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ORIGINAL ARTICLE ***Reproductive epidemiology***

Comparison of the obstetric and perinatal outcomes of children conceived from *in vitro* or *in vivo* matured oocytes in *in vitro* maturation treatments with births from conventional ICSI cycles

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STUDY QUESTION: Are the obstetric and perinatal outcomes of deliveries following *in vitro* maturation (IVM) cycles different from births generated from controlled ovarian stimulation (COS) cycles?

SUMMARY ANSWER: The obstetric and perinatal outcomes of births from IVM cycles are comparable with those of ICSI treatments, including the incidence of major and minor abnormalities.

WHAT IS KNOWN ALREADY: Only few and numerically small reports on the health of IVM children are currently available.

STUDY DESIGN, SIZE AND DURATION: Retrospective cohort study involving 196 babies born from IVM cycles carried out with different priming regimens. Of these children, 79 developed from oocytes matured *in vitro* after 30 h of culture, while 104 originated from oocytes found mature and inseminated on the day of recovery. Thirteen babies were obtained from embryos developed from both types of oocytes. Data of these births were compared with those of 194 children born from COS ICSI cycles performed during the same period (March 2004 to December 2011).

PARTICIPANTS/MATERIALS, SETTING AND METHODS: IVM cycles were done in the absence of gonadotrophin administration or with FSH and/or HCG priming. All oocytes were inseminated by microinjection. ICSI and ICSI cycles were chosen as a control group to exclude possible influences of the insemination technique. Couples in which maternal age was >39 years or affected by azoospermia were excluded to rule out major parental effects.

MAIN RESULTS AND THE ROLE OF CHANCE: In single births, gestational age at delivery was comparable, but birthweight was significantly higher ($P = 0.009$) in children from IVM cycles (3091 $\pm$ 669 versus 3269 $\pm$ 619 g). In a separate analysis of the IVM group, comparing singleton births derived with certainty from oocytes matured *in vitro* ($n = 71$) or *in vivo* ($n = 74$), no statistically significant differences were observed in terms of birthweight (3311 $\pm$ 637 versus 3194 $\pm$ 574 g, respectively) and gestational age (38.9 $\pm$ 2.4 versus 38.4 $\pm$ 2.1 weeks, respectively). In twin births, gestational age was lower in IVM cycles, while weight at birth was comparable (ICSI, 2432 $\pm$ 540 g; IVM, 2311 $\pm$ 577 g). In single births, major and minor abnormalities were 2 (1.4%) and 6 (4.1%) in the ICSI group and 0 (0.0%) and 8 (5.2%) in the IVM category, respectively. In twin children, major and minor abnormalities were 1 (2.2%) and 2 (4.3%) in ICSI babies and 0 (0.0%) and 2 (4.6%) in IVM cycles, respectively.

LIMITATIONS AND REASONS FOR CAUTION: The study is the largest conducted so far. Nevertheless, it is limited by its retrospective nature and the fact that most births of IVM treatments derived from oocytes found mature at recovery in cycles primed with HCG. A more comprehensive appraisal of the health status of IVM children will demand larger prospective studies.

WIDER IMPLICATIONS OF THE FINDINGS: The study is consistent with previous reports suggesting a possible role of standard ovarian stimulation in determining a reduced birthweight in children born from COS cycles.

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Key words: oocyte *in vitro* maturation / gestation / birth / birthweight / congenital abnormalities

Introduction

For more than three decades, assisted reproduction technologies (ARTs) have been increasingly used to assist infertile couples and, more recently, women requiring a fertility preservation treatment. This has led to the birth of ~4 million ART children. However, concerns have repeatedly been raised about possible adverse effects that intrinsic and extrinsic influences may exert on the development of the conceptus generated *in vitro*. However, collective evidence suggests that increased risks of obstetric complications and congenital abnormalities observed in IVF births can be largely attributed to inherent parents' characteristics (Romundstad *et al.*, 2008) or the occurrence of twin and higher order pregnancies derived from the transfer *in utero* of multiple embryos. Nevertheless, the possibility that new medical treatments or laboratory manipulations might alter the normal course of pre- and post-natal development cannot be ruled out *a priori*, imposing the necessity of further safety studies. In fully grown germinal vesicle (GV)-stage oocytes recovered from mid-antral follicles, the process of meiotic resumption and progression to the metaphase II (MII) stage can be obtained *in vitro*. Since the early 1990s (Trounson *et al.*, 1994), this approach, which requires minimal or no gonadotrophin administration, has been adopted clinically with the aim of reducing the disadvantages (costs, repeated monitoring) and the risks [namely ovarian hyperstimulation syndrome (OHSS)] of standard ovarian stimulation. *In vitro* maturation (IVM) is a delicate process during which the genetic and cytoplasmic integrity of the female germ cell might be compromised by suboptimal manipulation conditions (Barrett and Albertini, 2007, 2010; Son and Tan, 2010). Fears that IVM could introduce additional hazards in human ART have remained unaddressed, mainly because most of the estimated 2500 births derived from IVM cycles have not been reported in published studies or appropriately described. Only few and numerically small reports on the health of IVM children are currently available (Cha *et al.*, 2000, 2005; Mikkelsen, 2005; Söderström-Anttila *et al.*, 2005; Shu-Chi *et al.*, 2006; Buckett *et al.*, 2007). This lack of knowledge prompted us to assess the obstetric and perinatal outcomes of deliveries achieved in IVM treatments and compare them with births generated from controlled ovarian stimulation (COS) ICSI cycles.

Materials and Methods

Patients

The results of this study concern IVM and ICSI treatments carried out between when IVM was introduced at Biogenesi in March 2004 and December 2011. ICSI cycles were representative of the period of study. Couples had an indication for IVM or ICSI because of infertility due to male factor, tubal factor, stage I/II endometriosis, anovulation or unexplained infertility.

Normo-ovulatory patients potential candidates for IVM treatment were selected according to precise criteria previously established (Fadini *et al.*, 2009a). After informed consent, the choice of these patients to opt for IVM treatment as an alternative to COS was motivated by concerns about possible side effects of gonadotrophin stimulation, significant risk of OHSS or occurrence of OHSS in previous COS cycles.

IVM cycles

A baseline transvaginal ultrasound was carried out on Day 2 or 3 of a menstrual cycle to exclude the presence of ovarian cysts and assess endometrial thickness and antral follicle count. Antral follicles were defined as fluid-filled cavities <12 mm in diameter. A blood sample was also taken on the same day to determine basal estradiol (E_2) and FSH serum concentrations. The inclusion criteria were age <39 years, FSH <10 mIU/ml, E_2 <275 pmol/l and antral follicle count >5. Patients showing an ovarian functional cyst of >12 mm were excluded (Fadini *et al.*, 2009a). Cycles were carried out in the absence of gonadotrophin administration or with FSH and/or HCG priming, as described elsewhere (Fadini *et al.*, 2009a). If applicable, FSH priming involved administration of 150 IU/day for 3 days from Day 3 of cycle. All the women were monitored for follicular growth until a leading follicle of 10–12 mm in diameter and an endometrial thickness of >5 mm were observed. Under those conditions, oocyte retrieval was scheduled to occur within 24 h in non-primed or FSH only primed cycles or after 36–38 h in women primed with HCG (10 000 IU), with or without FSH priming. Maximal serum E_2 levels (mean $\pm$ SD) were 267 $\pm$ 259 pmol/l. Ultrasound-guided oocyte retrieval was performed using a single lumen aspiration needle (Ø17-gauge, 35 cm; Gynetics, Lommel, Belgium) connected to a vacuum pump (80–100 mmHg; Craft Pump, Rocket, Washington, UK). During oocyte collection, all the women received sedation with Propofol (Astra-Zeneca, Basiglio, Italy). Follicular aspirates, containing cumulus–oocyte complexes (COCs) were collected in a single 50-ml tissue culture flask containing 15 ml of pre-warmed flushing medium supplemented with heparin (Origio, Måløv, Denmark).

In vitro *maturation, insemination and embryo culture*

After collection, the follicular fluid was filtered through a 70-μm cell strainer (Becton-Dickinson, Buccinasco, Italy) and the retained COCs were washed twice. COCs were detected under a stereomicroscope. Oocytes with signs of mechanical damage or atresia were discarded. After recovery, COCs were transferred to a single-well petri dish containing 0.5 ml of IVM medium (Vial 2 of IVM system medium; Origio, Måløv, Denmark) supplemented with 0.075 IU/ml recombinant FSH (Merck Serono, Rome, Italy), HCG 0.10 IU/ml (Merck Serono) and 10% maternal

serum inactivated at 56°C or 10% synthetic serum substitute (Origio, Måløv, Denmark). Immature oocytes surrounded by compact cumulus cells were cultured at 37°C in a 5% CO_2 humidified atmosphere for 30 h. Oocytes associated with an expanded cumulus mass were cultured for 3–6 h and again observed under a stereomicroscope using the spreading technique to assess their meiotic stage. GV-stage oocytes were returned to maturation medium leaving intact the cumulus cell vestment. In the absence of a visible GV, COCs were treated with 80 IU/ml hyaluronidase solution (Sage, Pasadena, USA) to remove cumulus cells. Only oocytes showing the polar body 1 were used for insemination on the same day. GV or GV breakdown-stage oocytes recovered after cumulus cell removal were discarded. After 30 h of culture, all the remaining COCs were treated with 80 IU/ml hyaluronidase solution (Sage) to remove cumulus cells. MII-stage oocytes suitable for insemination were selected according to cytoplasmic characteristics such as granularity, presence of vacuoles or smooth endoplasmic reticulum aggregates and presence of dark zona pellucida. Fertilization was exclusively achieved by ICSI to circumvent possible hardening of the zona pellucida caused by IVM conditions, as shown in several experimental models. In addition, removal of cumulus cells to assess meiotic status following IVM makes standard IVF not recommended for *in vitro* matured oocytes. Fertilization was assessed 16–18 h after microinjection and confirmed by the presence of two pronuclei and two polar bodies. All the resulting fertilized eggs were individually placed in microdrops of IVF medium or ISM1 (Origio, Måløv, Denmark). Embryos were cultured until Day 2 or 3.

Endometrial preparation, embryo transfer and perinatal outcome

Under ultrasound scan guidance, embryo transfers were carried out 48 or 72 h after fertilization by using a soft catheter (Semtrac 5-2000 SET—Gynetics, Lommel, Belgium). All the women received oral E_2 haemihydrate supplementation at a dose of 6 mg/day starting on the day of oocyte retrieval. Luteal support was provided by intravaginal progesterone supplementation at 600 mg/day starting one day later. Pregnancy was tested 12–13 days after embryo transfer by quantitative definition of serum β-HCG. In case of pregnancy, estrogen and progesterone supplementation was continued until the 12th week of gestation.

A condition of pre-eclampsia was defined according to the guidelines of the Australasian Society for the Study of Hypertension in Pregnancy (ASSHP), a system capable of detecting both mild (isolated hypertension after 20 weeks) and severe (hypertension associated with one or more several other variables) forms of this pathology.

Diagnosis of congenital malformations at birth was performed on the basis of an investigation scheme designed by an especially trained neonatologist.

ICSI cycles

ICSI, but not standard IVF, cycles were chosen as a control group to limit possible influences of the insemination technique (microinjection), which might influence perinatal outcome *per se*, although marginally. Couples in whom maternal age was >39 or the male partner was affected by azoospermia were excluded, to rule out major parental effects. In ICSI cycles, pituitary down-regulation was achieved by a gonadotrophin-releasing hormone agonist long protocol (Decapeptyl 3.75 mg or 0.1 mg, Ipsen, Italy). Ovarian stimulation was carried out with rFSH (Merck Serono or Schering Plough, Italy), tailoring doses and duration of stimulation according to patient typology (Fadini *et al.*, 2009b). Final oocyte maturation was triggered with 10 000 IU human HCG (Merck Serono). Maximal serum E_2 levels (mean ± SD) were 1408 ± 906 pmol/l ($P < 0.0001$ in comparison with IVM cycles). Oocyte retrieval was performed 36–38 h after HCG administration. Oocyte manipulation to achieve fertilization, embryo culture and embryo transfer were conducted as described earlier.

Statistics

Absolute and percentage frequencies were used to describe categorical items of women and babies, while mean values and standard deviation were used for continuous characteristics. Student's *t*-test and Fisher's exact test were used to analyse the differences between ICSI and IVM data. Analysis of covariance was used to evaluate differences between treatments adjusted for differences between groups with respect to possible confounding variables such as maternal age, gestational age and gender. The Stata 9.0 (Stata Corporation, College Station, TX, USA) software was used to perform statistical analysis and a level of $P < 0.05$ was adopted for significance.

Results

From our IVM programme, 196 babies (153 single and 43 twin) were born during the period of study. In a twin pregnancy, one fetus affected by trisomy 21 was aborted. Of these children, 79 (71 singletons and 8 twins, 75 cycles) developed from oocytes matured *in vitro* after 30 h of culture, while 104 (74 singletons and 30 twins, 89 cycles) originated from oocytes found mature and inseminated on the day of recovery. Thirteen babies (8 singletons and 5 twins, 11 cycles) were obtained from transfers in which embryos derived from both types of oocytes were replaced. Classification of children according to type of priming showed that 23 (all singletons) were born from cycles performed without priming, 27 (19 singletons and 8 twins) from FSH-primed cycles, 15 (14 singletons and 1 from a twin pregnancy) from HCG-primed cycles and 131 (97 singletons and 34 twins) from FSH/HCG-primed cycles. Obstetric and perinatal outcomes of these births were compared with those of 194 children (148 single and 46 twin) born from COS ICSI cycles performed during the same period. Data from single and twin births were analysed separately.

Prior to treatment, the proportion of nulliparous women was 98.7 and 70.4% in the IVM and ICSI groups, respectively ($P < 0.0001$). Duration of infertility was comparable (3.0 ± 1.0 versus 3.1 ± 1.2 years in IVM and ICSI couples, respectively). Other women's characteristics are described in Tables I and II. In single births, maternal age (mean ± SD) was significantly higher in ICSI cycles (34.8 ± 3.0 versus 33.3 ± 3.2, $P < 0.0001$). This was not unexpected because a relatively younger age is one of the selection criteria of IVM patients in our programme. Before pregnancy, the BMI of ICSI women was moderately higher, but not significantly different at delivery. A polycystic ovary syndrome (PCO/PCOS) condition was more common ($P = 0.001$) in the IVM group (8/148, 5.4% versus 27/153, 17.7%). No further differences were found among other criteria of comparison.

In twin births, maternal age and BMI (prior to pregnancy and at delivery) were not significantly different. Similar to single births, PCO patients were more numerous in IVM cycles (1/23, 4.3% versus 4/22, 18.2%, 18.6% versus; $P = 0.035$). Other parameters were comparable between ICSI and IVM patients.

Table III summarizes the characteristics of ICSI and IVM children. In single births, gestational age at delivery was comparable, but birthweight was significantly higher ($P = 0.009$) in IVM children (3091 ± 669 versus 3269 ± 619 g). Such a difference was still present after adjusting data for maternal age, gestational age and gender by analysis of covariance. In addition, further analysis comparing the study babies with naturally conceived children born in North-East Italy (SMILA

Table I Age, BMI (pre- and post-delivery) and PCO/PCOS condition of women delivering single or twin children in ICSI and IVM cycles.

	Single			Twin		
	ICSI ($n = 148$)	IVM ($n = 153$)	*P*-value	ICSI ($n = 23$)	IVM ($n = 22$)	*P*-value
Maternal age (years)	34.8 ± 3.0	33.3 ± 3.2	<0.0001	35.2 ± 2.7	35.4 ± 3.1	NS
BMI pre- pregnancy (kg/m^2)	22.3 ± 3.8	21.6 ± 3.0	0.047	22.1 ± 2.3	22.2 ± 3.2	NS
BMI at delivery (kg/m^2)	27.1 ± 4.0	26.4 ± 3.3	NS	27.3 ± 2.8	27.6 ± 3.7	NS
PCO/PCOS	8 (5.4%)	27 (17.7%)	0.001	1 (4.3%)	4 (18.2%)	0.038

P-values from Student's *t*-test (continuous variables) or Fisher's exact test (proportions).

Table II Characteristics, present before or manifested during pregnancy, of women delivering single or twin children in ICSI and IVM cycles.

	Single			Twin		
	ICSI ($n = 148$)	IVM ($n = 153$)	*P*-value	ICSI ($n = 23$)	IVM ($n = 22$)	*P*-value
Smoking	4 (2.7%)	9 (5.9%)	NS	0 (0%)	0 (0%)	NS
Drugs	0 (0%)	0 (0%)	NS	0 (0%)	0 (0%)	NS
Pre-existing hypertension	1 (0.7%)	0 (0%)	NS	0 (0%)	0 (0%)	NS
Pre-gestational diabetes	1 (0.7%)	0 (0%)	NS	0 (0%)	0 (0%)	NS
Uterine cavity abnormalities	0 (0%)	1 (0.7%)	NS	0 (0%)	0 (0%)	NS
Urinary tract infections	2 (1.4%)	7 (4.6%)	NS	3 (13.0%)	6 (27.3%)	NS
Pre-eclampsia	1 (0.7%)	5 (3.3%)	NS	1 (4.4%)	0 (0%)	NS
Gestational diabetes	6 (4.1%)	9 (5.6%)	NS	0 (0%)	0 (0%)	NS
Placental abruption	0 (0%)	0 (0%)	NS	0 (0%)	1 (4.5%)	NS

P-values from Fisher's exact test.

Neonatal Standards for North-East Italy, 1996) adjusted for gender revealed overall different proportions of small, appropriate and large for gestational age babies in IVM and ICSI cycles (Table IV). However, comparing singleton IVM births derived with certainty from oocytes matured *in vitro* ($n = 71$) or *in vivo* ($n = 74$), no statistically significant differences were observed in terms of birthweight (3311 ± 637 versus 3194 ± 574 g, respectively), gestational age (38.9 ± 2.4 versus 38.4 ± 2.1 weeks, respectively) and minor abnormalities (4.2 versus 6.7%, respectively; no major abnormalities were found in children from IVM cycles, as described below). By analysing collectively all single births irrespective of the type of treatment, the weight of babies from PCO and non-PCO patients was not significantly different (3059 ± 703 versus 3198 ± 640 g, respectively). However, a lower weight at birth was found in PCO cases in the ICSI (2478 ± 850 versus 3127 ± 644, $P = 0.004$), but not in the IVM (3231 ± 564 versus 3277 ± 628, $P =$ ns), group (data not shown in table).

In twin births, gestational age was lower in IVM cycles (37.0 ± 2.6 versus 35.3 ± 2.7 weeks, $P = 0.02$), while weight at birth was comparable (ICSI, 2432 ± 540 g; IVM, 2311 ± 577 g). Analysis of twin IVM births on the basis of the type of oocyte (i.e. matured *in vitro* or *in vivo*) was not carried out because the resulting subgroups were too small. The birthweight of twin babies from all PCO patients was not different from that of children of non-PCO mothers (2476 ± 197 and 2360 ± 588 g, respectively). Separate analysis for the two treatment typologies indicated no birthweight difference in PCO and non-PCO twin births, both in IVM (2451 ± 216 versus 2279 ± 629 g, respectively) and, unlike single deliveries, ICSI (2575 ± 21 versus 2425 ± 552 g, respectively) cycles (data not shown in table).

Other perinatal characteristics were largely similar in ICSI and IVM children. In particular, in ICSI and IVM single births, values of Apgar at 1 min (mean ± SD) were 9.1 ± 0.8 and 9.1 ± 1.1, respectively. Major and minor abnormalities (Table V) were 2 (1.4%) and 6 (4.1%) in the ICSI group and 0 (0.0%) and 8 (5.2%) in the IVM category, respectively.

In twin children (Table III), a lower value of Apgar at 1 min was found in the IVM group (9.0 ± 1.1 versus 8.3 ± 2.0, $P = 0.02$). Major and minor abnormalities (Table V) were 1 (2.2%) and 2 (4.3%) in ICSI babies and 0 (0.0%) and 2 (4.6%) in IVM births, respectively.

Conclusions

In IVM cycles, oocytes are retrieved from antral follicles that have not developed to the pre-ovulatory stage and usually require maturation in extra-corporeal conditions. This has generated safety concerns about

Table III Characteristics at birth of single and twin children born from ICSI and IVM treatments.

	Single			Twin		
	ICSI (n = 148)	IVM (n = 153)	*P*-value	ICSI (n = 46)	IVM (n = 43)	*P*-value
Gender						
M	65 (43.9%)	74 (48.4%)	NS	22 (47.8%)	24 (55.8%)	NS
F	83 (56.1%)	79 (51.6%)		24 (52.2%)	19 (44.2%)	
Birthweight (g)	3091 ± 669	3269 ± 616	0.009	2432 ± 540	2311 ± 577	NS
Birthweight (g)						
>2500	132 (89.2%)	142 (92.8%)	NS	25 (54.5%)	16 (37.2%)	NS
1500–2500	12 (8.1%)	8 (5.2%)		18 (39.1%)	24 (55.8%)	
≤1500	4 (2.7%)	3 (2.0%)		3 (6.5%)	3 (7.0%)	
Gestational age (weeks)	38.5 ± 2.5	38.6 ± 2.3	NS	37.0 ± 2.6	35.3 ± 2.7	0.02
Preterm births	21 (14.2%)	26 (17.0%)	NS	10 (43.5%)	15 (68.2%)	NS
Delivery						
Vaginal	95 (64.2%)	99 (64.7%)	NS	3 (13.0%)	5 (22.7%)	NS
Caesarean	53 (35.8%)	54 (35.3%)		20 (87.0%)	17 (77.3%)	
Apgar 1	9.1 ± 0.8	9.1 ± 1.1	NS	9.0 ± 1.1	8.3 ± 2.0	0.02
Apgar 5	9.9 ± 0.4	9.9 ± 0.5	NS	9.7 ± 0.7	9.6 ± 0.7	NS
Intracranial hemorrhage	1 (0.7%)	0 (0%)	NS	2 (4.3%)	1 (2.3%)	NS
Seizures	0 (0%)	0 (0%)	NS	0 (0%)	0 (0%)	NS
Sepsis	0 (0%)	0 (0%)	NS	0 (0%)	0 (0%)	NS
Respiratory distress syndrome	1 (0.7%)	1 (0.7%)	NS	3 (6.5%)	2 (4.5%)	NS
Mechanical ventilation	3 (2.0%)	2 (1.3%)	NS	3 (6.5%)	6 (14.0%)	NS
Condition of infant at time of delivery						
Live	148 (100.0%)	153 (100.0%)	NS	46 (100.0%)	43 (100.0%)	NS

In IVM cycles, children born from oocytes matured *in vitro* or *in vivo* were considered as a single group. Separate analysis of such IVM categories is reported in the 'Results' section. Comparison of gestational age between ICSI and IVM children was made by adopting $n = 23$ and $n = 22$ as the number of deliveries, respectively, because such a parameter is not an independent observation in each couple of twins. *P*-values are from Student's *t*-test for continuopus variables and Fisher's exact test for proportions.

Table IV Proportion of small, appropriate and large for gestational age singleton children in IVM and ICSI groups ($P = 0.004$, Fisher's exact test).

Size for gestational age	ICSI (n = 148)	IVM (n = 153)
Small	17 (11.5%)	4 (2.6%)
Appropriate	114 (77.0%)	122 (79.7%)
Large	17 (11.5%)	27 (17.7%)

Data were adjusted for gender and banded according to values based on naturally conceived children born in North-East Italy (SMILA Neonatal Standards for North-East Italy, 1996).

the health status of the conceptus, based on the credence that insufficient growth *in vivo* and/or maturation *in vitro* may compromise developmental mechanisms. Data on IVM babies are scant. The largest study published so far on this subject involved 55 IVM, 217 IVF and 160 ICSI babies (Buckett *et al.*, 2007). In these births, odds ratio risks of congenital abnormalities in comparison with spontaneously conceived children were 1.42, 1.21 and 1.69, respectively. Other studies published on IVM children included even smaller numbers (Table VI), overall indicating good perinatal health status, although with sporadic cases of major and minor abnormalities (Cha *et al.*, 2000, 2005; Mikkelsen, 2005; Söderström-Anttila *et al.*, 2006; Shu-Chi *et al.*, 2006). In particular, in 17 pregnancies of PCOS women who gave rise to 20 children, Cha *et al.* (2000) reported no premature deliveries or congenital abnormalities. The mean birthweight of these children was 3.0 ± 0.4 kg, but single and twin pregnancies were not analysed separately. Subsequently, Cha *et al.* (2005) described another series of 24 births, reporting mean birthweights of 3252 ± 516 and 2361 ± 304 g in single and twin pregnancies, respectively, and two major congenital anomalies (5.3%). However, it is not clear whether the two sets of data were at least partially overlapped, the period of the first study (1995–1998) being included in that of the second (1995–2001). Mikkelsen (2005) reported 47 births (45 singletons and 2 twins) from IVM cycles. Preterm delivery affected one singleton and one twin pregnancy. Median weight was 3720 g, while possible congenital anomalies were not described. In 40 singletons and 3 sets of twins, Söderström-Anttila *et al.* (2006) observed normal birthweight (3550 ± 441 and 2622 ± 194 g in singletons and twins, respectively), a rate of 5% of pre-term births in singletons, good overall perinatal outcome and normal neuropsychological development at 2 years of age. Finally, Shu-Chi *et al.* (2006) compared 21 IVM children (17 singletons and two sets of twins) of PCOS mothers with an equivalent

Table V Major and minor birth defects in single and twin children born from ICSI and IVM treatments.

	Single			Twin		
	ICSI (n = 148)	IVM (n = 153)	P-value	ICSI (n = 46)	IVM (n = 43)	P-value
Major birth defects	Intestinal malformations with multiple intestinal obstruction (1)	No defects detected	NS	Multiple defects (1)	No defects detected	NS
	Thyroid agenesis (1)					
Minor birth defects	Club foot (4)	Wolff Parkinson White syndrome (1)	NS	Club foot (1)	Left pielectasy (1)	NS
	Hypospadias (1)	Left pielectasy (1)		Two blood vessels in umbilical cord (1)	Polydactyly (1)	
	Dextroversion (isolated dextrocardia with situs solitus) (1)	Labiopalatoschisis associated with hypospadias (1)				
		Sinus pilonidalis (1)				
		Choanal stenosis (1)				
		Controlateral cryptorchidism (1)				
		Club foot (1)				
		Leg pseudoarthrosis (1)				

Numbers of cases are indicated in brackets. In the twin ICSI group, the case of multiple defects involved pulmonary bronchodysplasia, hypothyroidism, bilateral kidney hypoplasia, skeletal dysplasia, encephalopathy and metabolic disease.

Table VI Birthweight, major congenital abnormalities and pre-term births in children born from IVM treatments and derived from previously published studies.

	Total number of children (twins)		Singletons birthweight (g)		Major congenital abnormalities (%)		Preterm deliveries	
	IVM	Control	IVM	Control	IVM	Control	IVM	Control
Cha *et al.* (2005)	24 (4)	n.a.	3252 ± 516	n.a.	2 (8.3)	n.a.	3 (13.6)	n.a.
Mikkelsen (2005)	47 (2)	n.a.	3720	n.a.	0 (0)	n.a.	2 (4.4)	n.a.
Söderström-Anttila *et al.* (2006)	46 (6)	n.a.	3550 ± 441	n.a	0 (0)	n.a.	4 (9.3)	n.a.
Shu-Chi *et al.* (2006)	21 (4)	21 (0)	3074 ± 488	3133 ± 287	0 (0)	0 (0)	2 (9.5)	n.a.

number of spontaneously conceived babies. Gestational age at birth and birthweight were comparable between the IVM and the control groups. Likewise, mental and psycho-motor development index scores assessed between 6 and 24 months were not different. To contribute to the knowledge base in this field, we performed the present study in which the obstetric and perinatal outcomes of IVM cycles were assessed and compared with those of ICSI treatments. The study is limited by its retrospective nature and the fact that most births of IVM treatments derived from oocytes found mature at recovery in cycles primed with HCG. Nevertheless, our analysis, the largest conducted so far, collectively suggests that the health of IVM and that of ICSI babies are comparable. In particular, in all births from IVM cycles, the frequency of all congenital abnormalities, 0% major and 5.1% minor, was not superior to that of the ICSI group and lower than the one (11.4%) reported in naturally conceived children in Italy (Boldrini *et al.*, 2011). A significant aspect of our findings concerns the birthweight of IVM babies, which was on average 178 g higher than that of the ICSI children. However, in a separate analysis of IVM cycles, comparing singleton births derived with certainty from oocytes matured *in vitro* or *in vivo*, no statistically significant differences were observed in terms of birthweight and gestational age. Comparing IVM and ICSI babies, Buckett *et al.* (2007) did not report difference in weight, perhaps as an effect of diverse patient population or sample size. The weight difference emerging from our data was independent of women age, duration of gestation and gender, and was not explained by a different condition of parity. Therefore, it is possible that this is an effect of treatment difference. Studies in animal models have suggested that under certain conditions IVM may somehow influence the health of the conceptus and the developing pregnancy. Especially in the cow, pregnancies from *in vitro* matured oocytes and *in vitro* produced embryos may be affected by increased fetal and placental weight, or aberrant development of fetal skeletal muscles and placental vessels (Farin *et al.*, 2006). It is suspected that these manifestations derive from deregulation of imprinted genes controlling fetal and placental growth, caused by inappropriate culture conditions during oocyte maturation or embryo development (Li, 2002). Therefore, IVM births should be thoroughly investigated to rule out an increased incidence of imprinting disorders. However,

experiments in the mouse question the hypothesis that oocyte IVM can perturb the conceptus developmental plan, as indicated by the fact that the long-term health of mice generated from *in vitro* matured oocytes is virtually unaffected (Eppig *et al.*, 2008). With regard to the babies born from single pregnancies of our IVM programme, some context needs to be considered before a relatively higher birthweight is a health concern, without overlooking the possibility of deregulation of imprinting mechanisms. Firstly, our study was still small and retrospective. It should also be considered that there is very well-established literature to suggest that ART babies are born lighter than naturally conceived babies. Secondly, the difference in birthweight between single IVM and ICSI babies may not represent a perturbation of IVM conditions on fetal development, but rather an undesirable influence of conventional ovarian stimulation on perinatal outcome. In fact, differences in the ICSI and the IVM approaches may be found not only in the diverse modalities by which oocyte maturation is achieved, but also in ovarian stimulation regimes, involving pituitary down-regulation and intense gonadotrophin stimulation in ICSI cycles and no or minimal use of gonadotrophins in IVM treatments. A retrospective study of Pelinck *et al.* (2010) seems to point towards an effect of standard ovarian stimulation on perinatal outcome. Similar to the present case, by comparing single births from modified natural cycle IVF and COS IVF, it was found that the birthweight of natural cycle children was higher of 134 g, despite no differences in pregnancy duration, proportion of prematurity and rate of low birthweight. Also, Pelinck and colleagues reported that the birthweight of natural cycle IVF children was comparable with that of naturally conceived children. If and how COS could influence birthweight in an ART cycle remains a matter of speculation. A large analysis based on over 32 000 singleton IVF births offers evidence that maternal weight, maternal height and duration of infertility, but not intensity and duration of ovarian stimulation, affect birthweight (Griesinger *et al.*, 2008). These data, together with the analysis of Romundstad *et al.* (2008) involving the health of sibling ART and naturally conceived children, reassure the safety of ovarian stimulation. However, a detrimental effect of ovarian stimulation regimens might be associated with high estrogen levels derived from the growth of multiple large follicles, as suggested by the observation of a negative correlation between the concentrations of this hormone and birthweight in ART treatments (Mitwally *et al.*, 2006). In this respect, it is important to note that in IVM cycles, estrogen levels remain within the physiological range irrespective of a possible FSH priming (Lin, 2003), a circumstance confirmed also by our data. Interestingly, in the present study, the birthweight of children of PCO/PCOS patients was lower in ICSI cycles in comparison with patients with morphologically normal ovaries. This is in agreement with the assumption that high estrogen levels, which occur frequently in stimulated cycles of PCO/PCOS patients, affect the perinatal outcomes of ART children. Such a difference in birthweight was not observed in our IVM cycles, in which also PCO/PCOS patients do not experience increased estrogen levels (Lin, 2003).

Assuming that a hyperestrogenic condition is detrimental to the perinatal outcomes of stimulated IVF cycles, it is not clear whether this influence is exerted directly on oocyte quality or through an indirect effect on endometrial receptivity. Experiences gained with frozen embryo replacements are suggestive of the latter hypothesis. In fact, it has been reported that the birthweight of children obtained from fresh embryo replacements is lower in comparison with frozen embryo transfers (Wennerholm *et al.*, 2009), in which the endometrium is not exposed to estrogen levels comparable with those generated in stimulated cycles involving several follicles. In principle, GnRH analogues, used to achieve pituitary down-regulation, might also interfere with endometrial receptivity, as suggested by the expression of GnRH receptors in endometrial cells (Cheng and Leung, 2005). However, a study of Klemmt *et al.* (2009) is not consistent with this hypothesis, in the light of *in vitro* experiments in which GnRH analogues were unable to affect endometrial decidualization and blastocyst invasion of endometrial cells. The entire question of a possible detrimental influence of stimulation regimens on endometrial receptivity remains complex, controversial and far from being resolved, warranting further investigation.

In summary, this study indicates that the obstetric and perinatal outcomes of births from IVM cycles are comparable with those of ICSI treatments, including the incidence of major and minor abnormalities. Our evidence is also consistent with previous reports suggesting a possible role of standard ovarian stimulation in determining a reduced birthweight in children born from COS cycles. A more comprehensive appraisal of the health status of IVM children will demand larger prospective studies, especially to rule out that the different birthweight of IVM children is originated by imprinting disorders.

Authors' roles

R.F.: coordination, study design, clinical tasks and critical discussion. M.M.: study design, clinical tasks and critical discussion. T.G.: clinical tasks and critical discussion. M.D.C.: study design, laboratory tasks and critical discussion. E.D.P.: statistics, study design and critical discussion. A.S.: statistics, study design and critical discussion. M.S.: statistics, study design and critical discussion. R.C.: clinical tasks and critical discussion. G.C.: coordination, study design, manuscript drafting and critical discussion.

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Conflict of interest

None declared.

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