The effect of legislation on outcomes of assisted reproduction technology: lessons from the 2004 Italian law

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Objective: To evaluate the effect of the 2004 Italian regulations (insemination of ≤3 oocytes/cycle, transfer of all embryos, prohibition of embryo cryopreservation) on outcomes of assisted reproduction treatment (ART).
Design: Case-control study.
Setting: The Center of Reproductive Medicine, Arcispedale Santa Maria Nuova, Reggio Emilia, Italy.
Patient(s): Women undergoing ART for the first time.
Intervention(s): Comparing outcomes of ART between 2 years before (n = 900) and after (n = 936) the law's implementation (March 10, 2004).
Main Outcome Measure(s): Rates of fertilization, pregnancy, "take-home baby," and multiple pregnancies.
Result(s): During the pre-law period, statistically significantly more patients reached embryo transfer (odds ratio 1.9; 95% CI, 1.5, 2.5), and embryo transfer rate per cycle was statistically significantly higher (3.1 ± 1.7 vs. 2.2 ± 0.7), but the overall transfer of good embryos was lower (OR 0.6; 95% CI, 0.5, 0.8). The pregnancy rates per aspiration cycle were similar between the periods, but the pregnancy rate per embryo transfer and birth rate with at least one liveborn baby per embryo transfer were statistically significantly lower in the pre-law period (OR 0.7; 95% CI, 0.5, 0.9). The multiple births rate was not different between the two periods.
Conclusion(s): In contrast to prior pessimistic expectations, the obligation to transfer all available embryos produced from ≤3 inseminated oocytes neither reduced success rates of ART nor increased the multiple births rate. (Fertil Steril® 2008;89:854–9. ©2008 by American Society for Reproductive Medicine.)

Key Words: Assisted reproduction, IVF, ICSI, outcomes, legislation

Since the early days of assisted reproduction technology (ART), it has been apparent that more transferred embryos may improve success rates but, at the same time, may increase the likelihood of an untoward outcome such as multiple gestations (1, 2). Consequently, intensive research has sought a golden path that would lead to the number of transferred embryos associated with an acceptable success rate of ART without increasing the risk of having multiples. The recommended number of transferred embryos in many ART protocols currently aims to reduce the risk of high-order multiples (triplets or more), but not the number of twins (3). As a result, a policy of elective single embryo transfer (eSET) has been subsequently introduced, and resulted in the expected significant reduction of all multiple pregnancies (4, 5).

From a public health perspective, reduction of the number of multiples by reducing the number transferred embryos to as low as eSET has been a welcomed approach, as little doubt exists about the contribution of multiple pregnancies to perinatal morbidity and mortality (2). In addition, such a preventive policy is certainly better than the potential available "cure" in the form of multifetal pregnancy reduction, with its related risks of total pregnancy loss and residual maternal psychological morbidity (6). However, whether such policies are suitable for all patients, in particular for those of advanced age and/or those with repeated failures, is a matter of continued controversy, and is the basis of most current modus vivendi guidelines issued by infertility experts and organizations worldwide.

From the patient's perspective, some compensatory measures for the potential reduction in success rates are required. The first and foremost means is a continuous improvement of the quality of the transferred embryos and the uterine condition for implantation (7). This goal may be achieved anywhere, but the second means, namely, to increase the number of cycles that are reimbursed by the insurance in case of ART failure, cannot obviously be implemented everywhere. Finally, but not the least important, freezing of surplus embryos with subsequent transfer in a spontaneous or induced cycle will increase the cumulative pregnancy rates with a concomitant predictable reduction of costs and risks of ovarian stimulation and ovum pickup.

As the controversy about the appropriate number of transferred embryos continues, a new Italian law, based on the ideological premise of protecting every early, preimplantation embryo, became operative in March 10, 2004 (8, 9). In terms of clinical ART practice, the new Italian legislation

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restricted the number of fertilized oocytes in each cycle to three, obliged the subsequent transfer of all resulting embryos, and prohibited freezing of surplus embryos (10–12). Three problems with the new Italian law were immediately identified, involving social issues, human rights, and the application of ART (12–14). The intuitive critique about this legislation was that it is expected to reduce success rates of ART with a concomitant increase of multiple pregnancies. Counterintuitively, however, a multicenter survey from seven Italian ART institutions that compared the results of the first 3 months following the new law with those from the same period during the previous year found that the success rates of in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) were practically unchanged by the new ART legislation; however, the prohibition of embryo freezing was expected to reduce the cumulative success rate (11).

We compared outcomes of clinical pregnancies of 1 year before and after the legislation and observed a lower spontaneous loss rates in the latter period (12). However, the conclusions from the survey (11) as well as our study might be limited because the observation period was relatively short, centers were not accustomed to the new regulations, the participating institutions in the survey used different ART protocols, and the population of both studies included a case-mix of patients with and without previous unsuccessful IVF/ICSI treatments. Therefore, the purpose of this study was to compare the results of a single ART center from the first 2 years after the new Italian reproductive legislation with the preceding 2 years, in patients without previous IVF/ICSI treatment.

MATERIALS AND METHODS

This study was carried out in our nonprivate infertility clinic at Arcispedale Santa Maria Nuova, at Reggio Emilia, Italy. This ART service had always some unique features related to constraints prohibiting disposal as well as freezing of surplus embryos (15, 16). Moreover, only cleavage-stage embryos are transferred without assisted hatching. The population included in the study comprised mainly (>95%) women of Italian origin, none of whom had had previous attempts with ART. The patients underwent IVF with and without ICSI using fresh sperm. Follow-up on outcome was available for all patients who underwent IVF, but two patients undergoing ICSI during the pre-law period were lost to follow-up verification and were excluded from the analysis. The data include detailed information related to the ART procedures as well as obstetric variables, and were prospectively collected, regularly updated, and evaluated using the Microsoft Excel (Microsoft Corporation, Redmond, WA).

We compared the results of ART cycles that reached the aspiration stage performed during the 2 years following the new Italian legislation (March 10, 2004, to March 9, 2006) with the previous 2 years (March 10, 2002, to March 9, 2004). During the study and control periods, we used the same ovarian stimulation and same ovum pickup methods. However, we gradually changed the in vitro culture media during the post-law period: During the pre-law period and during the first post-law period, we used the same culture media, whereas 1 year after the law, a different culture media was used. The change in culture media was made purely for financial reasons. The decision of whether to perform IVF or ICSI was made upon sperm quality analysis after ovum pickup. Excellent sperm counts were defined by the following characteristics: sperm count $\geq$20 M/mL, progressive motility of $\geq$30%, and normal forms (strict criteria) found in >4%. Quality of oocytes for IVF/ICSI was defined as "good" by finding dispersion of the cumulus cells, presence of the corona radiate with homogenous distribution of the cells, and a clear cytoplasm without granules. Good quality embryos were defined according to the classification developed by Veeck (17). In this classification system, grading ranges from I to V as follows: grade I, blastomeres of equal size and no fragmentation; grade II, blastomeres of equal size, with minor cytoplasmic fragmentation occupying less than 10%; grade III, blastomeres of distinctly unequal size and variable fragmentation, but no more than 30%; grade IV, blastomeres of equal or unequal size, and variable fragmentation from 30% to 50%; and grade V, embryos with few blastomeres of any size and severe fragmentation occupying at least 50% of surface. Good quality embryos were defined with scores I, II, and III.

Before implementation of the new reproductive law, we inseminated up to four oocytes in women <35 years old, $\geq$5 oocytes in women 35 to 37 years, and all available oocytes in women aged $\geq$38 years and transferred all available embryos. In contrast, after the new reproductive legislation, we were obliged to inseminate no more than three oocytes and to transfer all available embryos. Out of necessity, surplus oocytes were frozen and submitted to subsequent fertilization in future cycles, using the method previously described elsewhere (18).

In the analysis, we evaluated the effect of the new Italian reproductive legislation on fertilization, pregnancies, "take-home baby," and multiple pregnancies rates, in the entire population as well as separately in IVF and IVF/ICSI cases. We also compared outcomes by age group (<35, 35 to 37, 38 to 39, and 40 to 41 years). We used True Epistat Software (Math Archives, Round Rock, TX) to compare frequencies by the Fisher's exact tests. We derived odds ratio (OR) and Corenfield's 95% confidence intervals (CI). Continuous variables were compared by Student's *t*-test; $P<.05$ was considered statistically significant. The ethics committee of the hospital approved the study.

RESULTS

There where 900 first-time ART patients (484 without and 416 with ICSI, respectively) in the 2 years before the new Italian reproductive law, and 936 first-time ART patients (521 and 415, without and with ICSI, respectively) in the 2 years after the new Italian reproductive law. The preceding period had significantly fewer patients >38 years old. The proportion of IVF/ICSI cycles was similar in the two periods.

The ART characteristics are shown in Table 1. A total of 2484 and 1705 embryos were transferred before and after the law, respectively. Irrespective of the ART method, a statistically significantly higher embryo transfer (ET) per cycle rate was observed before the implementation of the law. In contrast, the overall transfer of good embryos was higher after the implementation of the law, and this was statistically significant among IVF as well as among ICSI cycles.

The outcome characteristics before and after implementation of the 2004 law are shown in Table 2. There was no statistically significant difference between the frequencies of chemical, ectopic, and clinical intrauterine pregnancies, for a total of 157 and 198 pregnancies, before and after the implementation of the 2004 law, respectively. Similarly, there were no interperiod differences between the frequencies of singleton, twins, and triplets among the clinical intrauterine pregnancies. The pregnancy rate per patient started (i.e., per aspiration) was similar between the periods. However, the pregnancy rate as well as the births with at least one liveborn baby ("take-home baby") per ET rate were statistically significantly higher after the implementation of the new Italian reproductive legislation.

The comparison of outcomes by maternal age groups (Table 3) indicates that outcomes were practically unchanged before and after the new Italian reproductive legislation.

To evaluate the potential effect of the change of culture media, we compared the outcomes between the first and second years after the law. We did not find any statistically significant differences (data not shown).

DISCUSSION

Practice guidelines are usually issued by census groups of medical authorities and are designed to help clinicians in making the appropriate decision for their patients. Thus, the new Italian infertility rules, issued by legislative authorities, were anticipated to be against the best interest of the infertile women (11, 14). Specifically, the limit of three fertilized oocytes for transfer in each cycle, the obligation to transfer all produced embryos, and the prohibition of cryopreservation of surplus embryos were expected to decrease the likelihood of achieving pregnancy, to increase the number and complexity of procedures per pregnancy, to increase the risk of multiple gestations, and thus to infringe upon basic human rights and the proper application of ART (11). These policies further restricted our protocols by not allowing for tailoring the number of transferred embryos according to our judgment, which had been a priori restricted by the unique clinical environment in our service. Moreover, the prohibition of sperm and oocyte donation also denies fertility to many couples who are in need for gamete donation, and the

TABLE 1

Cycle characteristics before and after the new Italian reproductive legislation.

	Before	After	Statistics
Patients started	900	936	
IVF	484	521	
ICSI	416 (46.2)	415 (44.3)	
Age >38 years	181/900 (20.1)	231/936 (24.7)	0.8 (0.6, 0.9)
IVF	103/484 (21.3)	136/521 (26.1)	0.8 (0.6, 1.0)
ICSI	78/416 (18.8)	95/415 (23.0)	0.8 (0.5, 1.1)
ET cycles (/patients)	791/900 (87.9)	741/936 (79.2)	1.9 (1.5, 2.5)
IVF	404/484 (83.5)	389/521 (74.7)	1.7 (1.2, 2.4)
ICSI	387/416 (93.0)	352/415 (84.8)	2.4 (1.5, 3.9)
Age >38 years (/ET cycles)	154/791 (19.5)	172/741 (23.2)	0.8 (0.6, 1.0)
IVF	88/404 (21.8)	95/388 (24.5)	0.8 (0.6, 1.2)
ICSI	66/387 (17.1)	77/352 (21.9)	0.7 (0.5, 1.1)
Transferred embryos (total)	2484	1705	
IVF	1271	931	
ICSI	1213 (48.8)	774 (45.4)	
ETs/cycle	3.1 ± 1.7	2.2 ± 0.8	$P<.05$
IVF	3.1 ± 1.7	2.2 ± 0.8	$P<.05$
ICSI	3.1 ± 1.7	2.2 ± 0.7	$P<.05$
Good quality embryos (total)	2103/2484 (84.7)	1525/1705 (89.4)	0.6 (0.5, 0.8)
IVF	1058/1271 (83.2)	829/931 (89.0)	0.6 (0.5, 0.8)
ICSI	1045/1213 (86.2)	696/774 (89.9)	0.7 (0.5, 0.9)

Note: Categorical data are presented as n/N (%) and continuous data as mean ± SD. Statistics are shown as OR (95% CI).

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TABLE 2

Outcomes of ART cycles before and after the new Italian reproductive legislation.

	Before	After	Statistics
Total pregnancies	157	198	
IVF	78	111	
ICSI	79	87	
Chemical	34/157 (21.7)	40/198 (20.2)	1.1 (0.6, 1.9)
Ectopic	1/157 (0.6)	6/198 (3.0)	0.2 (0.02, 1.7)
Clinical, intrauterine	122/157 (77.7)	152/198 (76.8)	1.0 (0.6, 1.8)
Singletons (/clinical pregnancies)	75/122 (61.5)	111/152 (73.0)	0.6 (0.3, 1.0)
Singleton births	57/75 (76.0)	86/111 (77.5)	0.9 (0.4, 1.9)
Twins (/clinical pregnancies)	39/122 (32.0)	38/152 (25.0)	1.4 (0.8, 2.5)
Twin births	25/39 (64.1)	27/38 (71.1)	0.7 (0.2, 2.1)
Singleton births	11/39 (28.2)	8/38 (21.1)	1.5 (0.5, 4.8)
Triplets (/clinical pregnancies)	8/122 (6.6)	3/152 (2.0)	3.5 (0.8, 17.0)
Triplet births	4/8 (25.0)	2/3 (66.7)	
Twin births	2/8 (25.0)	—	
Singleton births	1/8 (12.5)	1/3 (33.3)	
Pregnancies/ET cycles	157/791 (19.8)	198/741 (26.7)	0.7 (0.5, 0.9)
Births ≥1 baby/patients started	100/900 (11.1)	124/936 (13.2)	0.8 (0.6, 1.1)
Births ≥1 baby/ET cycles	100/791 (12.6)	124/741 (16.7)	0.7 (0.5, 0.9)
Singleton births	69/100 (69.0)	95/124 (76.7)	0.7 (0.3, 1.3)
Multiple births	31/100 (31.0)	29/124 (23.4)	1.5 (0.8, 2.8)

Note: Categorical data are presented as n/N (%). Statistics are shown as odds ratio (95% confidence interval). Differences between pregnancies and births arise from abortions.

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prohibition of preimplantation diagnosis cannot avoid transfer of genetically malformed embryos in patients with a known genetic disorder.

Our study follows the study of Ragni et al. (11) and our own preliminary observations (12) on the effect of the new Italian law on outcomes of ART cycles. In contrast to the Ragni group, who found that the new legislation did not significantly influenced the rate of success of IVF/ICSI cycles, we found that the limitation of up to three embryo transfers per cycle, irrespective of the patient's age, actually improved the pregnancy per ET rate as well as the take-home baby per ET rate by 30%. Moreover, although the multiple birth rates remain quite high (>20%), the obligation to re-implant all available embryos did not change the multiple birth rate between the two periods. The discrepancy between our current findings and those of Ragni et al. (11) are due to two main differences in study design. First, our data come from a single center whereas Ragni et al. collected data from seven centers with different ovarian stimulation and ART protocols; second, the data in the study of Ragni et al. were collected during a 3-month period whereas our study includes data from 2 years. Thus, the observations of Ragni et al. may represent the "acute" reaction to the imposed changes on Italian infertility centers, but our observations represent the adaptation to the new ART regulations.

The conclusions from our study are of special importance and are not limited to the Italian population. Three observations may apply to a broader perspective. First, our study shows that limiting the number of transferred embryos to three has little effect on the multiple pregnancy rate; specifically, transferring 2.2 ± 0.8 or 3.1 ± 1.7 embryos per cycle makes no difference in terms of the multiple birth rate. Particularly, there was no reduction of triplet births.

The second and intriguing observation is the combination of a significant reduction in ET cycles concomitant with improvement in embryo quality observed after the law implementation. This resulted in more pregnancies per ET cycles and in significantly higher take-home baby rates following the implementation of the new Italian reproductive legislation. The improved outcomes are possibly explained by the change of culture media used in the second period leading to the observed higher quality of the embryos (see Table 1). However, our comparison of outcomes obtained by using the different culture media does not support a significant effect on embryo quality. Alternatively, the need to select only the three best oocytes for insemination may have improved the overall quality of the embryos.

Our data indicate that statistically significantly fewer patients underwent ET in the post-law period; that is, 21% of the patients who started IVF in the post-law period did not continue with ET. This apparently high frequency might be explained by the fact that we perform oocyte retrieval even when ovarian response is inadequate; therefore, we had

TABLE 3

ART results before and after the new Italian reproductive legislation.

	Before	After
Pick-up cycles	900	936
<35 years	421 (46.8)	379 (40.5)
35–37 years	239 (26.6)	258 (27.6)
38–39 years	138 (15.3)	163 (17.4)
40–41 years	102 (11.3)	136 (14.5)
Clinical pregnancies	122/900 (13.6)	152/936 (16.2)
<35 years	65/421 (15.4)	66/379 (17.4)
35–37 years	37/239 (15.5)	52/258 (20.2)
38–39 years	17/138 (12.3)	25/163 (15.3)
40–41 years	3/102 (2.9)	9/136 (6.6)
Births ≥1 baby/ pick-up cycles	100/900 (11.1)	124/936 (13.2)
<35 years	53/421 (12.6)	53/379 (14.0)
35–37 years	31/239 (13.0)	48/258 (18.6)
38–39 years	13/138 (9.4)	19/163 (11.7)
40–41 years	3/102 (2.9)	4/136 (2.9)
Births ≥1 baby/ET	100/791 (12.6)	124/741 (16.7)
<35 years	53/378 (14.0)	53/305 (17.3)
35–37 years	31/210 (14.8)	48/220 (21.8)
38–39 years	13/117 (11.1)	19/116 (16.3)
40–41 years	3/86 (3.5)	4/100 (4.0)

Note: Categorical data are presented as n/N (%).

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more cases with fewer oocytes and/or low quality oocytes that eventually did not result in ET. Moreover, because of the legislative obligation to inseminate no more than three oocytes, we inseminated the "best" oocytes in terms of morphology, but one can never be sure if these are indeed the best that could result in embryos. In simple terms, the obligation to inseminate no more than three oocytes might have caused our high percentage of cases without ET.

Finally, the results for women older than 39 years (see Table 3) show a dramatic decrease in clinical pregnancies and take-home baby rates compared with younger women. This drop in success rates in patients over 39 years is in accord with numerous observations worldwide, but nonetheless reiterates the need for earlier access to ART facilities. This important aspect comes from another Italian law directive (not evaluated in this study), which states that ART should be an end-stage procedure after exhausting all other available non-ART options. For example, according to the law, a 37-year-old infertile woman with endometriosis should first undergo surgical treatment, then allow 6 to 12 months to achieve a spontaneous conception, then attempt 4 to 6 trials of ovulation induction (with or without intrauterine insemination) before she can be admitted for ART. It is possible that such a long interval reduces the overall chance of a given patient to achieve pregnancy; however, it is currently unknown how long the time interval is between the diagnosis of infertility and the first ART cycle and how many patients are penalized by this delay in terms of success rates. It is also unknown how long the interval is between the time when ART is indicated and the first ART cycle, and if this delay in access to ART is related to success rates.

From the results of this study, we are unable to comment about the potential reduction in cumulative pregnancy rates because, as opposed to some of the patients in the study of Ragni et al. (11), we did not perform embryo cryopreservation either before or after the new Italian law. However, the poor results of our oocyte cryopreservation program (18), used routinely after the implementation of the Italian law, suggest that oocyte freezing in its present state is a poor alternative to embryo freezing. Thus, the statutory prevention of embryo freezing and the poor results of oocyte cryopreservation are likely to decrease the cumulative pregnancy rates and to increase the number of ART procedures needed to obtain fresh embryos. This, in turn, is likely to increase the waiting list for access to an ART program, which will further increase maternal age and further decrease the success rates.

It follows that, despite the similar results from the periods 2 years before and after the new Italian law, the long-term effects of the legislation should be carefully monitored. In the meantime, some modifications of the law directed at the older age group should be considered.

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