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Comparative birth weights of singletons born after assisted reproduction and natural conception in previously infertile women

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BACKGROUND: The possible interference of assisted reproduction techniques (ART) with epigenetic reprogramming during early embryo development has recently sparked renewed interest about the reported lower birth weight among infants born as a consequence of infertility treatments. However, the latter finding so far has relied on the comparison of the birth weight of infants conceived with ART to general population data. A more appropriate comparison group should involve pregnancies in infertile women after natural conception. Therefore, we compared neonatal birth weight data of infants born after various ART treatments, including intrauterine insemination (IUI), with those of previously infertile women achieving pregnancy after sexual intercourse. METHODS: Between August 1996 and March 2004 the data of all infertile women presenting in the infertility unit of the University Women's Hospital of Basel, Switzerland, were collected prospectively, adding up to 995 intact pregnancies and deliveries. The birth weight of all infants resulting from 741 singleton pregnancies were analysed with regard to the patients' characteristics, the occurrence of complications during pregnancy and the type of infertility treatment with which the pregnancies were achieved. RESULTS: Comparison of duration of pregnancy and birth weight of infants born after infertility treatment confirms a shorter pregnancy span and a lower mean birth weight in infants born after IVF and ICSI. If women with pregnancies after ART deliver before term, neonatal birth weight is significantly lower. CONCLUSIONS: There is a specific effect of ART, mainly IVF and ICSI, on both shortening the duration of pregnancy and lowering neonatal birth weight. Both these parameters seem to be interrelated consequences of some modification in the gestational process induced by the infertility treatment. Freezing and thawing of oocytes in the pronucleate stage had a lesser impact on pregnancy span and on neonatal birth weight.

Key words: Cryopreservation/ICSI/IVF/parental genomic imprinting/vanishing twin

Introduction

The birth weight of infants conceived with either IVF or ICSI has been reported to be significantly lower than that of those conceived naturally, not only in multiple pregnancies but also in singleton pregnancies (Doyle *et al.*, 1992; D'Souza *et al.*, 1997; Isaksson and Tiitinen 1998; Hansen *et al.*, 2002; Schieve *et al.*, 2002; Katalinic *et al.*, 2004). The conclusions of these analyses were based on comparison with population-based delivery registers or with deliveries after natural conception during the same time interval. However, pregnancies in previously infertile women are characterized by a higher incidence of complications, particularly preterm delivery (Tan *et al.*, 1992; Rufat *et al.*, 1994; De Geyter, 1994; Wang *et al.*, 2002; Thomson *et al.*, 2005), which may result in an overestimation of the detrimental effects of IVF and ICSI on neonatal birth weight. Therefore, the birth weight of infants resulting from pregnancies achieved by natural conception in women previously suffering from infertility should constitute a better comparison group than overall population-based data.

Exact information about differences in neonatal infants' birth weight after various methods of infertility treatment are important in the light of the current discussion about abnormalities in parental genomic imprinting during assisted reproduction (Gosden *et al.*, 2003; Niemitz and Feinberg, 2004). This is exemplified by an unexpectedly high number of cases with Beckwith–Wiedemann's syndrome (De Baun *et al.*, 2003) and Angelman's syndrome (Cox *et al.*, 2002; Ørstavik *et al.*, 2003), both conditions linked to epigenetic errors, among children conceived with assisted reproductive techniques (ARTs). Based on evidence from animal models, the lower birth weight of the offspring may be caused either by the altered maternal endocrine environment due to the hormonal treatment preceding ART (Ertzeid and Storeng, 1992) or by the culture conditions during manipulation of the gametes and embryos (Khosla *et al.*, 2001;

Mann *et al.*, 2004). More recently, ICSI has been implicated in the slower growth of early embryos (Dumoulin *et al*., 2005).

The present analysis utilizes a data set of infertile couples presenting in a single infertility unit. Between August 1996 and March 2004 all details about each pregnancy and delivery were collected prospectively, including those couples achieving pregnancy naturally. The aim was to determine whether birth weight differences occur in neonatal infants born after various methods of assisted reproduction. In contrast to most earlier studies, the birth weight of neonatal infants born after sexual intercourse in women with a history of clinical infertility was used for comparison.

Material and methods

Between August 1996 and March 2004 the data for each individual infertile couple presenting in the Division of Gynecological Endocrinology and Reproductive Medicine at the University Women's Hospital of Basel, Switzerland, were entered prospectively in a locally made database (FertiMed; P. Belloni, Grellingen, Switzerland). In addition, details of all pregnancies including those induced by various ARTs and those occurring in previously infertile couples during natural conception, were recorded prospectively. Information about incident complications during pregnancy and details about the deliveries and the neonates were collected in each individual case by writing a letter to the referring gynaecologist and/or by repeated screening of the delivery list of the University Women's Hospital of Basel. The computer programme allowed continuous follow-up of all cases and the recording of the incoming information. Formal permission from our local ethics committee was obtained for the collection of these data.

Infertility was defined as not having achieved pregnancy during at least 1 year of unprotected intercourse. The duration of infertility was known in all couples. Natural conception was defined as having achieved pregnancy with the use of neither insemination nor any other form of ART. Although the majority of all pregnancies in this group arose without any medical intervention, some couples received medication to normalize ovarian function, including prolactin-lowering agents (2.7%), l-thyroxin for subclinical hypothyroidism (3.6%), clomiphene citrate (1.6%) or metformin (1.8%), according to the individual needs of each patient. Some couples (14.7%) were treated at some time with antibiotics, usually tetracyclines, to cure various genital infections, such as *Ureaplasma urealyticum*, *Mycoplasma hominis* or *Chlamydia trachomatis*. Other couples achieved pregnancy after surgical repair of tubal pathology (1.6%) or after removal of uterine polyps (2.0%). None of the couples achieving pregnancy after sexual intercourse were treated with gonadotrophins during their conception cycle.

All couples treated with intrauterine insemination (IUI) received exogenous gonadotrophins in order to stimulate follicular growth and to induce ovulation according to established protocols described in detail elsewhere (De Geyter *et al.*, 1996). Recombinant FSH was used in 86.5% of all treatment cycles with IUI, urinary menopausal gonadotrophins in the remainder. In 46% of all cycles treated with IUI more than three follicles developed and supernumerary follicles were aspirated by transvaginal, ultrasound-guided aspiration for the prevention of multiple pregnancies (De Geyter *et al.*, 1996, 1998).

IVF and ICSI were exclusively performed after controlled ovarian hyperstimulation using either long-term down-regulation (90.6% of all deliveries after IVF or ICSI) with a single dose of a long-acting GnRH agonist (triptorelin acetate; Decapeptyl Retard, Ferring, Wallisellen, Switzerland) or using a multiple-dose GnRH antagonist (ganirelix; Orgalutran, Organon, Pfäffikon, Switzerland) approach (9.4% of all deliveries after IVF or ICSI). Urinary menopausal gonadotrophins were used for controlled ovarian hyperstimulation in 56.2% of all treatment cycles with IVF or ICSI leading to pregnancy and delivery, whereas recombinant FSH was used in the others. Oocytes and embryos were cultured under paraffin oil in a commercially available culture medium supplemented with human serum albumin (Universal IVF-Medium with phenol red; MediCult, Berlin, Germany). Embryo transfer into the uterine cavity of the recipient was always performed within 48 h after oocyte collection or after thawing of the oocytes previously stored frozen at the pronucleate stage.

The Swiss legislation regulating ART stipulates that not more than three embryos are to be replaced in one single treatment cycle and that all supernumerary oocytes in progression to fertilization are to be stored frozen at the pronucleate stage (Senn *et al.*, 2000). Therefore, freezing and thawing were exclusively done in that particular stage of development. Freezing of pronucleate oocytes was performed in a computerized open freezing system (CTE920; Kryotechnik Erlangen, Erlangen, Germany) following a slow-freezing protocol using dimethyl sulphoxide (DMSO) and propanediol as cryoprotectants in varying concentrations. Thawing was performed using a rapid thawing protocol. Replacement of embryos resulting from the thawing of pronucleate oocytes was usually performed after treatment with clomiphene citrate (84.4% of all deliveries after freezing and thawing). In women with anovulatory cycles refractory to clomiphene citrate, the endometrium was prepared with estradiol valerate and secretory transformation was achieved with both estradiol valerate and vaginal micronized progesterone (Utrogestan; Laboratoire Golaz, Fribourg, Switzerland).

The level of statistical significance was calculated by χ^2 analysis and analysis of variance (ANOVA) as indicated. Neonatal birth weight was distributed normally, whereas differences in the duration of pregnancy expressed in weeks were analysed non-parametrically. The level of statistical significance was set at 5% and the level of sufficient statistical power at 70%. Statistical analysis was performed with Statgraphics 5 Plus (Manugistics, Rockville MD, USA).

Results

A total of 995 deliveries of previously infertile women were recorded during the entire observation period. Among the couples achieving pregnancy after sexual intercourse, the data of 19 deliveries were missing (1.9%) and in 18 cases information about the course of the pregnancy was incomplete (1.8%). These cases were omitted from further analysis. The pregnancy and delivery data of all treatments with IUI, IVF, ICSI and freezing/thawing were complete.

In order to examine the exclusive effect of various conception methods on birth weight, all possible factors interfering with the aim of the study, such as multiple pregnancies or all infants' major malformations, were excluded from further analysis. Therefore, data of 125 twin (12.6%) and eight triplet pregnancies (0.8%) were excluded as well as a neonatal death case and seven cases of infants with major malformations (0.7%). From the initial 995 deliveries, the data of 817 could be analysed (82.1%) and are presented in Figure 1. Obviously, the neonatal birth weight depends to a large extent on the duration of the pregnancy. Therefore, in order to be able to analyse homogeneous cohorts of data, variance checks of the birth weights after sexual intercourse and those after assisted reproduction, including IUI, IVF, ICSI and freezing/thawing (jointly denominated 'ART'), using Bartlett's test were performed. Based upon the variance check results, only the data of the

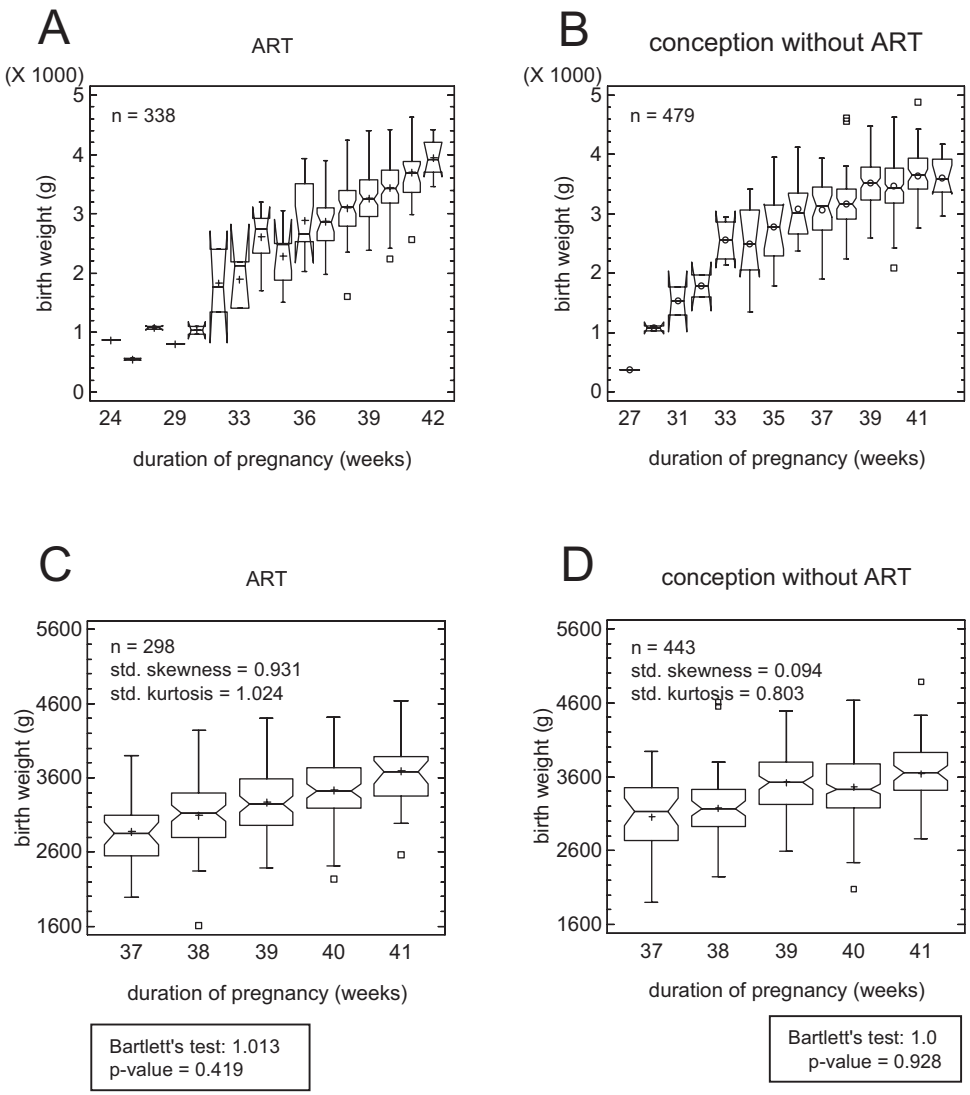

Figure 1. Box-and-whisker plots of infant birth weight (A) in 338 singleton deliveries arising from IUI, IVF, ICSI and freezing/thawing of pronucleate oocytes (jointly designated 'ART'), (B) in 479 singleton deliveries arising from natural conception following sexual intercourse (designated 'no ART'), (C) in 298 selected singleton deliveries after ART and (D) 443 singleton deliveries after no ART. The length of each box represents the interquartile range, both boxes together representing 50% of the data. The whiskers represent the outer 1.5 interquartile range between the upper and lower quartiles. The median is visualized as a notch in the box, the mean by the symbol +, whereas outliers outside the 1.5 interquartile range are presented by the symbol □. The normal distribution of neonatal birth weight data is given by standardized skewness and standardized kurtosis within the range of –2 to +2 (C and D) The selection of delivery data from week 37 to week 41 is motivated by the homogeneity of the values, as given by a variance check using Bartlett's test and the need to exclude complications of pregnancy-related confounders of neonatal birth weight.

pregnancy weeks 37–41 were considered suitable for further analysis (Figure 1). Further analysis could thus be performed with the data of 741 deliveries: 443 (59.8%) after natural conception with sexual intercourse and 298 (40.2%) after ART. Singleton neonatal birth weight after sexual intercourse and natural conception was significantly higher than after IVF, ICSI or after freezing and thawing of oocytes in the pronucleate stage between the 37th and 39th weeks of pregnancy ($P < 0.01$) but not at term (Figure 2).

The roles of various factors that were thought to potentially influence the newborn infants' birth weight and the duration of pregnancy are presented in Table I. We found the following determinants to influence neonatal birth weight significantly in singleton pregnancies at term: the presence or absence of a previous pregnancy ($P < 0.0001$), intrauterine growth retardation as diagnosed during the course of the pregnancy ($P < 0.0001$) and hypertensive disease during pregnancy ($P < 0.0001$). The presence of a vanishing twin during early pregnancy and of a placenta praevia also determined the infant's weight at birth ($P < 0.05$). Delivery was more likely to be preterm in the presence of various factors: a vanishing twin during early pregnancy ($P < 0.01$), fetal growth retardation ($P < 0.01$), placenta praevia ($P < 0.01$) and premature rupture of the membranes ($P < 0.02$).

In order to determine the obstetrician's decision on the timing of the delivery among the patients having conceived after sexual intercourse (no ART) and those after ART, the duration of pregnancy was plotted against the mode of delivery (Figure 3). There were no differences in the mode of delivery among all groups.

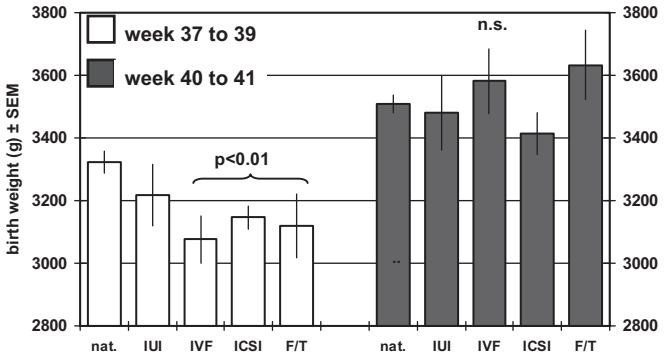

Figure 2. Neonatal birth weight is plotted against conception mode at delivery during pregnancy weeks 37–39 (empty columns) and at delivery during pregnancy weeks 40–41 (black columns). The abbreviation nat. means natural conception after sexual intercourse; F/T means freezing and thawing. Neonatal birth weight after IVF, ICSI and F/T was significantly lower than after natural conception only if delivery occurred during pregnancy weeks 37–39 ($P < 0.01$, ANOVA).

As the presence of a vanishing twin during early pregnancy was associated both with lower birth weight and with a shorter duration of pregnancy, the effect of the obstetrician's decision on inducing labour or on the choice of the delivery mode was assessed (Table II). Although the neonatal birth weight was significantly lower in vanishing twin pregnancies at delivery between weeks 37 and 39 compared with delivery at weeks 40–41, there were no differences in the incidence of labour induction or in the mode of delivery.

The selected cohort of deliveries was grouped according to the method used to support conception, including natural conception after sexual intercourse, IUI, IVF, ICSI, and freezing and thawing of oocytes in the pronucleate stage (Table III). Using the natural conception group for reference, the age of the mother was significantly higher after IVF and after freezing/thawing ($P < 0.001$), the age of the father was significantly higher after IUI, IVF and ICSI ($P < 0.01$) and the duration of infertility was significantly higher after IVF, ICSI and freezing/thawing ($P < 0.0001$). A vanishing twin during early pregnancy was diagnosed exclusively after treatment with gonadotrophins, albeit significantly more often among women treated with IVF, ICSI and frozen/thawed oocytes ($P < 0.0001$). In this highly

Table I. Neonatal birth weight (in g, mean ± SEM) and duration of pregnancy (in weeks, ±95% confidence interval) in 741 singleton deliveries as related to various confounding factors co-determining neonatal birth weight

Influencing factor	Birth weight (g)	No.	Pregnancy week	Counterpart	Birth weight (g)	No.	Pregnancy week
Mother's age <34 at delivery (years)	3344.3 ± 26.1	344	39.3 ± 0.1	Mother's age >34	3381.5 ± 24.3	397	39.3 ± 0.1
Father's age <36 at delivery (years)	3333.4 ± 26.1	344	39.3 ± 0.1	Father's age >36	3390.6 ± 24.3	397	39.3 ± 0.1
Duration of infertility <20 months	3343.1 ± 39.2	337	39.3 ± 0.1	>20 months	3381.8 ± 24.1	404	39.3 ± 0.1
Male infants	3392.1 ± 24.8	382	39.2 ± 1.1	Female infants	3334.3 ± 25.5	359	39.2 ± 1.1
No previous pregnancy	3296.8 ± 25.1[a]	386	39.1 ± 0.1	Previous pregnancy	3437.5 ± 25.5[a]	355	39.2 ± 0.1
No smoking	3373.6 ± 19.1	640	39.2 ± 0.0	>10 cigarettes/day	3304.7 ± 48.2	101	39.3 ± 0.1
Vanishing twin	3164.6 ± 91.3[b]	28	38.6 ± 0.2[f]	Absent	3372.1 ± 18.1[b]	713	39.2 ± 0.1[f]
Gestational diabetes	3359.9 ± 18.3	41	39.2 ± 0.0	Absent	3438.1 ± 75.7	700	39.3 ± 0.1
Recorded fetal growth retardation	2505.0 ± 136.3[c]	12	38.3 ± 0.3[g]	Absent	3378.4 ± 17.5[d]	729	39.3 ± 0.0[g]
Hypertensive disease	3033.3 ± 77.7[d]	38	38.9 ± 0.2	Absent	3382.1 ± 18.1[e]	703	39.3 ± 0.0
Uterine bleedings	3286.3 ± 60.5	64	39.0 ± 0.1	Absent	3371.6 ± 18.6	677	39.3 ± 0.0
Placenta praevia	2970.7 ± 182.7[e]	7	38.0 ± 0.4[h]	Absent	3368.0 ± 17.8[f]	734	39.3 ± 0.0[h]
Premature uterine contractions	3249.4 ± 93.2	27	38.9 ± 0.2	Absent	3368.6 ± 18.1	714	39.3 ± 0.0
Premature rupture of membranes	3154.2 ± 197.8	6	37.8 ± 0.5[i]	Absent	3366.0 ± 17.9	735	39.3 ± 0.0[i]

[a–e]Statistically significant differences in neonatal birth weight.[a, c, d]$P < 0.0001$;[b, e]$P < 0.05$.
[f–i]Statistically significant differences in duration of pregnancy:[f, g, h]$P < 0.01$;[i]$P<0.02$ (Kruskal–Wallis test).

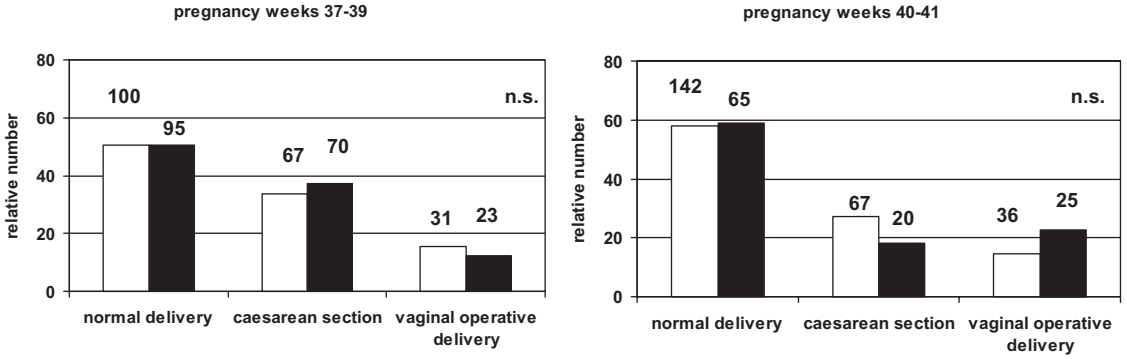

Figure 3. Effect of obstetrician's choice of delivery mode on birth weight and the timing of delivery. White columns represent women having a singleton delivery after sexual intercourse followed by natural conception; black columns represent singleton deliveries after ART. None of the differences was statistically significant (χ^2 analysis).

Table II. Neonatal birth weight (in g) and mode of delivery in singleton pregnancies at term in previously infertile women, after ART and in singleton pregnancies initially diagnosed with vanishing twins after ART

Duration of pregnancy (weeks)	Pregnancy after intercourse		Pregnancy after ART		Pregnancy with vanishing twin	
	37–39	40–41	37–39	40–41	37–39	40–41
Number of cases	198	280	134	101	21	7
Birth weight (g) (± SEM)	3322.3 ± 33.3[a]	3483.8 ± 28.0[a]	3199.3 ±41.4[b]	3389.2 ± 47.6[b]	3055.5 ±103.8[c]	3492.1 ± 179.1[c]
Induction of labour (%)	11 (5.6%)	31 (11.1%)	14 (10.4%)	9 (8.9%)	6 (28.6%)	2 (28.6%)
Normal delivery (%)	100 (50.6%)	166 (59.3%)	68 (50.7%)	55 (54.5%)	9 (42.9%)	4 (57.1%)
Operative vaginal delivery (%)	31 (15.7%)	41 (14.6%)	16 (11.9%)	20 (19.8%)	5 (23.8%)	2 (28.6%)
Caesarean section (%)	67 (33.8%)	73 (26.1%)	50 (37.3%)	26 (25.7%)	7 (33.3%)	1 (14.3%)

[a]$P < 0.001$;[b]$P < 0.01$;[c]$P < 0.05$.

Table III. Distribution of confounding factors of neonatal birth weight among the five different conception modes in 741 singleton deliveries

	No ART	IUI	IVF	ICSI	Frozen/thawed
Number of deliveries at term	443	37	56	147	58
Population characteristics					
Age of mother, years (± SEM)	33.2 ± 0.2	33.7 ± 0.8	35.4 ± 0.6[a]	33.8 ± 0.4	35.5 ± 0.6[a]
Age of father, years (± SEM)	35.9 ± 0.3	37.1 ± 1.0[b]	38.3 ± 0.8[b]	37.0 ± 0.5[b]	37.5 ± 0.8
Duration of infertility, months (± SEM)	45.2 ± 1.6	43.5 ± 5.5	55.1 ± 4.5[c]	55.8 ± 2.8[c]	72.1 ± 4.4[c]
Delivery mode (% of all per method of conception)					
Normal delivery	242 (54.6)	26 (70.3)	23 (41.1)	84 (57.1)	27 (46.6)
Operative vaginal delivery	67 (15.1)	5 (13.5)	11 (19.6)	21 (14.3)	11 (19.0)
Caesarean section	134 (30.2)	6 (16.2)	22 (39.3)	42 (28.3)	20 (34.5)
Sex of infant (% of all)					
Number of male infants	232 (53.4)	20 (54.1)	29 (51.8)	71 (48.3)	30 (51.7)
Number of female infants	211 (47.6)	17 (45.9)	27 (48.2)	76 (51.7)	28 (48.3)
General determinants of neonatal birth weight (% of all)					
Previous pregnancy	243 (54.8)	14 (37.8)	19 (33.9)	48 (32.7)	31 (53.4) (51.7)
Induction of labour	39 (8.8)	3 (8.1)	7 (12.5)	20 (13.6)	4 (6.9)
Smoking ≥10 cigarettes daily	56 (12.6)	4 (10.8)	6 (10.7)	21 (14.3)	14 (24.1)
Vanishing twin	0	2 (0.3)	9 (1.2)[d]	12 (1.6)[d]	5 (0.7)[d]
Gestational diabetes	30 (0.7)	0	5 (8.9)	5 (3.4)	1 (1.7)
Recorded fetal growth retardation	2 (0.5)	0	2 (3.6)	2 (1.4)	6 (10.3)
Hypertensive disease	22 (5.0)	0	5 (8.9)	5 (3.4)	6 (10.3)
Uterine bleeding	32 (7.2)	6 (16.2)	6 (10.7)	14 (9.5)	6 (10.3)
Placenta praevia	3 (0.7)	0	1 (1.8)	3 (2.0)	0
Premature uterine contractions	13 (2.9)	1 (2.7)	2 (3.6)	9 (6.1)	2 (3.4)
Premature rupture of membranes	3 (0.9)	0	0	1 (0.7)	1 (1.7)

[a–c]Significant differences compared with 'no ART' group:[a]$P < 0.001$;[b]$P < 0.01$;[c]$P < 0.0001$ (ANOVA).
[d]Significant difference compared with 'no ART' group ($P < 0.0001$) (χ^2 analysis).

selected group of singleton deliveries at term the incidence of the pregnancy-related complications was similar among all five infertility treatment modalities (Table III).

The most significant variable influencing birth weight was the presence or absence of a previous pregnancy. Therefore, the infant's birth weight and the duration of pregnancy were grouped accordingly (Table IV) and both parameters were subsequently allocated to one of the following conception modes: natural conception after sexual intercourse, IUI, IVF, ICSI and transfer of embryos derived from frozen-thawed pronucleate oocytes. In women without a previous pregnancy the duration of pregnancy was significantly shorter after IVF and ICSI ($P < 0.05$) but there were no significant differences in the neonatal birth weight among the various groups. Due to the splitting of the data into many rather small groups, statistical power was calculated for each comparison and proved to be sufficient only in the ICSI groups and in the IVF group (for the latter only with all data combined). In women with a previous pregnancy both the neonatal birth weight and the duration of pregnancy were significantly lower after ICSI than after sexual intercourse ($P < 0.01$ and $P < 0.02$ respectively). Taking all women together, both the birth weight and the duration of pregnancy were significantly shorter after IVF and ICSI than in women with natural conception after sexual intercourse ($P < 0.001$). If delivery took place between weeks 37 and 39, the neonatal birth weight in women conceiving after IVF, ICSI and after freezing and thawing of oocytes was significantly lower than after natural conception ($P < 0.01$), but not if delivery took place at week 40 or 41 (Figure 2).

Discussion

For many years the birth weight of neonatal infants born after IVF or ICSI has been suggested to be lower not only in multiple births but also in singleton deliveries (Doyle *et al.*, 1992; D'Souza *et al.*, 1997; Hansen *et al.*, 2002; Schieve *et al.*,

Table IV. Neonatal birth weight (g) and duration of pregnancy (weeks) in previously infertile women with a total 741 singleton deliveries after different methods of conception

	No previous pregnancy	Previous pregnancy	All
Conception after sexual intercourse			
Number	200	243	443
Duration of pregnancy (± 95% CI)	39.3 ± 0.1	39.5 ± 0.1	39.4 ± 0.1
Mean birth weight (± SEM)	3347.8 ± 33.8	3489.5 ± 30.5	3425.5 ± 22.8
IUI			
Number	23	14	37
Duration of pregnancy (± 95% CI)	39.1 ± 0.2	39.2 ± 0.3	39.1 ± 0.2
Mean birth weight (± SEM)	3282.4 ± 99.6	3355.7 ± 127.1	3310.1 ± 78.8
Statistical power (%)	15.6	24.9	41.1
IVF			
Number	37	19	56
Duration of pregnancy (± 95% CI)	38.9 ± 0.2[a]	39.1 ± 0.3	39.0 ± 0.2[d]
Mean birth weight (± SEM)	3191.9 ± 78.5	3409.0 ± 109.8	3265.5 ± 64.0[g]
Statistical power (%)	48.7	20.1	71.3
ICSI			
Number	99	48	147
Duration of pregnancy (± 95% CI)	39.0 ± 0.1[b]	38.8 ± 0.2[c]	38.9 ± 0.1[e]
Mean birth weight (± SEM)	3243.1 ± 48.0	3224.2 ± 68.6[f]	3237.0 ± 39.5[h]
Statistical power (%)	63.2	98.1	99.8
Frozen/thawed early zygotes			
Number	27	31	58
Duration of pregnancy (± 95% CI)	39.4 ± 0.2	39.0 ± 0.2	39.2 ± 0.2
Mean birth weight (± SEM)	3284.6 ± 91.9	3404.5 ± 85.4	3348.7 ± 63.0
Statistical power (%)	13.5	16.8	23.1

The statistical power of the comparison of the neonatal birth weight after infertility treatment with that of children born after sexual intercourse was calculated based on the hypothesis that the latter is higher than the former (one-tailed test).
[a–e]Statistically significant differences compared with duration of pregnancy after sexual intercourse:[a, b]$P < 0.05$;[c]$P < 0.01$;[d, e]$P < 0.001$ (Kruskal–Wallis test).
[f, g, h]Statistically significant differences compared with birth weight after sexual intercourse:[f]$P < 0.02$;[g, h]$P < 0.001$.

2002). In addition to these epidemiological findings in the human, data resulting from animal research have also demonstrated a lower weight in fetuses resulting from superovulation of female mice (Elmazar *et al.*, 1989; Ertzeid and Storeng, 1992; Van der Auwera and D'Hooghe, 2001). Transfer of embryos resulting from superovulated animals to foster mother animals resulted in fetuses or newborn pups with normal weight (Elmazar *et al.*, 1989; Van der Auwera and D'Hooghe, 2001), suggesting that the modified hormonal environment created by superovulation is the cause of the observed differences rather than suboptimal culture conditions or rescue of abnormal follicles during superovulation. An alternative explanation for the observed differences in birth weight is given by changes in parental genomic imprinting during *in vitro* culture of gametes and embryos. Experiments with mouse embryos have demonstrated that *in vitro* culture conditions, particularly the presence of serum in the medium, can indeed alter the imprinting profile of some genes involved in subsequent fetal development and in reduced weight of mouse fetuses (Khosla *et al.*, 2001). Trophectodermic tissue seems to be more frequently affected by culture condition-related changes in imprinted gene expression than the preimplantation embryo itself (Mann *et al.*, 2004). Parental genomic imprinting is the process by which maternal and paternal alleles are differentially silenced or selected by the methylation and demethylation respectively of DNA and/or histones through the action of various enzymes, collectively denominated methyltransferases.

Pregnancies in previously infertile women are characterized by a significantly higher incidence of obstetric complications (Tan *et al.*, 1992; De Geyter, 1994; Rufat *et al.*, 1994; Wang *et al.*, 2002; Thomson *et al.*, 2005). Therefore, comparison of birth weight data in the offspring of previously infertile women conceiving naturally may constitute a more appropriate control group than data taken from population-based birth registries. Furthermore, the comparison of neonatal birth weights after IVF and/or ICSI with the outcome of alternative infertility treatments may provide some information about the mechanisms involved, as suggested by Wang *et al.* (2002). The effect of a vanishing twin during early pregnancy on subsequent neonatal birth weight and on the health status of neonate has been reported recently (Pinborg *et al.*, 2005).

Both the duration of pregnancy and the neonatal birth weight were significantly reduced after IVF and ICSI, but not after ovulation induction combined with IUI. In infants born after IUI the birth weight and the duration of pregnancy were also low compared with the pregnancies achieved naturally, but the differences did not reach statistical significance as the sample size was underpowered. From Figures 1 and 2 it appears that the lower birth weight in ART pregnancies, including those after freezing and thawing of oocytes in the pronucleate stage, is mainly found among women with delivery before term. In this population a normal birth weight seems to be achieved only at term of pregnancy. None of these differences found in this highly selected population are caused by the obstetrician's decision to induce labour or by the mode of delivery. Therefore, the lower birth weight and the earlier delivery in pregnancies after ART must be considered to be interrelated consequences of some modification caused by the infertility treatment. Similar findings were presented earlier in a study focusing on a subset of couples with unexplained infertility, in whom the neonatal birth weight was

lower after superovulation compared with the birth weight of the infants of infertile couples having achieved pregnancy naturally (Isaksson and Tiitinen, 1998). The observation that after a vanishing twin during early pregnancy neonatal birth weight is significantly lower in those women having delivery after a shorter duration of pregnancy but not in those with a pregnancy at term adds to the idea that a similar mechanism is involved.

The technique of ICSI has been implicated as causing a higher number of cells in male embryos in the blastocyst stage compared with female embryos in the blastocyst stage (Dumoulin *et al.*, 2005). This difference was not found in embryos resulting from IVF. Other studies have not demonstrated any difference in neonatal birth weight after IVF or ICSI (Bonduelle *et al*., 2005). A shorter duration of pregnancy in IVF and ICSI has been reported earlier and was attributed to the high age of the mother and the role of obstetric emergencies (Wang *et al*., 2002). If IUI is performed without pretreatment with gonadotrophins the duration of pregnancy and neonatal birth weight were similar compared with IVF (De Sutter *et al.*, 2005). However, if IUI was preceded by treatment with gonadotrophins in a majority of the patients, neonatal birth weight was significantly lower compared with women having delivery after natural conception (Nuojua-Huttunen *et al.*, 1999). In our data the neonatal birth weight after ovulation induction with gonadotrophins and IUI was also lower than in the comparison group, but due to the small size of the populations studied the difference did not reach statistical significance. Therefore, the results from the present analyses are inconclusive as to whether hormonal stimulation of the ovaries leads to a shorter pregnancy span or lower neonatal birth weight.

Despite the higher mean age of the mothers and the extensive manipulation of their zygotes and embryos, the mean birth weight of the children born after freezing and thawing of oocytes at the pronucleate stage was remarkably similar to the mean birth weight of children born after natural conception. Only if delivery took place before term was the infant's birth weight significantly lower than those arising from natural conception. The collection of oocytes after stimulation of the ovaries, freezing at the pronucleate stage and replacement in a virtually untreated menstrual cycle mimics to some extent the condition in which embryos collected from superovulated mice were replaced into foster mice resulting in normal weight foetuses or newborn pups (Elmazar *et al.*, 1989; Van der Auwera and D'Hooghe, 2001). The weight reduction of mouse fetuses has been attributed to the action of elevated progesterone levels (not the estrogen levels) caused by the preceding superovulation (Safro *et al.*, 1990; Ertzeid and Storeng, 1992). In contrast to the mouse model, high luteal phase progesterone levels are indicative of an intact pregnancy in human pregnancies achieved with assisted reproduction (Ioannidis *et al.*, 2004). Nevertheless, pregnancies arising during a luteal phase modified by controlled ovarian hyperstimulation may well give rise to a shorter duration of pregnancy or lower neonatal birth weight.

Freezing and thawing of oocytes at the pronucleate stage is performed during a period of highly active demethylation as part of intensive epigenetic reprogramming (Reik *et al.*, 2001, 2003). During this phase of development both the maternal genome and histones in pronucleate stage oocytes are becoming methylated, whereas the paternal genome rapidly loses methylated DNA. One of the cryoprotectants used for the freezing process, DMSO, has been shown to interact with the main enzyme involved in epigenetic reprogramming, methyltransferase (Yokochi and Robertson, 2004). DMSO is known to enhance the methylating effect of some but not all methyltransferases. The net effect of DMSO may have normalized the imprinting process during freezing and thawing of the oocytes at the pronucleate stage.

Evidence for a shorter duration of pregnancy and a lower birth weight in neonates conceived with ART has now become overwhelming. More experimental work has to be carried out to identify the molecular mechanisms responsible for the observed differences in neonatal birth weight. The late effects of COH on subsequent placenta formation or subtle changes in epigenetic reprogramming during manipulation of the zygotes and embryos *in vitro* may both be responsible. Although imprinted genes represent only a minimal fraction of all genes in the mammalian genome (~0.1%), the equilibrium between paternally and maternally derived alleles, either activated or repressed by epigenetic processes, determines the transfer of maternal resources to the offspring (Barlow, 1997). In general, paternally derived embryonic alleles are selected to demand more resources from the pregnant mother than maternally derived alleles (Moore and Reik, 1996). Therefore, both the shorter duration of pregnancy and the reduced neonatal birth weight could be caused by inactivation of paternal alleles during the imprinting process. If the shorter duration of pregnancy and the lower birth weight after IVF and ICSI can be attributed to a distinct defect of epigenetic phenomena at work during parental imprinting, then this defect may have late effects on the individual itself (Maher, 2005) and has the potential to be passed down to other generations (Mills and Moore, 2004).

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Conflict of interest statement

None declared.

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