004*
Baseline-Estradiol (pg/mL)		43.50+15.94	43.18+12.48[6,8,9]	37.45+14.50[6,10,11,13]	45.04+17.25[10]	49.46+18.46[8,11]	51.12+15.36[9,12]	<0.001*
Antral follicle count		6.0 (3.5-8.5)	6.0 (4.5-8.0)	6.0 (4.0-9.0)	6.0 (4.0-8.0)	6.0 (4.0-8.0)	6.0 (4.0-9.0)	0.394
TSH (µIU/mL)		2.05+0.93	2.08+0.86	2.16+1.01	2.28+1.26	2.30+1.27	2.20+1.11	0.445
Prolactin (ng/mL)		15.22+7.58	14.85+7.62	15.97+5.75	16.36+8.74	18.70+12.18	17.01+10.12	0.128
Male age (years)		35.75+4.43	35.74+3.42	35.94+4.39	35.36+4.46	34.73+4.35	35.49+4.52	0.576
TPMSC (million)		17.85+10.50	24.62+12.42	25.45+18.27	32.74+14.89	32.13+15.02	22.94+10.91	0.089
Stimulation protocol (%)	Long	57.8%	47.0%	22.1%	15.5%	10.4%	10.2%	0.082
	Antagonist	41.9%	52.6%	77.1%	82.1%	87.5%	88.2%	
	Microdose	0.3%	0.4%	0.8%	2.4%	1.1%	1.6%	
Duration of stimulation (days)		10.35+1.61[3,4]	10.24+1.21[7,8]	10.26+1.71[10,11]	9.22+1.48[3,7,10,14]	9.39+1.42[4,8,11,15]	10.09+1.82[8,14,15]	<0.001*
Gonadotropin dose (IU)		2044.85+968.91[5]	2193.51+824.78[8,9]	2237.09+955.20[10,11,12]	1940.45+860.81[10,14]	1811.15+785.66[8,11]	1543.83+716.63[5,9,12,14]	<0.001*
Estradiol levels on day hCG (pg/mL)		2040.06+938.55	2168.32+1145.84[6,7]	1778.42+1014.95[6]	1801.97+1297.08[7]	1898.94+1159.32	1754.85+114.63	0.025*
Progesterone levels on day hCG (pg/mL)		0.93+0.41	0.85+0.40	0.78+0.31	0.81+0.44	0.78+0.35	0.85+0.39	0.112
Endometrial thickness on day hCG (mm)		10.59+1.84	10.41+1.69	10.40+1.70	10.09+1.78[13]	10.75+1.74[13]	10.59+1.58	0.008*

BMI: body mass index; FSH: follicle stimulating hormone; LH: luteinizing hormone; TSH: thyroid stimulating hormone; TPMSC: total progressive motile sperm count; hCG: human chorionic gonadotropin

** Statistically significant (1=2012 vs 2013), (2=2012 vs 2014), (3=2012 vs 2015), (4=2012 vs 2016), (5=2012 vs 2017), (6=2013 vs 2014), (7=2013 vs 2015), (8=2013 vs 2016), (9=2013 vs 2017), (10=2014 vs 2015), (11=2014 vs 2016), (12=2014 vs 2017), (13=2015 vs 2016), (14=2015 vs 2017), (15=2016 vs 2017).*

difference was found between the groups in terms of female BMI, smoking status, etiology of infertility, TSH, prolactin levels, AFC, male age, TPMSC, stimulation protocol, progesterone levels on hCG administration day, and transfer day ($p>0.05$), female age, infertility period, baseline FSH, LH, E2 levels, duration of stimulation, total gonadotropin dose required, peak E2 level, and endometrial thickness on hCG administration day were statistically significant ($p<0.05$).

The ART outcomes of the participants are given in Table II. While a statistically significant difference was found in fertilization rate ($p<0.05$), the following were comparable between the groups ($p>0.05$): number of retrieved and MII oocytes, 2 pronuclei, day of transfer, embryo transfer technique, single vs. multiple embryo transfer, rates of the Grade-I embryo, biochemical and clinical pregnancy, live birth, ectopic pregnancy, and miscarriage.

Discussion

The current study found that over time, the age and duration of infertility decreased during the years of the study, baseline ovarian hormonal levels increased, and thus ovarian reserve decreased. Rising infertility rates have now become a global problem. Rapid developments in technology and industry have led to exposure to harmful chemicals, an intense work pace may cause emotional and mental stress, lifestyle and nutritional habits have changed, and women often postpone having a baby due to their professional careers (1,2,4,15). With the increase in infertility rates, the number of ART centers, and the number of IVF-ICSI cycles in these centers for infertile couples, have also increased. In June 2012, our ART center was opened as a tertiary institution referral center for Konya and middle Anatolia. In August 2012, staffed by a certified physician and an embryologist, it started accepting patients. Our center provided treatment services for infertile couples with a physician and an embryologist in 2012 and 2013; two physicians and two embryologists in 2014 and 2015; and a physician and two embryologists in 2016 and 2017. Following a decision of the Ministry of Health, the ART center was moved to the City Hospital in May 2017, and IVF-ICSI cycles could not be performed for the rest of that year. It seems that the number of IVF-ICSI cycles per physician over the years increased in line with the literature (8,16,17). Additionally, the number of ART centers increased from four in Konya in 2012 to eight in 2017.

In the current literature, studies in which IVF-ICSI cycles were evaluated retrospectively (8,18) showed that the age and duration of infertility decreased over the years, and baseline FSH, LH, and E2 increased relatively. We observed similar findings in our study. From these results, we can say that with the increased accessibility of infertile couples to treatment, the duration of their infertility decreases. Studies have shown that

Table II: *Laboratory and reproductive outcome parameters of the patients*

		2012 (Group 1) (n=67)	2013 (Group 2) (n=135)	2014 (Group 3) (n=192)	2015 (Group 4) (n=285)	2016 (Group 5) (n=175)	2017 (Group 6) (n=94)	p
Number of oocytes retrieved		9.26+4.96	10.24+5.71	9.22+5.80	8.36+5.68	8.94+5.50	8.30+5.54	0.068
Number of MII oocytes		7.24+3.65	8.04+4.67	7.71+4.57	6.80+4.61	7.03+4.23	6.56+4.35	0.073
2 Pronucleus		4.15+2.69	4.90+3.27	5.15+3.60	4.48+3.20	4.80+3.55	3.98+3.42	0.085
Fertilization rate (%)		55.73+26.27[3]	59.04+26.30[7]	66.95+24.74	68.70+28.72[3,7]	68.14+27.43	61.66+31.22	0.002*
Grade I embryo (%)		51.0%	58.9%	68.2%	62.6%	68.9%	72.4%	0.077
Number of embryo transfers (%)	Single	64.7%	79.4%	77.7%	79.6%	84.8%	85.5%	0.113
	Multiple	35.3%	20.6%	22.3%	20.4%	15.2%	14.5%	
The days of the embryo transfer (%)	2	17.6%	15.0%	2.5%	3.2%	3.1%	1.3%	0.124
	3	66.7%	74.8%	92.4%	90.0%	79.5%	82.9%	
	5	15.7%	10.2%	5.1%	6.8%	17.4%	15.8%	
The embryo transfer technique (%)	Easy transfer with a soft catheter	39.2%	39.3%	25.6%	19.3%	6.8%	1.3%	0.156
	After external guidance transfer	54.9%	53.3%	65.4%	76.1%	83.3%	94.7%	
	Difficult transfer with a stylet	5.9%	7.4%	9.0%	4.6%	9.8%	3.9%	
Clinical pregnancy rate (%)		38.1%	28.9%	36.9%	30.6%	38.7%	43.5%	0.141
Live birth rate (%)		22.2%	21.9%	27.9%	25.7%	29.6%	28.2%	0.682
Biochemical pregnancy rate (%)		14.0%	10.5%	19.3%	17.5%	17.8%	17.3%	0.402
Miscarriage rate (%)		6.3%	9.4%	5.8%	3.4%	5.9%	7.5%	0.832
Ectopic pregnancy rate (%)		-	3.1%	1.4%	1.1%	-	-	0.569

** Statistically significant (1=2012 vs 2013), (2=2012 vs 2014), (3=2012 vs 2015), (4=2012 vs 2016), (5=2012 vs 2017), (6=2013 vs 2014), (7=2013 vs 2015), (8=2013 vs 2016), (9=2013 vs 2017), (10=2014 vs 2015), (11=2014 vs 2016), (12=2014 vs 2017), (13=2015 vs 2016), (14=2015 vs 2017), (15=2016 vs 2017).*

with the delay of pregnancy, poor responder infertility increases (18). In line with the literature, we observed that when the baseline ovarian reserve hormone levels were taken into account, the ovarian reserve decreased and the rate of poor responders increased over time. While the long GnRH agonist protocol was preferred early in the treatment of the IVF-ICSI cycle, mild stimulation protocols have gained importance with the increase in poor responder rates (1,18). In our study, it was clearly seen that the preferred rate of GnRH antagonist protocols increased year by year, although it was not statistically significant. Cycle cancellation rate and total fertilization rate were reported in the literature as 5-7% and 1.5-4%, respectively (19,20), and the rates in our study were compatible with the literature.

The reasons for the relative increase in FR over the years may be advances in the ICSI technique, more optimal embryo culture media, and increased dexterity and experience of embryologists. When single and multiple ET were compared by year, no difference was found. A law enacted on March 6, 2010, made it mandatory to perform one ET in the first and second IVF-ICSI cycles in infertile women under 35 years of age, and two ET in the third and subsequent cycles in infertile women over 35. The law was intended to prevent multiple pregnancies and related adverse perinatal outcomes. This practice is followed under strict and regular control of ART centers (8). Since our ART center is a public institution, ET is carried out within the limits set by the law. When the CPR, LBR, miscarriage, and ectopic pregnancy rates are evaluated year over year, we can see increases in CPR and LBR that do not reach statistical significance; this is consistent with the literature (4,8,21). We hope that these rates will increase in time with a better understanding of the etiopathogenesis of infertility due to clinical studies and advances in ART.

This study's strength lies in its prototypical sample from central Türkiye from which results can be generalized to most of the country's population. The results of the study were compatible with the literature, showing that the treatment provided at the ART center is in line with up-to-date information and that the correct and appropriate algorithm has been implemented for infertile couples. However, the study is limited in that it was conducted in a single tertiary care institution and is retrospective in design, with data from a short period of six years, and also possibly procedures in the clinic and laboratory were performed by different people at different periods, which can affect the results.

In conclusion, infertile women in the reproductive age group benefit from ART to fulfill their fertility goals. Factors such as advanced age decrease the chances of success of the treatment. These negative repercussions should be considered by infertile couples who contemplate delaying pregnancy.

Declarations
Availability of data and materials: The data supporting this study is available through the corresponding author upon reasonable request.
Competing interests: The authors declare that they have no competing interests.
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Authors' contributions: ZOI and HAI raised the presented idea. HAI designed the study. ZOI conducted the analyses. HAI developed the first draft of the manuscript. All authors contributed to the writing of the paper, and have read and approved the final manuscript.

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