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Improved neonatal prognosis following restriction in the number of transferred embryos in assisted reproduction – single center yearly comparison from Turkey

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Summary

Purpose: To evaluate the impact of new legislation for assisted reproductive technology (ART) restricting the number of transferred embryos on neonatal prognosis of infants born after infertility treatments. *Materials and Methods:* Neonatal records of all live born infants in Ege University Maternity Ward were reviewed for 2006 and 2012. Neonatal outcome measures such as birth weight (BW), gestational age (GA), preterm birth (PTB), very low birth weight (VLBW), and neonatal intensive care unit (NICU) admission were evaluated. *Results:* Compared to 2006 percentage of newborns conceived by medically assisted reproduction (MAR) decreased from 14.6% to 5% in all live births, from 23.8% to 8.2% in NICU patients in 2012. The number of fetuses in the last pregnancy, frequency of intrauterine reductions, spontaneous pregnancy losses, antenatal bleeding, and premature delivery decreased. Percentage of multiples among MAR newborns (31.7% *vs.* 55.7%), twins from 51.4% to 30.9%, triplets from 4.3% to 0.8% all decreased significantly. Mean BW and gestational age increased resulting in decreased frequency of PTB and VLBW. Consequently Level 3 NICU admission rate significantly decreased from 44.3% to 22%. *Conclusion:* The new ART legislation in Turkey resulted in decreased rate of multiple births, prematurity and related complications, and NICU admissions in MAR newborns. However the twin rates are still high. Since uncontrolled ovulation stimulation and intrauterine insemination techniques are also associated with multiple births and unfavorable neonatal outcomes, these procedures deserve close monitorization.

Key words: Medically assisted reproduction; Newborn; Multiple; Morbidity; Legislation; Turkey.

Introduction

Advances in reproductive medicine were welcomed with great enthusiasm in Turkey and compensation of treatment costs by the national social security system has made assisted reproductive technology (ART) available to a large population. However, with the widespread use of ART, concerns about increased rate of multiple births and associated complications were raised [1]. Most ART centers in Turkey preferred to transfer more than one, sometimes up to five embryos, hoping to improve the pregnancy rates as shown in a study of the Turkish Society of Reproductive Medicine [2]. This practice resulted in increased risk of multiple births, prematurity, and related complications, causing a great rate of neonatal intensive care unit (NICU) admission in this population [3]. Experience in different countries has shown that with single embryo transfer (SET) in appropriate patient populations, it is possible to decrease the rate of multiple births without a significant decrease in pregnancy success rates [4-9].

In March 2010 the Turkish Ministry of Health announced new legislation for ART, favoring SET, regulating assisted reproductive treatments and funding of treatment costs [10]. According to this legislation a maximum of two cycles of assisted reproductive treatments are reimbursed by the government in women between the ages 23-39; only after having a report stating that all other treatment options have failed and at least one partner has paid his or her social security contributions for a consecutive period of five years. The number of embryos to be transferred is limited to one for the first two trials to women under the age of 35. Double embryo transfer is allowed after two unsuccessful trials before 35 years of age, and to women older than 35 years of age, starting from the first ART trial [10]. In this study the authors aimed to evaluate the effects of this new legislation on neonatal outcome in infants born after infertility treatments.

Materials and Methods

Medical records of all infants delivered at Ege University Hospital were reviewed for the two study periods, before and after the ART legislation. The first study period was between January 1st, 2006 to December 31st, 2006; and the second study period was between January 1st, 2012 to December 31st, 2012. Data about the mode of conception, pregnancy outcome, maternal and neonatal characteristics were recorded in addition to neonatal morbidity parameters, including respiratory distress syndrome (RDS), transient tachypnea of the newborn (TTN), patent ductus arteriosus

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Table 1. — *Distribution of study population in two study periods.*

	Group 1 (n=961)			Group 2 (n=2460)		
	Spontaneous	MAR	*p*	Spontaneous	MAR	*p*
All live births*	821 (85.4%)	140 (14.6%)		2337 (95%)	123 (5%)	
Discharged from newborn nursery*†‡	606 (89.2%)	73 (10.8%)	< 0.001	1823 (95.9%)	77 (4.1%)	< 0.001
Admitted to NICU*†‡	215 (76.2%)	67 (23.8%)		514 (91.8%)	46 (8.2%)	
Level 2§	143 (79.9%)	36 (20.1%)	0.025	243 (89%)	30 (11%)	< 0.001
Level 3§	191 (75.5%)	62 (24.5%)	< 0.001	179 (86.9%)	27 (13.1%)	< 0.001

*$p < 0.001$ for Group 1 *vs.* Group 2; †$p < 0.001$ for spontaneous *vs.* MAR in group 1; ‡ $p < 0.001$ for spontaneous *vs.* MAR in group 2;
§ $p < 0.005$ for percentage of MAR in Group 1 *vs.* Group 2.

Table 2. — *Characteristics of MAR pregnancies in study groups.*

	Group 1 2006 (n=140)	Group 2 2012 (n=123)	*p*
Maternal age, mean (SD)	32.92 (5.38)	32.15 (5.12)	0.245
Paternal age, mean (SD)	35.63 (5.11)	35.39 (4.96)	0.722
Childless years despite parental will, median (min-max)	7.16 (5.86)	5.76 (4.59)	0.067
Prior unsuccessful ART trial, number (%)	65 (46.4%)	49 (39.8%)	0.319
Number of fetuses, median (min-max)	2 (1-4)	1 (1-3)	< 0.001
Intrauterine reduction, number (%)	11 (7.9%)	3 (2.4%)	0.044
Spontaneous loss, number (%)	36 (25.7%)	14 (11.4%)	0.004
MAR type, Number (%)			
Over-stimulation and/or insemination	25 (17.6%)	15 (12.2%)	0.231
IVF, ICSI	115 (82.1%)	108 (87.8%)	

(PDA), intraventricular hemorrhage (IVH), necrotizing enterocolitis (NEC), sepsis, and mortality for infants admitted to the NICU at Ege University Children's Hospital. Medically assisted reproduction (MAR) was defined as reproduction brought about through ovulation induction, controlled ovarian stimulation, ovulation triggering, ART procedures, and intrauterine, intracervical, and intravaginal insemination with semen of husband/partner or donor [11]. Local ethical committee approval was obtained for the study.

Statistical Package for the Social Sciences 19.0 was used for data analyses. Normally distributed data were summarized as mean ± SD and percentages, whereas non-normally distributed data were given as median (min-max). Chi-square test, Fisher exact test, independent samples t-test (with 95 % confidence interval) and Mann Whitney U tests were performed to determine differences between the study groups as appropriate. All *p* values < 0.05 were considered significant.

Results

Frequency of infertility treatment

Nine hundred and sixty-one live born infants from 2006 (Group 1) and 2,460 live born infants from 2012 (Group 2) were enrolled to the study (Table 1). Percentage of newborns conceived by MAR decreased from 14.6 % to 5 % in all live births; and from 23.8 % to 8.2 % in infants admitted to the NICU (all *p* values <0.001). In 2006, 20.1% of patients cared in the Level 2 NICU and 24.5% of patients cared in the Level 3 NICU were newborns from MAR pregnancies; whereas in 2012 these percentages decreased to 11% in Level 2 NICU and 13.1% in Level 3 NICU (p = 0.009 and 0.002, respectively).

Characteristics of the parents and MAR procedures

Maternal and paternal ages were similar in MAR and spontaneous pregnancies (Table 2). In 2012 infertile couples appeared to apply for fertility treatments earlier, with a shorter duration of childless years, but this difference was not statistically significant. The number of fetuses in the last pregnancy decreased in 2012 compared to 2006 ($p <$ 0.001, Table 2). Intrauterine reduction procedures were applied less frequently and also the frequency of spontaneous losses decreased in 2012. IVF and ICSI constituted a higher percentage in fertility treatments, but this trend did not reach statistical significance.

Pregnancy complications

In 2012 maternal follow-up visits for both spontaneous and ART pregnancies increased in number due to continuing improvements in healthcare delivery in Turkey. However, for both study periods; MAR pregnancies had increased risk of complications (such as gestational diabetes, antenatal bleeding, preterm birth, premature rupture of membranes-PROM) when compared to spontaneous pregnancies (Table 3). However the frequency of antenatal bleeding, cerclage application, hospitalization, administration of tocolytics, and antenatal steroids together with preterm delivery decreased in MAR pregnancies in 2012.

Neonatal prognosis

In 2006, infants conceived by MAR had lower mean gestational age and mean birth weights (BWs) and lower

Table 3. — *Antenatal complications of spontaneous and MAR pregnancies in study groups.*

Complications	Group 1 (n=961)			Group 2 (n=2460)		
	Spontaneous (n=821) n (%)	MAR (n=140) n (%)	*p*	Spontaneous (n=2337) n (%)	MAR (n=123) n (%)	*p*
Preeclampsia/eclampsia	34 (4.4)	14 (10)	0.012	97 (4.2)	9 (7.3)	0.106
Gestational DM	68 (8.3)	31 (22.1)	<0.001	197 (8.4)	16 (13)	0.017
Abruptio placenta	3 (0.4)	0 (0)	1.000	4 (0.2)	0 (0)	1.000
Placenta previa	8 (1)	1 (0.7)	1.000	40 (1.7)	2 (1.6)	1.000
Placental insufficiency	5 (0.6)	0 (0)	1.000	12 (0.5)	0 (0)	1.000
Chorioamnionitis	3 (0.4)	1 (0.7)	0.468	8 (0.3)	0 (0)	1.000
Antenatal bleeding*	81 (9.9)	34 (24.3)	< 0.001	96 (4.1)	12 (9.8)	0.010
Premature birth*	140 (17.1)	83 (59.3)	< 0.001	221 (9.5)	41 (33.3)	< 0.001
PROM	56 (6.8)	17 (12.1)	0.037	115 (4.9)	17 (13.8)	< 0.001
Regular follow up*	750 (91.4)	132 (94.3)	0.317	2293 (98.1)	122 (99.2)	0.725
Hospitalization*	118 (14.4)	74 (52.9)	< 0.001	166 (7.1)	33 (26.8)	< 0.001
Cerclage*	12 (1.5)	11 (7.9)	< 0.001	18 (0.8)	3 (2.4)	0.084
Tocolysis*	101 (12.3)	69 (49.3)	< 0.001	101 (4.3)	21 (17.1)	< 0.001
Antenatal steroids*	89 (10.8)	74 (52.9)	< 0.001	141 (6)	26 (21.1)	< 0.001

DM: diabetes mellitus, UTI: urinary tract infection, PROM: premature rupture of membranes
*$p < 0.001$ for Group 1 *vs.* Group 2 MAR pregnancies

Table 4. — *Neonatal characteristics of infants born from spontaneous and MAR pregnancies.*

Neonatal characteristics	2006			2012		
	Spontaneous (n= 821)	MAR (n=140)	*p*	Spontaneous (n= 2337)	MAR (n=123)	*p*
Birth weight (g), Mean (±SD)*	3016.82 (828)	2293.68 (788)	< 0.001	3076.84 (654)	2613.42 (719)	< 0.001
Gestational age (week), Mean (±SD)*	37.02 (3.19)	34.11 (3.36)	< 0.001	37.63 (2.49)	35.81 (2.64)	< 0.001
Apgar score at 1st min, Mean (±SD)	8.50 (1.6)	7.91 (1.8)	< 0.001	8.12 (1.1)	7.65 (1.3)	< 0.001
Apgar score at 5th min, Mean (±SD)	9.72 (0.9)	9.54 (0.8)	0.051	9.37 (0.8)	9.08 (0.9)	< 0.001
C/S, *n* (%)*	648 (78.9)	132 (94.3)	< 0.001	1524 (65.2)	105 (85.4)	< 0.001
Multiple births, *n* (%)*	54 (6.6)	78 (55.7)	< 0.001	83 (3.6)	39 (31.7)	< 0.001
Singleton	767 (93.4)	62 (44.3)	< 0.001	2254 (96.4)	84 (68.3)	< 0.001
Twins	49 (6)	72 (51.4)		82 (3.5)	38 (30.9)	
Triplets	5 (0.6)	6 (4.3)		1	1 (0.8)	
Premature birth (< 37 weeks), *n* (%)*	211 (25.7)	110 (78.9)	< 0.001	519 (21.8)	65 (52.8)	< 0.001
Premature birth (< 32 weeks), *n* (%)*	67 (8.2)	28 (20)	< 0.001	73 (3.1)	7 (5.7)	0.117
VLBW (<1500 gr), *n* (%)	57 (6.9)	28 (20)	< 0.001	66 (2.8)	10 (8.1)	0.004
SGA, *n* (%)	13 (1.6)	3 (2.1)	0.717	41 (1.8)	2 (1.6)	1.000
Congenital anomaly, *n* (%)	51 (6.2)	4 (2.9)	0.165	183 (7.8)	11 (8.9)	0.607
Need for neonatal care, *n* (%)	215 (26.2)	67 (47.9)	< 0.001	514 (22)	46 (37.4)	< 0.001
Level 3*	191 (23.3)	62 (44.3)	< 0.001	179 (7.7)	27 (22)	< 0.001
Level 2	143 (17.4)	36 (25.7)	0.025	243 (0.4)	30 (24.4)	< 0.001
CPAP/N_IMV, *n* (%)	40 (4.9)	19 (13.9)	< 0.001	73 (3.1)	14 (11.4)	0.029
Surfactant, *n* (%)*	59 (7.2)	26 (18.6)	< 0.001	58 (2.5)	2 (1.6)	0.767
Mechanical ventilation, *n* (%)*	74 (9.0)	28 (20)	< 0.001	86 (3.7)	6 (4.9)	0.461
Phototherapy, *n* (%) *	194 (23.6)	65 (46.4)	< 0.001	157 (6.7)	15 (2.2)	< 0.001
RDS, *n* (%)*	56 (6.8)	25 (17.9)	< 0.001	66 (2.8)	8 (6.5)	< 0.001
TTN, *n* (%)	33 (4)	14 (10)	0.005	50 (2.1)	13 (10.6)	0.005
Pneumonia, *n* (%)	12 (1.2)	4 (2.9)	0.273	48 (1.6)	2 (1.6)	1.000
PDA, *n* (%)*	27 (3.3)	11 (7.9)	0.017	38 (1.6)	2 (1.6)	1.000
Sepsis, *n* (%)	20 (2.4)	5 (3.6)	0.394	52 (2.2)	5 (4.1)	0.028
NEC, *n* (%)	8 (1)	2 (1.4)	0.646	19 (0.8)	0(0)	0.205
IVH, *n* (%)*	31 (3.8)	13 (9.3)	0.008	31 (1.3)	3 (2.4)	0.452
BPD, *n* (%)	8 (1.0)	3 (2.1)	0.207	13 (0.6)	0(0)	1.000
Mortality	23 (2.8)	6 (4.3)	0.418	38 (1.6)	2 (1.6)	1.000

C/S: cesarean section; VLBW: very low birth weight; SGA: small for gestational age; CPAP: continuous positive airway pressure;
N-IMV: nasal intermittent ventilation; RDS: respiratory distress syndrome; TTN: transient tachypnea of newborn; PDA: patent ductus arteriosus; NEC: necrotizing enterocolitis; IVH: intraventricular hemorrhage; BPD: bronchopulmonary dysplasia; *$p<0.005$ for comparison of MAR babies in Group 1 *vs.* Group 2.

Apgar scores and were delivered by cesarean section (C/S) more frequently when compared to spontaneously conceived newborns (Table 3). These babies needed NICU admission and respiratory support therapies more frequently. Patent ductus arteriosus (PDA) and intraventricular hemorrhage (IVH) were more common, but mortality rates were similar.

In 2012, mean BWs, mean gestational ages, and Apgar scores were lower and frequencies of C/S, multiple births, preterm birth (PTB), very low birth weight (VLBW) and NICU admissions were higher in MAR infants compared to spontaneously conceived infants (Table 4). RDS, TTN, and non-invasive ventilation were more common; but the need for surfactant and invasive mechanical ventilation therapies and mortality rates were similar to spontaneously- conceived infants.

Comparing neonatal characteristics of newborns from MAR pregnancies in 2006 *vs.* 2012; the frequency of PTB and VLBW decreased and accordingly there was a significant increase in mean BWs and mean gestational ages in 2012. In 2012 the percentage of multiples in MAR infants was decreased significantly when compared to 2006 (31.7 % *vs.* 55.7 %, $p < 0.001$). The frequency of twins decreased from 51.4 % to 30.9 %; triplets from 4.3% to 0.8 %. Although the decrease in the need for total NICU hospitalization was not significant, Level 3 NICU admission rates significantly decreased in 2012 (22 % *vs.* 44.3 %, $p < 0.001$). A significant decrease in RDS frequency resulted in a decreased need for surfactant administration and mechanical ventilation. PDA and IVH were also seen less frequently in 2012.

Discussion

The authors have demonstrated that infertility treatments are associated with increased rates of multiple gestations and adverse neonatal outcome. However, in accordance with the new regulations, the number of fetuses in the last pregnancy has decreased, as did the rate of twins and triplets and neonatal outcome parameters improved in 2012.

Frequency of ART

The frequency of ART (ICSI/IVF) and non-ART (ovulation induction [OI] / intrauterine insemination [IUI]) infertility treatment utilization increased steadily throughout the world after the birth of the first successful IVF infant in the late 1970s. According to latest reports, ART contributed to 1% of all births in 2006 and 1.4% of births in 2009 in the United States [12-13]. The percentage of infants conceived through ART is above 3.0% in most of the Nordic countries; 4.9% in Denmark; and between 1.2% and 1.8% in Germany, France, UK, and Italy [14]. In the current study the percentage of newborns conceived through MAR (reproduction brought about through ovulation induction, controlled ovarian stimulation, ovulation triggering, ART procedures, and intrauterine, intracervical, and intravaginal insemination with semen of husband/partner or donor [11]) pregnancies among all live births, was significantly lower in 2012 than in 2006, 5% *vs.* 14.6%. When we exclude ovarian stimulation and intrauterine insemination procedures; the total percentage of ART (IVF and ICSI) among all live births was 4.3% for 2012 and 11.9% for 2006. The decrease in this rate is in parallel with the decreased number of multiple births after regulations restricting the number of transferred embryos in ART.

In 2006, MAR infants constituted 20.1% of Level 2 NICU patients and 24.5% of Level 3 NICU patients in the present center in a similar pattern with the multicenter data giving the ratios as 21% for Level 2 and 25% for Level 3 MAR infants [3]. The percentage of MAR infants among NICU patients decreased significantly to 11% for Level 2 and 13.1% for Level 3 in 2012 in the present center showing the positive effect of new legislation. Similarly, Guzoglu *et al.* have recently demonstrated that, after the new legislation, NICU utilization decreased in newborns conceived by IVF or ICSI [15].

Characteristics of the parents and MAR procedures

Parental characteristics were similar in the two study periods. However, after the new legislation favoring SET, the median number of fetuses in the last pregnancy was decreased in 2012 when compared to 2006, with median values of one *vs.* two, respectively. The significant decline seen in intrauterine reduction and spontaneous loss rates is a reflection of the decreased fetus number. United States data for 2009 gives the average number of embryos transferred as 2.1 among women aged < 35 years, 2.5 among women aged 35–40 years, and 3.0 among women aged > 40 years [13]. The 11th European IVF-Monitoring Report of the European Society on Human Reproduction and Embryology (ESHRE), comprising 33 European countries and 1,029 clinics showed the total percentage of SET 21.4%, double embryo transfer (DET) 53.4%, triple embryo 22.7%, ≥ four embryos 2.5%, together with twin birth rate of 21.3%, and triplet rate of 1% [14]. The same ESHRE registry data from infertility clinics of Turkey shows SET ratio of 1.5%, DET 24.1%, three embryos 52.8%, ≥ four embryos 11.4% resulting in higher ART multiple rates as 32.9% for twins and 4.1% for triplets in 2007 before the 2010 legislation [14]. Kutlu *et al.* have reported significantly decreased multiple pregnancy rates without causing a significant decline in the pregnancy rates after the new legislation [16].

Pregnancy complications

Pregnancy complications were seen more frequently in MAR pregnancies in both study periods. However in the second study period, perinatal care of all mothers improved significantly. Together with better antenatal care decreased rate of multifetal pregnancies, resulted in a decreased frequency of antenatal bleeding and premature delivery, necessitating C/S, hospitalization, and treatments such as cerclage and tocolytics less frequently in these MAR pregnancies.

Neonatal outcome for MAR infants

Mean BW of 2,293 (±788) grams for MAR babies increased to 2,613 (±719) grams in 2013, in accordance with the increased mean gestational ages from 34.1 (3.3) weeks to 35.8 (2.6) weeks in 2012.

The frequency of premature birth (< 37 weeks) decreased from 78.9% to 52.8% and very preterm birth (< 32 weeks) from 20% to 5.7%. In USA, the percentage of preterm(< 37 weeks) and very preterm (< 32 weeks) newborns among infants conceived with ART were 33.4% and 6.1%, respectively, whereas in the general birth population these ratios were 12.2% and 2% in 2009 [13]. D'Angelo *et al.* have recently investigated outcomes of pregnancies among women who used ART, ovulation stimulation, *vs.* spontaneous pregnancies [17]. They have reported that the prevalence of adverse infant outcomes increased with the use of more intensive treatment, giving the rate of preterm birth (< 37 weeks gestation) as highest among the ART group (16.1%), followed by the medication-only group (11.0%), and lowest in spontaneous pregnancy group (8.0%). The frequency of very preterm infants has reached reported frequencies from developed countries, but the frequency of near term infants is still high in accordance with the still high twin rates.

In the present center, 20% of MAR babies were born as VLBW infants in 2006, whereas in 2012 this percentage decreased to 8.1%. In the United States, 6% of ART infants and 1% of general birth population were VLBW [13].

After the new ART legislation favoring SET, the percentage of MAR babies from multiple gestations decreased from 55.7% to 31.7%. The frequency of twin births was 51.4% *vs.* 30.9% and triplet rates were 4.3% *vs.* 0.8%. The percentage of multiple pregnancies varied between 15-40% in ART pregnant [18, 19]. In the United States, among 31,582 ART newborns, twin rate was 44.5% and triplet rate was 9.3% between 1997-2000 [20]. Again in USA in 2006, 1% of all births were ART newborns; but 17% of twins and 38% of triplets or higher order multiples were conceived with ART [12]. In 2009 data of USA, among ART infants 47% were born as multiple-birth infants, compared with only 3% of infants among the general birth population [13].

Yayla and Baytur evaluated the epidemiology of multiple births in Turkey, in 2003-2004; and reported that 76% of twins and 90% of triplets was achieved with ART (for twins; spontaneous: 24.15%, OI-IUI: 31.70%, IVF-ICSI: 44.15%; for triplets; spontaneous: 10%, OI-IUI: 27.50%, IVF-ICSI: 62.5%). They concluded that ART may be responsible in 75% of multiple pregnancies [21]. ESHRE registry data from Turkey shows ART multiple rates as 32.9% for twins and 4.1% for triplets in 2007 [14].

The limitation of this study was to reflect only the pediatricians' point of view about the effect of new ART legislation on neonatal outcomes. The data recorded for this study was obtained from the medical records of the newborns, reflecting the statements of the family regarding the mode of conception and pregnancy follow-up. Possibility of families to withhold information about details of conception results is a limitation of estimating the real frequency of fertility treatments. The present authors also do not know the number of embryos transferred, but only the number of fetuses in the last pregnancy. They did not have the ART center records available to us to evaluate the number of unsuccessful ART trials. Therefore, determining the change in the pregnancy success rates with the new ART guidelines was beyond the scope of this study.

Since the most important factor being responsible of multiple pregnancies is the number of embryos transferred; it is obvious that limiting the number of embryos to be transferred by the new ART legislation in Turkey resulted in a decreased rate of multiple births, prematurity, and related complications in MAR babies. However, the twin rates are still high and near term deliveries remain to be frequent, compared to European and USA data. These findings indicate ongoing high DET rates and uncontrolled infertility treatments in Turkey. Since twin gestations still cause maternal and neonatal risks, the goal of infertility treatments should be one healthy child. Single-embryo transfer should be promoted more and ovulation stimulation medication use needs to be closely monitored.

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