Impact of the mandatory age-based single-embryo transfer legislation in Turkey on outcome of *in vitro* fertilization: a multicentre study

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أثر تشريع النقل الإجباري لجنين واحد استناداً إلى العمر في تركيا على حصيلة الإخصاب في المختبر: دراسة متعددة المراكز

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الخلاصة: أجرى الباحثون هذه الدراسة في تركيا لتقييم أثر تشريع النقل الإجباري لجنين واحد على أساس العمر، وما أدى إليه من ازدياد في نقل الأجنة المجمّدة المذابة على حصائل الراغبات في الإخصاب في المختبر. وقد شملت الدراسة استخدام جنين احد ونقل الأجنة المجمّدة المذابة والنقل المزدوج للأجنة في 5632 سيّدة بعد صدور التشريع، إلى جانب استخدام الإخصاب التقليدي في المختبر وأسلوب نقل الأجنة المجمدة المذابة لدى 6029 سيّدة قبل صدور التشريع. واتضح أن المعدل التراكمي للحمل بعد صدور التشريع كان أقل بقليل (38.2٪) مما كان عليه قبل صدور التشريع (42٪)، دون أن يكون لذلك أهمية إحصائية يُعْتَدُّ بها. وكان معدل الحمل بجنين واحد عند نقل جنين واحد وفي الإخصاب التقليدي في المختبر متشابهاً لدى المجموعتين (37.8٪ مقابل 38.7٪)، بينما كان معدل الحمل المتعدد أعلى بمقدار يُعْتَد به إحصائياً قبل صدور التشريع (13.7٪)، مقارنة بما أصبح عليه بعد صدور التشريع (0.3٪). وبالنسبة لنقل الأجنة المجمدة المذابة، فإن عدد الدورات كان أعلى بعد صدور التشريع (862) مما كان عليه قبل التشريع (616). وهكذا اتضح للباحثين أن نقل جنين واحد يعطي نتائج مشابهة للإخصاب التقليدي في المختبر. ويرى الباحثون أنه من أجل خفض عدد الأحمال المتعددة دون إحداث انخفاض يُعْتَد به إحصائياً في معدلات الحمل، فإن نقل الجنين الواحد قد يمثّل استراتيجية ناجحة.

ABSTRACT This study in Turkey evaluated the impact of age-based mandatory single-embryo transfer (SET) legislation with the subsequent increase in frozen–thawed embryo transfer (FT-ET) on pregnancy outcome of *in vitro* fertilization (IVF) patients. SET, FT-ET and double-embryo transfer were used in 5632 patients after legislation, while traditional IVF and FT-ET approach was used in 6029 patients before legislation. The cumulative pregnancy rate after legislation was slightly lower (38.2%) than before legislation (42.0%) but not significantly so. The single pregnancy rate for SET and traditional IVF were similar between the 2 groups (37.8% versus 28.7%), while multiple pregnancy rates were significantly higher before than after legislation (13.7% versus 0.3%). For FT-ET, the number of cycles was significantly higher after legislation (862 versus 616). SET yielded similar results to traditional IVF. In order to reduce multiple pregnancies without significantly decreasing pregnancy rates, SET might be a successful strategy.

Impact de la loi turque rendant obligatoire le transfert d'un embryon unique en fonction de l'âge sur l'issue de la fécondation in vitro : étude multicentrique

RÉSUMÉ La présente étude menée en Turquie a évalué l'impact de la loi rendant obligatoire le transfert d'un embryon unique en fonction de l'âge et de l'augmentation consécutive des transferts d'embryons congelés-décongelés sur l'issue de la grossesse des patientes bénéficiant d'une fécondation in vitro. Le transfert d'un embryon unique, le transfert d'embryons congelés-décongelés et le transfert de deux embryons ont été réalisés chez 5632 patientes après l'entrée en vigueur de la loi, tandis que l'approche traditionnelle par fécondation in vitro et par transferts d'embryons congelés-décongelés a été utilisée chez 6029 patientes avant le vote de cette loi. Le taux de grossesses cumulé après l'application de la nouvelle loi était légèrement inférieur (38,2 %) au taux avant la nouvelle loi (42 %), mais la différence n'était pas très importante. Les taux de grossesses simples par transfert d'un embryon unique et par fécondation in vitro traditionnelle étaient similaires entre les deux groupes (37,8 % contre 28,7 %), tandis que les taux de grossesses multiples étaient significativement supérieurs avant l'application de la loi (13,7 % contre 0,3 %). Pour le transfert d'embryons congelés-décongelés, le nombre de cycles était nettement supérieur après la nouvelle loi (862 contre 616). Le transfert d'un embryon unique a généré des résultats similaires à la fécondation in vitro. Afin de réduire le nombre de grossesses multiples sans réduire le taux de grossesses de manière significative, la politique du transfert d'un embryon unique peut représenter une stratégie efficace.

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Introduction

Since the success of the first *in vitro* fertilization (IVF) birth in 1978, IVF became a source of hope for many infertile couples around the world. To increase the success rates of pregnancy, for many years multiple embryo transfers were preferred. However, during recent decades, the multiple pregnancy rate, especially the twin pregnancy rate, has been increasing as a result of assisted reproduction technologies [1,2]. Multiple pregnancies are the most common complication associated with IVF treatment. It is not only a financial, medical and ethical burden on couples, it is considered a high-risk pregnancy for both the mother and infants, due to the relative increased incidence of maternal, perinatal and neonatal morbidity and mortality [1–5]. Between 2005 and 2006 in Europe 19.9% of IVF live births were still twins, while the natural incidence was 1%–1.5% when conceived spontaneously [3,4]. This is important for infertility specialists and also for obstetricians, neonatologists and primary health care providers due to the risks involved and the increased costs to the health service.

The most successful way to decrease twin pregnancies in IVF is to transfer only 1 embryo [6]. The single-embryo transfer (SET) approach is becoming more viable, and several randomized studies have been performed in which transfer of a single embryo was compared with the transfer of 2 embryos [7]. Yet health care commissioners in several countries do not know exactly how alternative embryo transfer strategies will affect health service costs, pregnancy rates and twin pregnancy rates over time in different subgroups of women [5]. Because of the risks mentioned above, in March 2010 the Turkish Ministry of Health legislated to make SET mandatory (for the first 2 IVF cycles) for women in Turkey who are aged 35 years and younger. For women who are older than 35 years, double-embryo transfer (DET) is permitted in all IVF cycles. After 2 failed IVF cycles, regardless of age, DET is permitted. In all cases, if additional good-quality embryos are available, they can be frozen and used in the future.

The aim of this study was to evaluate the impact of this age-based mandatory legislation on pregnancy outcomes, regardless of medical history or the etiology of IVF (male factor, ovulatory factor, infertility duration, etc.).

Methods

The Department of Obstetrics and Gynaecology at Istanbul University School of Medicine coordinated this multicentre, retrospective study, in which the results of 7 IVF centres in Turkey were included.

Sample

Group 1 included IVF patients from the year before the aforementioned mandatory legislation (6029 patients, treated between March 2009 and February 2010). During this period, patients (regardless of age) received a maximum number of 3 high-quality embryos. The clinician on a case-by-case approach decided the exact number of transferred embryos.

Group 2 included IVF patients treated after the March 2010 mandatory legislation (5632 patients, treated between March 2010 and February 2011). Within group 2, patients ≤ 35 years old received only a single embryo in their first 2 IVF attempts regardless of their medical history, duration of infertility or ethology of infertility. Patients ≤ 35 years old received a maximum number of 2 embryo transfers only when they had a history of 2 failed IVF cycles. Patients > 35 years old received a maximum number of 2 embryo transfers regardless of their previous IVF failures, medical history, duration of infertility or ethology of infertility. The mandatory legislation mentioned above also involved frozen–thawed embryo transfers (FT-ET).

The age, demographic features and body mass index (BMI) were adjusted and matched for both groups. To achieve demographic standardization between the 2 groups, patients with extreme BMI (< 18 and > 35 kg/m^2) and/or age (< 18 and > 39 years) were excluded from the study. In all the centres, only good-quality embryos were transferred. Good-quality embryos were defined by their morphologic features and included embryos with < 20% fragmentation and 4–6 cells at day 2, 6 and 10 cells at day 3. In both groups, the numbers of single and multiple pregnancies and number of FT-ETs were recorded.

Ovarian stimulation and IVF/ICSI procedures

Each centre was allowed to follow its own local stimulation protocol according to the medical history of the patient, including age and levels of antral follicle count, follicle-stimulating hormone and body mass index. Hence, ovarian stimulation was performed using an antagonist or agonist protocol. Stimulation was performed with recombinant follicle-stimulating hormone (Gonal-F, Serono, Puregon, Organon) or urinary-derived follicle-stimulating hormone (Menopur, Ferring). Oocyte retrieval was performed via vaginal ultrasound guidance, 36 hours after human chorionic gonadotropin (hCG) administration. Fertilization was performed by standard intra-cytoplasmic sperm injection (ICSI) depending on the experience of each centre. Progesterone was administered intramuscularly or vaginally daily, from the time of oocyte retrieval until the time of a negative pregnancy test or until 2 weeks after a positive pregnancy test.

Embryo culture, transfer, cryopreservation and frozen embryo transfer

Oocytes and embryos were cultured in a ready-to-use commercially available medium: IVF medium (Irvine Scientific). After micro-injection, oocytes were incubated for 14–16 hours at 37 °C and 5% CO_2 within an early cleavage medium. The embryos were transferred on the 2nd or 3rd day via ultrasound guidance. Embryo morphology was scored from grade 1 to 4. Grade 1 embryos contained 6–8 blastomeres and did not have multi-nucleation or fragmentations. Cryopreservation of additional embryos was performed on the same day of the fresh embryo transfer, depending on their morphological aspect. Cryopreservation was performed on day 3 embryos. The criteria for cryopreservation of embryos were at least 7 or 8 cells, > fragmentation 10% and no multinucleated blastomeres.

Each centre was allowed to follow its own FT-ET protocol as well. The same FT-ET protocol was applied in SET and DET groups in each centre.

Outcomes

Pregnancy was initially detected by rising serum hCG concentration in 2 consecutive assays at least 12 days after embryo transfer. The pregnancy was defined as clinical if fetal cardiac activity was visualized by ultrasonography in the 7th week of gestation. The clinical pregnancy rate was calculated as the ratio between the number of pregnancies after fresh embryo transfers and the number of oocyte pick-ups.

Statistical analysis

All data were analysed with the use of the *SPSS for Windows* software, version 16.0. Data are presented as means and standard deviation (SD) or percentages The Shapiro–Wilk W-test was used to identify whether the variables were normally distributed. The differences between groups were assessed by using unpaired *t*-tests for parametric data and Mann–Whitney U-test for nonparametric data. Statistical significance was defined as $P < 0.05$.

Results

An overview of the groups is presented in Table 1. The total pregnancy rate in group 2 (after legislation) (38.2%) was slightly lower than for group 1 (before legislation) (42.0%), but this difference was not statistically significant ($P > 0.05$). Single pregnancy rates were 37.8% for SET in group 2 and 28.7% for traditional IVF in group 1. The multiple pregnancy rate was significantly lower for SET in group 2 (0.3%) than for traditional IVF in group 1 (13.7%) ($P < 0.05$).

As far as FT-ET was concerned, the proportion of transfers was significantly higher in group 2 (862/5632, 15.3%) than group 1 (616/6029, 10.2%) ($P < 0.05$). There was no significant difference in the number of FT-ET single pregnancies comparing group 2 (28.0%) and group 1 (25.4%) ($P > 0.05$). However, there was a decrease in the number of multiple pregnancies as a result of FT-ET in group 2 (4.9%) and group 1 (14.4%) ($P < 0.05$).

Table 1 Pregnancy rates (based on positive beta human chorionic gonadotropin or fetal cardiac activity) in women undergoing *in vitro* fertilization in group 1 (before embryo-transfer legislation) and group 2 (after embryo-transfer legislation)

Groups	No. of patients treated	Pregnancy rate (per transfer)							
		β-HCG positive		Fetal cardiac activity positive				Total	
				Single pregnancies		Multiple pregnancies			
		No.	%	No.	%	No.	%	No.	%
Fresh embryo transfer									
Group 1	5413	2577	47.6	1552	28.7[a]	740	13.7[c]	–	–
Group 2:									
SET	2997	1349	45.0	1134	37.8[a]	10	0.3[c]	–	–
DET	1773	809	45.6	536	30.2	190	10.7	–	–
FT-ET									
Group 1	616	322	52.2	157	25.4[b]	89	14.4[d]	–	–
Group 2	862	396	45.9	242	28.0[b]	43	4.9[d]	–	–
Total									
Group 1	6029	–	–	1709	28.3	829	13.7	2538	42.0
Group 2	5632	–	–	1912	33.9	243	4.3	2155	38.2

[a]P > 0.05 group 1 traditional IVF versus group 2 SET; [b]P > 0.05 FT-ET group 1 FT-ET versus group 2 FT-ET; [c]P < 0.05 group 1 traditional IVF versus group 2 SET; [d]P < 0.05 group 1 FT-ET versus group 2 FT-ET.

FT-ET = frozen-thawed embryo transfer; SET = single-embryo transfer; DET = double-embryo transfer; IVF = in vitro fertilization; β-HCG = beta human chorionic gonadotropin.

Table 2 Pregnancy rates of fresh embryo transfer (based on positive beta human chorionic gonadotropin or fetal cardiac activity) in women undergoing *in vitro* fertilization in group 1 (before embryo-transfer legislation) and in group 2 (after embryo-transfer legislation)

Groups	No. of embryo transfers	Pregnancy rate			
		β-HCG positive (embryo transfers per cycle)		Fetal cardiac activity positive (embryo transfers per cycle)	
		No.	% (95% CI)	No.	% (95% CI)
Group 1	5413	2577	47.6[a] (36.5–52.4)	2292	42.9[a] (31.2–48.3)
Group 2					
SET	2997	1349	45.1 (32.4–51.6)	1144	38.2 (28.1–47.5)
DET	1773	809	45.6 (29.2–39.8)	726	40.9 (30.3–44.5)
Total	4770	2158	45.2[a] (37.3–61.2)	1870	39.2[a] (29.8–46.4)

[a]P > 0.05 SET and DET group 2 versus traditional IVF group 1.
SET = single-embryo transfer; DET = double-embryo transfer; β-HCG = beta human chorionic gonadotropin; CI = confidence interval.

When successful pregnancy outcome of only fresh embryo transfers were compared between SET and DET in group 2 (after legislation) and traditional IVF in group 1 (before legislation), the differences were non-significant (β-HCG positive rate: 45.2% versus 47.6%, clinical pregnancy rate: 39.2% versus 42.9%) ($P > 0.05$) (Table 2).

When we divided group 2 into SET and DET subgroups, β-HCG positive and clinical pregnancy success rates of only fresh-embryo transfers showed no statistically significant difference (β-HCG positive rate: 45.1% versus 45.6%, clinical pregnancy rate: 38.2% versus 40.9%) ($P > 0.05$) (Table 2).

Comparison of single and multiple pregnancies of fresh embryo transfer on SET and DET in group 2 indicated a significant increase in the DET subgroup (clinical pregnancy rate: 0.9% versus 26.2%) ($P < 0.05$) (Table 3).

Discussion

In this large, retrospective, multi-centre study, transferring 1 fresh embryo according to the age-based mandatory legislation did not result in a substantially lower rate of pregnancies than transferring embryos according to the decision of the clinician before the legislation. In addition, the use of SET resulted in a significant reduction in the rate of multiple pregnancies.

SET is effective in reducing the rate of twin pregnancies following IVF. In 6 published randomized controlled trails comparing SET with DET, rates of multiple pregnancies were significantly lower after SET [8–14]. Six of these trials were conducted using predominantly cleavage-stage embryos, those cultured *in vitro* for 2 or 3 days. A Cochrane review including 4 of these trials found an odds ratio of 23.55 (95% CI 8.00–69.29) for multiple pregnancy following DET compared with SET [15].

A French study demonstrated that a high cumulative clinical pregnancy rate (69.8%) and delivery rate (54.7%) could be obtained after a single oocyte pick-up and the transfer of only 1 fresh embryo [16]. These results were not lower than the cumulative pregnancy rate (64.3%) and delivery rate (49.0%) observed when 2 fresh embryos were transferred after IVF and ICSI in a similar population with the same embryo quality, in routine clinical practice. Their results can encourage IVF teams to routinely practise SET in selected populations and should increase the percentage of patients in favour of SET, which was only 35.0% in their study. Infertility specialists should also correctly inform patients about the realistic chances of obtaining a child after SET and on the incidence of potential

Table 3 Pregnancy rate of single and multiple pregnancies of fresh embryo transfer on sub-groups of women undergoing *in vitro* fertilization in group 2 (after embryo-transfer legislation)

Groups	No. of clinical pregnancies	Pregnancy rate			
		Single pregnancies (per clinical pregnancy)		Multiple pregnancies (per clinical pregnancy)	
		No.	% (95% CI)	No.	% (95% CI)
Group 2					
SET	1144	1134	99.1[a] (0–5.1)	10	0.9[a] (0–4.8)
DET	726	536	73.8[a] (17.3–31.2)	190	26.2[a] (23.4–41.2)

[a]P < 0.05 SET group 2 versus DET group 2.
SET = single-embryo transfer; DET = double-embryo transfer; CI = confidence interval.

unfavourable effects of twin deliveries after DET. The introduction of SET has substantially reduced this risk [12,17].

A Finnish study reported similar results for SET and DET for women aged 36–39 years, indicating that SET may also be applied in this age group [18]. Thus, SET could be recommended for women up to 39 years old. It also seems that the embryo quality is an important factor to be considered. Moreover, a number of studies underlined the importance of the whole embryo cohort quality, showing that the availability of several embryos of good quality was associated with higher implantation and pregnancy rates [19–21]. In addition, in another study, cumulative multiple live birth rates were significantly lower in the SET compared with the DET group [22].

A systematic review and meta-analysis of 6 randomized controlled trials comparing SET with DET of cleavage stage embryos published by Gelbaya et al. revealed cumulative multiple birth rates (CLBRs) that ranged from 35.8–46.3% but showed no statistically significant difference between the 2 groups [23]. Moreover, CLBRs decreased significantly from a range of 13.1%–41.2% in the DET group to 0%– 0.8% in the SET group. In general, CLBRs following IVF has been reported as between 45% and 55%. Maternal age has been shown to reduce these rates significantly, as has preimplantation genetic diagnosis. On the contrary, techniques mostly used to decrease the chance of multiple births, such as elective SET and traditional IVF, do not affect CLBRs while achieving a significant reduction in the rates of multiple pregnancy [24].

A potential hindrance to the use of SET is the patient's concern that the chance of pregnancy will be reduced. On the other hand, a change in attitude among patients may be possible with proper patient consultation. SET can be more easily accepted when there is insurance coverage for IVF. If patients pay for IVF, they might prefer more embryo transfers to maximize their chance of having a child. In Turkey, the social insurance covers up to 2 IVF cycles. Therefore, many patients want more than 1 embryo transfer to increase their chances. This factor usually results in discordance between the patient's wishes and clinician's legal responsibility. Moreover, in Turkey FT-ET is not covered separately by the social insurance (it is a part of the patient's 2 IVF cycle coverage). Our study findings suggest that FT-ET following an unsuccessful SET attempt yields similar pregnancy outcomes to fresh embryo transfer, while helping to keep multiple pregnancy incidence low, as indicated in a recent opinion review of reproductive medicine societies [25]. Hence, a separate social/private insurance coverage for FT-ET may be considered to increase the support to SET.

Finally, this study has some inherent limitations. As stated previously, patients generally have a strong belief that transferring more embryos will improve their probability of pregnancy despite the well-recognized risks associated with twin deliveries. Hence, before the age-based mandatory legislation, statistically significant prospective studies to compare SET and DET were difficult to design in Turkey. Therefore, with this study we mainly tried to evaluate the impact of the age-based mandatory legislation of SET.

Conclusions

This multi-centre study clearly highlights that the SET strategy achieves pregnancy rates that were no lower than the rates after transfer of 2 fresh embryos. These optimistic results may influence the decision of eligible couples around the world in favour of SET along with the FT-ET option. In addition, it may convince practitioners of the necessity to apply carefully structured embryo transfer legislation at least in a well-selected population of patients.

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