Ultrasound Obstet Gynecol 2022
Published online in Wiley Online Library (wileyonlinelibrary.com). **DOI:** 10.1002/uog.24920.

Fetal neurosonography and infant neurobehavior following conception by assisted reproductive technology with fresh or frozen embryo transfer

M. L. BOUTET[1,2], E. EIXARCH[1,2,3], P. AHUMADA-DROGUETT[1,2], A. NAKAKI[1,2], F. CROVETTO[1,2,3], M. S. CÍVICO[2,4], A. BORRÁS[4], D. MANAU[2,4], E. GRATACÓS[1,2,3], F. CRISPI[1,2,3] and G. CASALS[2,4]

[1]*BCNatal, Fetal Medicine Research Center (Hospital Clínic and Hospital Sant Joan de Déu), Universitat de Barcelona, Barcelona, Spain;* [2]*Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain;* [3]*Centre for Biomedical Research on Rare Diseases (CIBER-ER), Madrid, Spain;* [4]*Assisted Reproduction Unit, Hospital Clínic de Barcelona, Universitat de Barcelona, Barcelona, Spain*

KEYWORDS: ART; cortical folding; fetal brain; *in-vitro* fertilization; IVF; mode of conception; neurodevelopment; neurosonography; prenatal imaging

CONTRIBUTION

What are the novel findings of this work?
Comparison of brain cortical folding in fetuses conceived by assisted reproductive technology (ART) and fetuses conceived spontaneously showed distinct differences, with reduced sulci depth and lower Sylvian fissure grading score in ART fetuses, and more pronounced changes in the fetuses conceived by ART using fresh embryo transfer (ET) than those conceived using frozen ET. ART infants also showed lower Ages and Stages Questionnaires scores, especially in the fresh ET group.

What are the clinical implications of this work?
Neurosonography is an appropriate tool to identify subtle brain differences among fetuses conceived by ART. These findings suggest the existence of *in-utero* brain reorganization associated with ART, and support neurodevelopmental follow-up in offspring conceived by ART.

ABSTRACT

Objective *We aimed to explore fetal cortical brain development by neurosonography in fetuses conceived by assisted reproductive technology (ART), including frozen and fresh embryo transfer (ET), compared with those conceived spontaneously (SC), and to investigate its association with infant neurobehavior at 12 months of age.*

Methods *This was a prospective cohort study of 210 singleton pregnancies, including 70 SC pregnancies, 70 conceived by* in-vitro *fertilization (IVF) following frozen ET and 70 conceived by IVF after fresh ET. Fetal neurosonography was performed at 32 ± 2 gestational weeks to assess cortical development. Sulci depths were measured offline and normalized by biparietal diameter (BPD). Ages and Stages Questionnaires (ASQ) were completed postnatally, at 12 ± 1 months of corrected age. Neurosonographic findings were adjusted by regression analysis for maternal age, ethnicity, parity, fetal sex and fetal-weight centile and gestational age at scan, and ASQ scores were adjusted for maternal age, ethnicity, parity, educational level and employment status, gestational age at birth, breastfeeding, infant sex and infant age at the ASQ evaluation.*

Results *Overall, in comparison to the SC fetuses, fetuses conceived by ART showed statistically significant differences in cortical development, with reduced parieto-occipital sulci depth adjusted for BPD (mean ± SD: fresh ET, 12.5 ± 2.5* vs *frozen ET, 13.4 ± 2.6* vs *SC, 13.4 ± 2.6, P < 0.001), cingulate sulci depth adjusted for BPD (median (interquartile range (IQR)): fresh ET, 5.8 (4.2–7.4)* vs *frozen ET, 5.8 (4.1–7.5)* vs *SC, 6.5 (4.8–7.8), P = 0.001) and calcarine sulci depth adjusted for BPD (median (IQR): fresh ET, 13.5*

Correspondence to: Dr F. Crispi, BCNatal - Barcelona Center for Maternal–Fetal and Neonatal Medicine (Hospital Clínic and Hospital Sant Joan de Déu), IDIBAPS, University of Barcelona, and Centre for Biomedical Research on Rare Diseases (CIBER-ER), Sabino de Arana 1, 08028 Barcelona, Spain (e-mail: fcrispi@clinic.cat)

Accepted: 14 April 2022

(10.1–16.1) vs *frozen ET, 14.5 (12.1–15.8)* vs *SC, 16.4 (14.3–17.9),* P *< 0.001), together with lower Sylvian fissure grading score. Changes in cortical development were more pronounced in the fresh ET than in the frozen ET group. ART infants showed lower ASQ scores as compared to SC infants, particularly in the fresh ET group (mean ± SD global ASQ Z-score: fresh ET, −0.3 ± 0.4* vs *frozen ET, −0.2 ± 0.4* vs *SC, 0 ± 0.4,* P *< 0.001).*

Conclusions *Fetuses conceived by ART show a distinctive pattern of cortical development and suboptimal infant neurodevelopment, with more pronounced changes in those conceived following fresh ET. These findings support the existence of* in-utero *brain reorganization associated with ART and warrant follow-up studies to assess its long-term persistence. © 2022 The Authors. Ultrasound in Obstetrics & Gynecology published by John Wiley & Sons Ltd on behalf of International Society of Ultrasound in Obstetrics and Gynecology.*

INTRODUCTION

The number of pregnancies conceived by assisted reproductive technologies (ART) is increasing worldwide[1]. There is a growing interest in the neurodevelopment of people conceived by ART, and this has been studied mostly in children and adolescents. Some follow-up studies have suggested suboptimal neurodevelopment in ART offspring compared with the general population, although there are inconsistencies in the literature[2–4]. Fresh and frozen embryo transfer (ET) in *in-vitro* fertilization (IVF) cycles show different perinatal risk profiles[5–7], and some registry-based studies have revealed poorer neurologic results for those conceived using frozen compared with fresh ET in singleton pregnancies[8]. This inconsistency between previous studies reporting postnatal neurodevelopment in ART offspring might be explained partially by the influence of socioeconomic and educational levels during childhood and adolescence that limit direct comparisons with spontaneously conceived (SC) offspring.

Prenatal neurosonography enables accurate evaluation of fetal brain cortical folding, a surrogate marker of brain maturation, prior to any influence of postnatal factors. Interestingly, only two previous studies have explored the central nervous system in fetuses conceived by ART *vs* SC, with contradictory results, reporting differences between these groups in first-trimester brain volumes[9] and no differences during the second trimester[10]. However, no study has evaluated fetal brain cortical development in the third trimester of pregnancy, which would be the optimal time period in which to study prenatal brain maturation.

We aimed to explore fetal cortical brain development by neurosonography in fetuses conceived by ART, including frozen and fresh ET, compared with SC fetuses, and to investigate its association with infant neurobehavior at 12 months of age.

SUBJECTS AND METHODS

Study populations and protocol

We conducted a prospective cohort study of 210 singleton pregnancies between 2017 and 2020, including 70 SC pregnancies and 140 conceived by IVF after frozen ET ($n = 70$) or fresh ET ($n = 70$). All ART pregnancies were recruited from a single center during the first trimester (Assisted Reproduction Unit, Hospital Clínic de Barcelona), ensuring homogeneity among the study participants with respect to ovarian stimulation and endometrial preparation protocols, laboratory procedures and embryo culture conditions. In addition, a group of SC pregnancies was recruited during the third trimester from fertile couples (with a time-to-pregnancy of no longer than 12 months) who were attending the BCNatal Barcelona Center for Maternal Fetal and Neonatal Medicine (Hospital Clínic and Hospital Sant Joan de Déu). Enrolment of the SC population started 11 months after the beginning of recruitment of the ART participants, and each SC pregnancy was matched to both a fresh and a frozen ET pregnancy by maternal age (± 1 year) and gestational age at neurosonography (± 1 week). Pregnancies conceived after oocyte-donation cycles were not eligible for inclusion. Intrauterine infection, fetal malformation or chromosomal anomaly were considered exclusion criteria. Figure 1 summarizes the study population.

Maternal demographics, ART-related variables, obstetric variables and, following delivery, perinatal outcomes, were collected directly by patient interview and by review of medical records. Gestational age was calculated according to crown–rump length (CRL) measurement at first-trimester ultrasound examination[11]. Small-for-gestational age was defined as birth weight < 10th centile and large-for-gestational age as birth weight > 90th centile[12], according to local standards[13]. Preterm birth was defined as delivery prior to the 37th week of gestation. Pre-eclampsia was defined by new-onset hypertension (≥ 140 mmHg systolic blood pressure and/or ≥ 90 mmHg diastolic blood pressure, on two occasions at least 4 h apart, after 20 weeks' gestation) together with proteinuria (≥ 300 mg protein or protein/creatinine ratio ≥ 0.3 in 24-hour urine sample) or, in the absence of proteinuria, new onset of maternal thrombocytopenia, renal insufficiency, liver dysfunction, pulmonary edema or neurological features[14]. Gestational diabetes was defined as glucose intolerance with either onset or first recognition during pregnancy, and was diagnosed by means of a pathologic oral glucose tolerance test (usually indicated after determination of an altered fasting glucose or an altered glucose challenge test from the second trimester onwards, according to the National Diabetes Data Group (NDDG) guidelines[15]. Major neonatal morbidity was defined by the presence of at least one of the following: bronchopulmonary dysplasia, necrotizing enterocolitis, intraventricular hemorrhage, periventricular leukomalacia, retinopathy, persistent ductus arteriosus and sepsis. Minor neonatal morbidity was defined by the presence

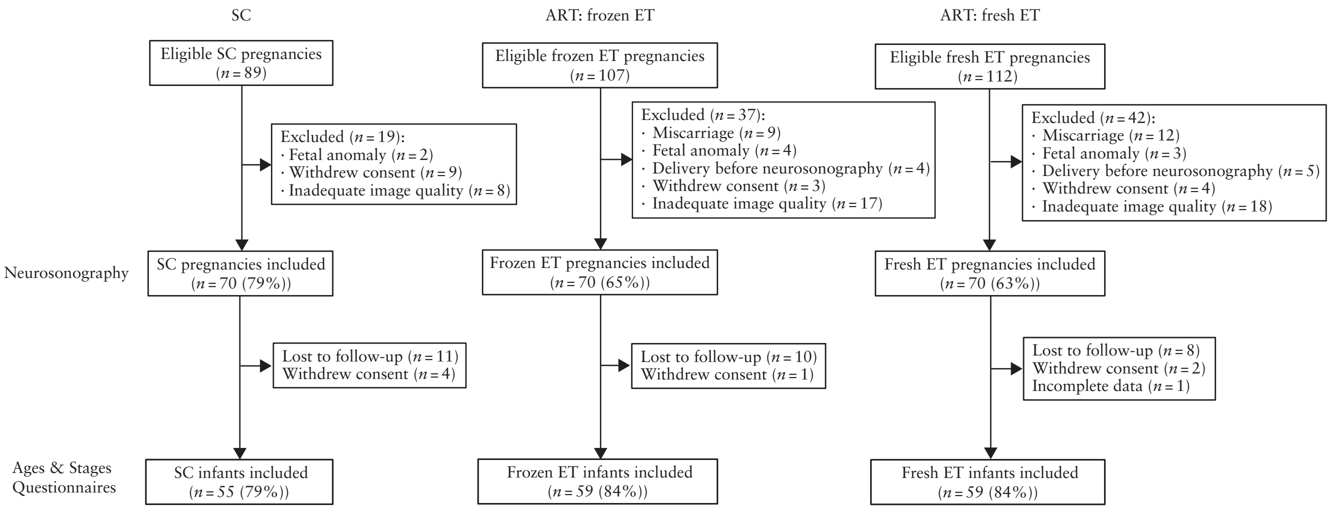

Figure 1 Flow diagram summarizing the study populations of pregnancies conceived spontaneously (SC) or using assisted reproductive technology (ART) with fresh or frozen embryo transfer (ET).

of at least one of the following: respiratory distress, hyperbilirubinemia and anemia. Perinatal mortality was defined by either intrauterine fetal death after 22 weeks of pregnancy or neonatal death within the first 28 days of age.

The study protocol included performance of fetal neurosonography in the third trimester and completion of Ages and Stages Questionnaires[16] (ASQ) at 12 months of corrected age. This study was conducted according to the Declaration of Helsinki for Medical Research involving Human Subjects[17]; the study protocol was evaluated and approved by the local ethics committee (HCB/2017/0714) and all participants provided their written informed consent.

ART protocols

Ovarian stimulation

The ovarian stimulation protocol for IVF and the gonadotropin doses were chosen according to the woman's age and ovarian reserve markers. Long agonist or antagonist protocols were used. Ovarian stimulation was achieved with daily doses of 150–300 IU recombinant follicle stimulating hormone (FSHr) (Gonal-F®; Merck-Serono S.A., Madrid, Spain), alone or with the addition of 75 IU recombinant luteinizing hormone (LHr) (Luveris®; Merck-Serono S.A.) or human menopausal gonadotropin (HMG) (Menopur®; Ferring SA, Madrid, Spain). Administration of human chorionic gonadotropin (hCG) (Ovitrelle®; 250 mg s.c., Merck-Serono S.A.) was indicated in the presence of two or more follicles $\geq$ 18 mm in diameter, with four or more follicles measuring $\geq$ 14 mm in association with a consistent rise in serum estradiol concentration. Ultrasound-guided transvaginal oocyte retrieval was performed 36 h after hCG administration.

ART laboratory procedures

After fertilization by IVF or intracytoplasmic sperm injection (ICSI), embryo culture was carried out in microdrops of Global Media (LifeGlobal, CooperSurgical, Målov, Denmark) under mineral oil at 37°C in an atmosphere of 6.5% carbon dioxide (CO_2) and 7% oxygen (O_2). Embryo quality was assessed according to the Asociación Española para el estudio de la Biología Reproductiva (ASEBIR) criteria[18]. The quality of blastocysts was assessed according to the criteria of Gardner *et al.*[19].

Vitrification and warming protocols of both cleavage embryos and blastocysts were performed using commercially available kits (Kitazato, Tokyo, Japan) according to the method described by Kuwayama[20]. After warming, embryos were cultured in Global Media containing 10% protein substitute supplement (LifeGlobal) until ET. Cleavage embryos with at least 50% of their cells intact immediately after warming and further development after a 24-h culture period were considered as surviving embryos and transferred. Survival of blastocysts was defined by their re-expanding or starting to re-expand within 2 h after warming.

In cases undergoing preimplantation genetic testing for monogenic defects, embryos underwent biopsy on day 3 and unaffected embryos were transferred or cryopreserved 2 days later at blastocyst stage. In those undergoing preimplantation genetic testing for aneuploidies, trophectoderm biopsy at blastocyst stage and subsequent vitrification was performed.

ET protocols

In pregnancies undergoing IVF with fresh ET, vaginal natural progesterone was started the morning after oocyte retrieval (200 mg per 8 h). Cleavage embryos were transferred on day 3 and blastocysts on day 5.

Frozen ET was performed either during the woman's natural cycle or using an endometrial preparation protocol. The choice of natural cycle depended on the regularity of the patient's menstrual cycle and their preference. For frozen ET with natural cycle, ultrasound surveillance was started on day 8–9 of the cycle (depending on the cycle duration) and, once the dominant follicle reached a mean diameter of 17–18 mm, daily follow-up was carried out until its disappearance, that day then being defined as day 0. Frozen ET with endometrial preparation was achieved using transdermal estrogen (Evopad 50 μg, three patches replaced every 72 h (Janssen, Toledo, Spain)) or oral estradiol valerate (Progynova, 2 mg every 8 h (Bayer, Barcelona, Spain)). Estrogen was started on the first day of the cycle and ultrasound monitoring was performed after 12–15 days of treatment. Vaginal natural progesterone (200 mg every 8 h (Progeffik®, Effik, Alcobendas, Spain or Utrogestan®, SEID, Barcelona, Spain)) was added when endometrial thickness was ≥ 7 mm on ultrasound. The first day of progesterone treatment was considered day 0. Cleavage embryos were thawed on day 3 and transferred on day 4, and blastocyst embryos were thawed and transferred on day 5. Supplementation with estrogens and progesterone was performed until the 12th week of pregnancy.

Pregnancy was diagnosed by a positive serum β-hCG test 12 days after ET and transvaginal ultrasound examination was performed in all pregnancies at 5–6 weeks of gestation.

Fetal neurosonography

Neurosonographic acquisition

A detailed two-dimensional neurosonographic examination was performed in all fetuses during the third trimester (32 ± 2 weeks), using a Voluson 730 Expert (GE Healthcare, Zipf, Austria) ultrasound machine. Structural brain normality was confirmed using a standardized protocol, following the International Society of Ultrasound in Obstetrics and Gynecology (ISUOG) guidelines[21]. This included axial planes (transventricular and transthalamic), obtained by a transabdominal approach, and coronal planes (transthalamic, transcaudate and transcerebellar), obtained by a transvaginal approach in fetuses with cephalic presentation and transabdominally in fetuses with breech presentation. We excluded patients with image quality that was insufficient for delineation of measurements (mainly due to fetal presentation or patient intolerance of the transvaginal approach, but also because of the presence of severe endometriosis, previous abdominopelvic surgery, high maternal body mass index or placenta previa).

Image processing, linear measurements and assessment of cortical folding

Measurements were performed offline, using OsiriX MD 12.0 imaging software (Pixmeo SARL, Geneva, Switzerland), by a single experienced examiner, who was blinded to the study group. To provide rigorous measurements that were perpendicular to the midline, a straight line lying along the interhemispheric fissure was traced in every plane, from frontal to occipital bone in axial views and from cranial to caudal bone in coronal views. Brain structures were measured according to previous studies[22–25]. Briefly, fissures and sulci depths were measured in millimeters (Figure 2) and values were corrected by dividing by the biparietal diameter and multiplying by 100 to normalize them according to head size[22,23]. Parieto-occipital sulcus depth was evaluated in a plane slightly cranial to the transventricular plane, where the full depth or triangle shape of the sulcus could be visualized, drawing a perpendicular line from the midline to the apex of the sulcus[23]. Cingulate sulcus depth was measured in the coronal transthalamic plane, drawing a perpendicular line from the midline to the apex of the sulcus. Calcarine sulcus depth was measured in the coronal view, using the transcerebellar plane, drawing a perpendicular line from the midline to the apex of the sulcus. All sulci measurements were performed excluding the cortex and only the side distal to the transducer was measured.

The degree of cortical development of the Sylvian fissure and parieto-occipital, cingulate and calcarine sulci was evaluated according to methodology described

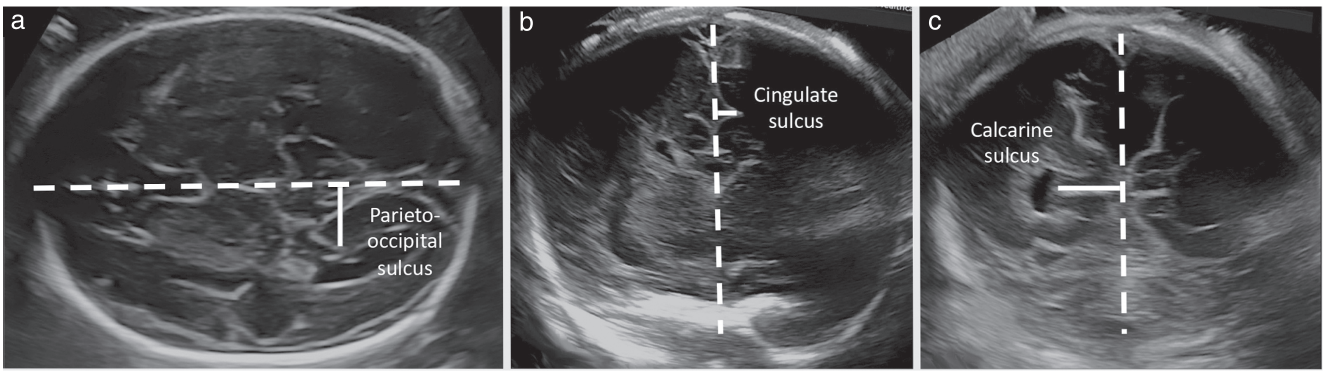

Figure 2 Ultrasound images demonstrating fetal neurosonographic measurements (solid lines): (a) axial transthalamic plane showing measurement of parieto-occipital sulcus depth; (b) coronal transthalamic plane showing measurement of cingulate sulcus; and (c) coronal transcerebellar plane showing measurement of calcarine sulcus depth. Dashed line corresponds to the interhemispheric fissure.

previously[26], assigning grading scores on a scale from 0 (no maturation) to 5 (maximum maturation).

For the sulci depth measurements, an intraobserver intraclass correlation coefficient (ICC) of 0.685–0.971 and an interobserver ICC of 0.773–0.917 were reported in our previous publication[24]. In the same report, the Cohen's kappa coefficient calculated for the sulci grading scores lay between 0.894 and 0.955 for intraobserver variability and between 0.765 and 0.906 for interobserver variability.

Postnatal ASQ assessment

Postnatal neurobehavior was assessed at a mean age of 12 ± 1 months of corrected age by means of a Spanish version of the ASQ (2nd edition), a first-level comprehensive screening program used widely to determine children's performance compared with standards taken from typically developing children of the same age, and validated for its application by both parents and primary caregivers[16,27]. The ASQ screening system comprises several questionnaires designed for use from 4 to 60 months, providing information on five different domains at each age: communication, personal-social, problem-solving, gross-motor and fine-motor skills. In most cases, these questionnaires identify accurately infants or young children who need further evaluation to determine whether they require early intervention services. For the current study, parents completed the questionnaires and emailed them to the researchers. In order to compare the ASQ score results among groups, we calculated Z-scores using the SC population as a reference group.

Statistical analysis

Data management and statistical analyses were performed using STATA 16 software (Statacorp, College Station, TX, USA). The prespecified study outcomes were fetal neurosonographic measurements and postnatal neurobehavioral scores per domain. The independent or exposure variable of interest was the mode of conception (spontaneous, ART with frozen ET, ART with fresh ET). Normal distribution of continuous variables was checked using the Shapiro–Wilk test and histograms. Descriptive statistics and results were expressed as mean (± SD), median (interquartile range) or *n* (%), as appropriate. After checking the fulfilment of each test's assumptions, comparisons among the study groups were made by ANOVA or Kruskal–Wallis tests with Bonferroni correction for continuous variables and Pearson's chi-square test for categorical variables. Neurosonographic findings between groups were adjusted for confounding factors (maternal age, ethnicity, parity, fetal sex and fetal weight centile and gestational age at scan) by multiple regression analyses. Normalized neurobehavioral *Z*-scores from each domain (communication, gross-motor, fine-motor, problem-solving and personal-social skills) and from the global performance (sum of the five domains) were calculated using the correspondent SC group mean and SD by means of the following formula: *Z*-score = (score – mean score for SC)/SD for SC. Neurobehavioral comparisons among groups were adjusted by linear regression for maternal age, ethnicity, parity, educational level and employment status, gestational age at birth, breastfeeding, infant sex and infant age at postnatal evaluation. Correlations between normalized neurosonographic and ASQ findings were investigated using the Spearman correlation coefficient, ρ. All reported *P*-values are two-sided. The significance level was set at 0.05 for all statistical tests.

Sample size calculation

Since there are no data on cortical brain assessment by ultrasound in ART compared with SC fetuses, the sample size was calculated based on a previous publication that acknowledged ultrasonographic changes in cortical folding between third-trimester fetuses with ventriculomegaly *vs* controls[24]. In a proportion of 1 : 1, for a two-sided 95% CI and 80% power, samples of 36 and 63 patients per experimental group were calculated for detecting a 15% difference in normalized calcarine and parieto-occipital sulci depths, respectively. Therefore, the study design aimed for an initial recruitment of at least 70 patients per study group.

RESULTS

Baseline and perinatal characteristics

Baseline characteristics (Table 1) and perinatal outcomes (Table 2) were similar among the study groups, with the exception of higher rates of nulliparity and induction of labor among the ART groups compared with the SC group and a higher proportion of Caucasian ethnicity in the fresh ET population. Regarding causes of infertility, there was a higher proportion of unexplained infertility in the fresh ET than in the frozen ET group, with no difference in the rates of endometriosis, tubal obstruction, male factor and preimplantation genetic diagnosis, nor in the rate of ICSI or the number of embryos transferred. There was a higher proportion of blastocysts transferred in the frozen ET than in the fresh ET group. Frozen ET was performed in programmed cycles in 78.6% ($n = 55$) of cases and in natural cycles in 21.4% ($n = 15$). There were no significant differences between groups regarding the gestational age at CRL assessment for pregnancy dating (mean ± SD: SC group, 12.5 ± 0.6 weeks *vs* ART frozen ET, 12.5 ± 0.6 weeks *vs* ART fresh ET, 12.5 ± 0.5 weeks, $P = 0.505$).

Fetal neurosonography

Gestational age and estimated fetal weight were similar at neurosonographic assessment in all three groups (Table 3). Cephalic dimensions were similar between groups. Both ART populations showed less profound cingulate and calcarine sulci depths compared with the

Table 1 Baseline and fertility characteristics in pregnancies conceived spontaneously (SC) or using assisted reproductive technology (ART) with fresh or frozen embryo transfer (ET)

		ART	
Characteristic	*SC* (n = 70)	*Frozen ET* (n = 70)	*Fresh ET* (n = 70)
Maternal characteristics			
Age (years)	34.4 (30.1–37.3)	35.8 (33.7–37.6)	35.9 (33.9–37.7)
Body mass index (kg/m^2)	23.2 (20.6–25.7)	23.4 (20.3–25.9)	23.1 (20.9–27.2)
Caucasian	48 (68.6)	58 (82.9)	63 (90.0)*
Nulliparous	40 (57.1)	58 (82.9)*	65 (92.9)*
Chronic hypertension	1 (1.4)	2 (2.9)	0
Cardiovascular disease	1 (1.4)	2 (2.9)	3 (4.3)
Diabetes	0	1 (1.4)	1 (1.4)
Autoimmune disease	3 (4.3)	3 (4.3)	2 (2.9)
Thyroid disease	2 (2.9)	2 (2.9)	5 (7.1)
Kidney disease	1 (1.4)	1 (1.4)	0
Epilepsy	1 (1.4)	0	1 (1.4)
Psychopharmaceutical exposure during pregnancy	3 (4.3)	1 (1.4)	3 (4.3)
Self-reported smoking habit during pregnancy	13 (18.6)	9 (12.9)	11 (15.7)
Self-reported alcohol intake during pregnancy	9 (12.9)	8 (11.4)	5 (7.1)
Self-reported drug use during pregnancy	0	0	0
University-level education	37 (52.9)	41 (58.6)	40 (57.1)
Employment rate	46 (65.7)	52 (74.3)	56 (80.0)
Fertility and ART characteristics			
Cause of infertility/indication for ART			
Unexplained	—	20 (28.6)	35 (50.0)†
Endometriosis	—	7 (10.0)	11 (15.7)
Tubal obstruction	—	7 (10.0)	7 (10.0)
Male factor	—	35 (50.0)	27 (38.6)
Preimplantation genetic diagnosis	—	4 (5.7)	5 (7.1)
Number of embryos transferred	—	2.0 (1.0–2.0)	2.0 (2.0–2.0)
Transfer of ICSI embryo	—	68 (97.1)	68 (97.1)
Transfer in blastocyst stage	—	41 (58.6)	14 (20.0)†
Vanishing twin	—	29 (41.4)	14 (20.0)

Data are given as median (interquartile range) or *n* (%). *$P < 0.05$ *vs* SC. †$P < 0.05$ *vs* ART with frozen ET. ICSI, intracytoplasmic sperm injection.

Table 2 Perinatal characteristics in pregnancies conceived spontaneously (SC) or using assisted reproductive technology (ART) with fresh or frozen embryo transfer (ET)

		ART	
Characteristic	*SC* (n = 70)	*Frozen ET* (n = 70)	*Fresh ET* (n = 70)
Pregnancy complications			
Preterm delivery	2 (2.9)	5 (7.1)	2 (2.9)
Pre-eclampsia	2 (2.9)	7 (10.0)	2 (2.9)
Gestational diabetes	4 (5.7)	5 (7.1)	6 (8.6)
Placenta previa	0	1 (1.4)	0
Placental abruption	0	1 (1.4)	0
Aspirin exposure from first trimester onwards	9 (12.9)	10 (14.3)	4 (5.7)
Prenatal corticoid exposure	1 (1.4)	4 (5.7)	2 (2.9)
Delivery data			
Gestational age at birth (weeks)	40.0 (39.0–40.4)	39.5 (38.4–41.0)	40.2 (39.1–41.0)
Birth weight (g)	3280 (3030–3540)	3280 (3050–3550)	3235 (2876–3630)
Birth-weight centile	41 (19–67)	40 (18–60)	36 (11–64)
Birth weight < 10th centile	11 (15.7)	6 (8.6)	12 (17.1)
Birth weight > 90th centile	4 (5.7)	5 (7.1)	5 (7.1)
Induction of labor	24 (34.3)	40 (57.1)*	35 (50.0)*
Cesarean section	21 (30.0)	29 (41.4)	19 (27.1)
Female gender	37 (52.9)	28 (40.0)	39 (55.7)
Neonatal outcome			
Admission to neonatal intensive care unit	9 (12.9)	6 (8.6)	2 (2.9)
Minor neonatal morbidity	11 (15.7)	11 (15.7)	4 (5.7)
Major neonatal morbidity	0	0	0
Neonatal mortality	0	0	0

Data are given as *n* (%) or median (interquartile range). *$P < 0.05$ *vs* SC.

SC group, and the fresh ET group presented less profound parieto-occipital sulci depth compared with both the SC and the frozen ET groups. Lower cortical grading scores were also observed in both ART groups at the level of the Sylvian fissure and the parieto-occipital and calcarine sulci as compared with the SC population (Figure 3). Overall, differences in cortical development, as reflected in sulci depth, were more pronounced in the fresh ET compared with the frozen ET group (Table 3). These reported neurosonographic differences were statistically significant after adjustment for maternal age, ethnicity, parity, fetal sex and gestational age and estimated fetal weight centile at ultrasound scan.

Infant ASQ scores

Infant ASQ results were available in 173 cases (Figure 4). The fresh ET group presented lower global ASQ scores

Table 3 Fetal neurosonographic results in pregnancies conceived spontaneously (SC) or using assisted reproductive technology (ART) with fresh or frozen embryo transfer (ET)

		ART		
Characteristic	*SC* (n = 70)	*Frozen ET* (n = 70)	*Fresh ET* (n = 70)	P
Gestational age at scan (weeks)	31.6 (29.3–32.5)	31.2 (29.3–32.2)	30.5 (29.4–32.0)	0.192
EFW at scan (g)	1775 ± 435	1716 ± 340	1678 ± 346	0.325
EFW at scan (centile)	43 (20–79)	57 (33–82)	46 (21–72)	0.303
EFW < 10th centile	8 (11.4)	8 (11.4)	9 (12.9)	0.967
EFW > 90th centile	16 (22.9)	11 (15.7)	8 (11.4)	0.188
Cephalic presentation	60 (85.7)	64 (91.4)	58 (82.9)	0.315
Left laterality	42 (60.0)	45 (64.3)	39 (55.7)	0.585
Biparietal diameter (mm)*	78.3 ± 6.4	78.1 ± 4.5	78.4 ± 4.5	0.910
Occipitofrontal diameter (mm)*	102.9 ± 6.9	101.8 ± 5.3	101.1 ± 5.9	0.171
Head circumference (mm)*	289.8 ± 19.6	288.0 ± 14.4	286.8 ± 15.1	0.401
Normalized parieto-occipital sulcus depth*†	13.4 ± 2.6	13.4 ± 2.6	12.5 ± 2.5‡§	< 0.001
Normalized cingulate sulcus depth*†	6.5 (4.8–7.8)	5.8 (4.1–7.5)‡	5.8 (4.2–7.4)‡	0.001
Normalized calcarine sulcus depth*†	16.4 (14.3–17.9)	14.5 (12.1–15.8)‡	13.5 (10.1–16.1)‡§	< 0.001

Data are given as median (interquartile range), mean ± SD or *n* (%). **P*-values adjusted by maternal age, ethnicity, parity, fetal sex and gestational age and fetal weight centile at scan. †Data normalized by dividing by biparietal diameter and multiplying by 100. ‡$P < 0.05$ *vs* SC. §$P < 0.05$ *vs* ART with frozen ET. EFW, estimated fetal weight.

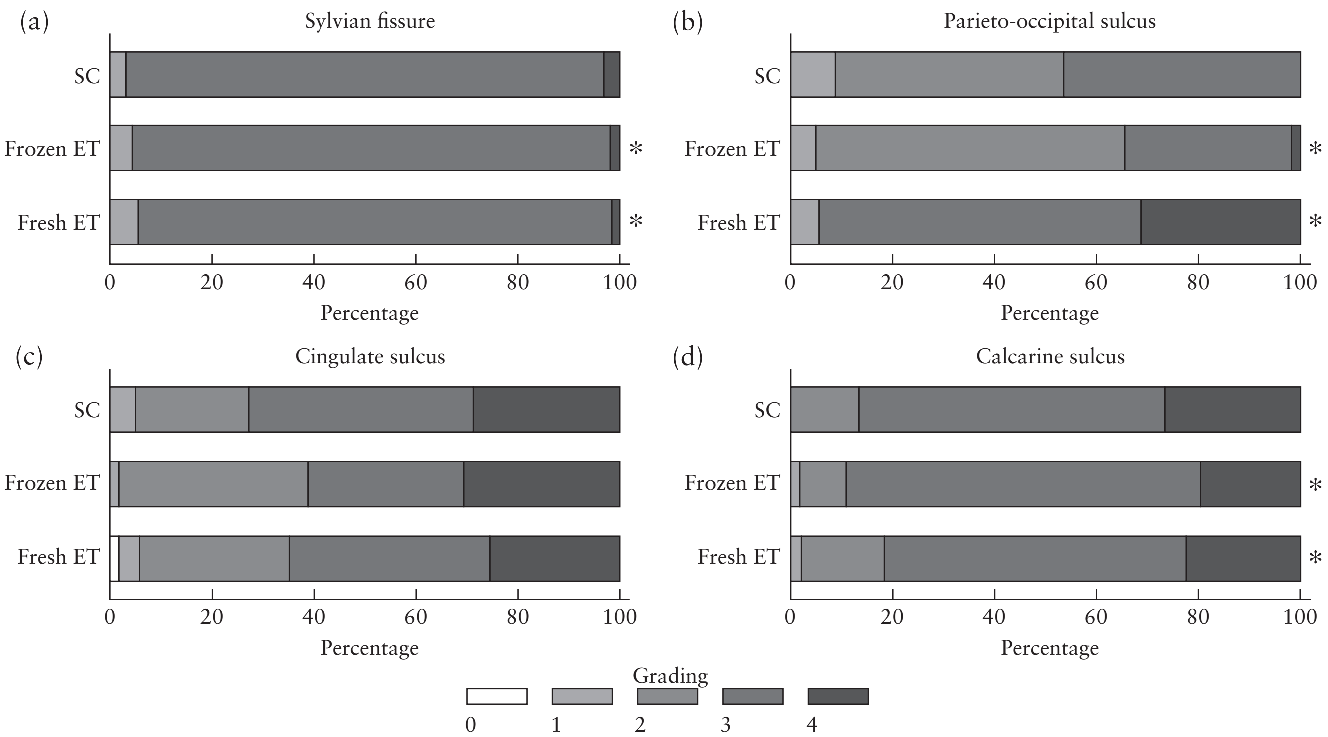

Figure 3 Distribution of main sulci and Sylvian fissure grading scores in fetuses of pregnancies conceived spontaneously (SC) or using assisted reproductive technology with fresh or frozen embryo transfer (ET): (a) Sylvian fissure; (b) parieto-occipital sulcus; (c) cingulate sulcus; and (d) calcarine sulcus. *P*-values adjusted for maternal age, ethnicity, parity, fetal sex, and gestational age and fetal weight centile at scan. *$P < 0.05$ *vs* SC.

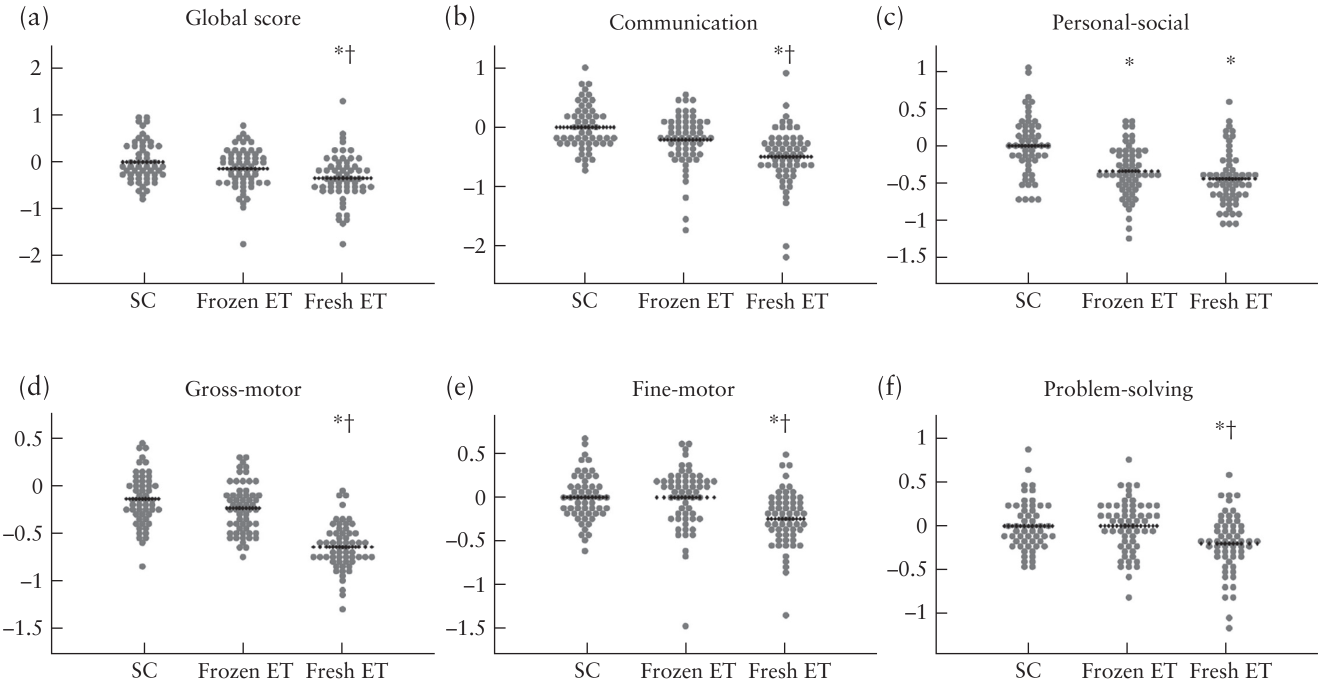

Figure 4 Adjusted *Z*-scores of infant Ages & Stages Questionnaire domains at 12 months of age among study groups according to mode of conception, either spontaneously (SC) ($n = 55$) or using assisted reproductive technology (ART) with fresh or frozen embryo transfer (ET) ($n = 59$ each): (a) global score; (b) communication skills; (c) personal-social skills; (d) gross-motor skills; (e) fine-motor skills; and (f) problem-solving skills. *Z*-score values and *P*-values adjusted for maternal age, ethnicity, parity, educational level and employment status, gestational age at delivery, breastfeeding, infant sex and infant age at evaluation. Individual (●) and median (◆) values are shown. *$P < 0.05$ *vs* SC. †$P < 0.05$ *vs* ART with frozen ET.

as compared with the SC and the ART with frozen ET populations (mean ± SD global ASQ *Z*-score: fresh ET, −0.3 ± 0.4 *vs* frozen ET, −0.2 ± 0.4 *vs* SC, 0 ± 0.4, $P < 0.001$). Moreover, the fresh ET group showed significantly lower scores for communication, motor and problem-solving skills compared with both the SC group and the frozen ET group. Both groups of infants conceived by ART showed significantly lower scores compared with the SC group in the personal-social domain. Results were statistically significant after adjustment for maternal age, ethnicity, parity, educational level, employment status, breastfeeding, infant sex, gestational age at birth and infant age at postnatal evaluation. The distribution of cases with *Z*-scores below −2 SD from the spontaneously conceived study population mean is given according to domain in Table S1.

Fetal neurosonographic cortical features and infant global ASQ scores showed a weak but statistically significant positive correlation in the overall population with (parieto-occipital sulcus depth: $\rho = 0.23$, $P = 0.003$; cingulate sulcus depth: $\rho = 0.21$, $P = 0.008$; calcarine sulcus depth: $\rho = 0.32$, $P < 0.001$) and within the ART population (parieto-occipital sulcus depth: $\rho = 0.22$, $P = 0.017$; cingulate sulcus depth: $\rho = 0.20$, $P = 0.032$; calcarine sulcus depth: $\rho = 0.29$, $P = 0.001$). In addition, there were statistically significant positive correlations between these parameters and each of the ASQ domains, except for the cingulate sulcus in motor and problem-solving areas (Table S2).

DISCUSSION

We report third-trimester neurosonographic results in fetuses conceived by ART which suggest association with a distinctive pattern of cortical development, along with suboptimal infant neurobehavior at 12 months of age. To our knowledge, this is the first study assessing prenatal brain cortical development in ART offspring. We report less profound parieto-occipital, cingulate and calcarine sulci depths, together with lower cortical grading scores, in ART fetuses.

Cortical folding is a complex process of prenatal brain organization, in which the smooth brain surface evolves into a system of sulci and gyri associated with rapid cortical expansion in functional areas in ventricular and subventricular zones[28]. Parieto-occipital, cingulate and calcarine sulci increase in depth with gestational age[23,26] and the Sylvian fissure undergoes a process of operculization[29]. Changes in cortical development have been described in fetuses with growth restriction (FGR), congenital heart defects (CHD) and non-severe ventriculomegaly. Whereas FGR, with or without pre-eclampsia, has been associated with only reduced Sylvian fissure depth[25,30,31], fetuses with CHD[32] or isolated ventriculomegaly[24,33] have been reported to experience a widespread suboptimal cortical folding process, with shallower sulci depths and delayed operculization, similar to that observed in our ART population.

Our reported differences in fetal cortical development in ART pregnancies correlated weakly with suboptimal neurobehavior in infancy, in communication, personal-social, problem-solving and motor domains. Complex functions at the cortical level are distributed across several neural networks[34]. The calcarine sulcus is part of the primary visual cortex. The parieto-occipital sulcus participates in visuospatial working memory[35]. The cingulate sulcus is involved in cognitive[36], motor[37], emotional and social-behavioral[38,39] processing; its deficiency has been described in psychiatric disorders such as schizophrenia, attention deficit hyperactivity disorder and autism spectrum disorder[40,41]. Therefore, the changes observed in our population in these structures may have contributed partially to the neurobehavioral features found at 12 months. Previous follow-up studies in infants and children conceived by ART showed inconsistent cognitive, psychomotor and behavioral results[42–47]. These inconsistencies may be explained by differences in the ART populations, the influence of cofactors such as infertility, multiple gestation, prematurity or other perinatal complications associated with ART[8,48–52], and the effect of different postnatal socioeconomic and educational levels[50]. Couples undergoing fertility treatment may differ intrinsically from those conceiving spontaneously in demographic characteristics such as age, educational level and socioeconomic position[50], and also in the way in which they encourage learning[53,54] and acknowledge health issues in their offspring[55]. As many factors influence neurodevelopment after birth, the uniqueness of the current study is our description of fetal brain changes *in utero*, occurring before exposure to postnatal influences.

It has been proposed that neurodevelopment in fetuses conceived by ART might be influenced by the underlying parental subfertility, ovarian stimulation and/or IVF procedures. The observed differences between our three study groups could have been triggered by their different intrauterine vasoactive and hormonal milieu[56–61], but also by changes in cardiac function: brain and heart development take place simultaneously *in utero* and often share morphogenetic programs[62]. Fetuses conceived by ART show differences in growth and cardiac shape and function compared with SC fetuses, fresh ET being associated with fetal smallness[6,63] and cardiac remodeling and dysfunction[64,65], and frozen ET with macrosomia[63], hypertensive disorders of pregnancy[5,66] and milder cardiac changes[65,67].

Interestingly, our findings were more pronounced in the fresh ET than in the frozen ET group, contrary to a registry-based study reporting a statistically higher risk of neurodevelopmental delay associated with ART (particularly ICSI using ejaculated sperm with fresh and frozen ET), with higher risk for ICSI with frozen ET, when restricting the analysis to singletons[8]. Nevertheless, no differences were reported between fresh and frozen ET when comparing academic performance in adolescents, in an uncontrolled follow-up study, in which, however, mild conditions may have been underrepresented[68]. A birth cohort study with a 2-year follow-up reported reassuring results for ART, but it was underpowered to detect differences between the fresh and frozen ET modalities[69].

Strengths and limitations

Among the strengths of our study is that it is the first to examine cortical development in fetuses conceived by ART. We present a well-phenotyped cohort from a single center, with all study groups having been included prospectively. All patients underwent a detailed neurosonographic examination to exclude any additional abnormality of the central nervous system, preventing the inclusion of conditions that could potentially bias our results. Another strength of this study is the use of both sulci depth measurements and grading of fissures and sulci, providing quantitative objective data along with cortical maturation status. To ensure high image quality and accurate assessment of cortical folding parameters, ultrasonographic measurements were performed offline, following a strict and reliable protocol, by a single trained neurosonographer who was blinded to the mode of conception. Finally, we adjusted for potential confounders in our models for both prenatal and postnatal outcomes.

Among the limitations of our study is that the potential contribution of infertility factors to outcome cannot be separated from the contribution of the ART procedure itself. Moreover, regarding embryonic stage at ET, there was a different proportion of blastocysts transferred in the two ART groups. We therefore performed a sub-analysis, excluding the cases with transfer at the blastocyst stage (Tables S3 and S4 and Figure S1) and, even though the power decreased, significant differences between the study groups remained. Furthermore, the frozen ET group itself was heterogeneous, with 15% of ET performed with the woman's natural cycle. The conditions associated with decreased fertility were likely to have been underdiagnosed in the SC population. The reported neurosonographic differences were subtle, with most outcomes lying within normal ranges; their postnatal persistence needs to be investigated. Due to technical reasons, particularly shadowing from the fetal skull, we measured only the side of the brain distal to the transducer, which could have biased our results in case of asymmetry. The ASQ used to assess postnatal performance at 12 months is mainly a screening tool, subject to reporting bias, and it was applied only once, rather than longitudinally. Finally, although the mass-significance effect was attenuated by applying Bonferroni correction to each comparison, we cannot exclude that some of the apparent differences could have been due to chance.

Conclusions

Fetuses conceived by ART showed a distinctive pattern of cortical development and suboptimal infant neurodevelopment at 12 months, with more pronounced changes in those conceived following fresh ET. Our results provide new evidence for the existence of *in-utero* brain reorganization associated with ART and for the importance of neurodevelopmental follow-up and assessment of the long-term consequences in ART offspring.

ACKNOWLEDGMENTS

We thank the study participants for their personal time and commitment to this project. This project was partially funded with support from the Erasmus+ Programme of the European Union (Framework Agreement number: 2013-0040). This publication reflects the views only of the author, and the Commission cannot be held responsible for any use, which may be made of the information contained therein. Additionally, the research leading to these results received funding from 'la Caixa' Foundation under grant agreement LCF/PR/GN18/10310003, the Instituto de Salud Carlos III (PI15/00130, PI16/00861, PI17/00675, PI18/00073, INT21/00027), cofunded by the European Union, Cerebra Foundation for the Brain Injured Child (Carmarthen, Wales, UK) and AGAUR 2017 SGR grant no 1531. E.E. received funding from the Departament de Salut under grant SLT008/18/00156.

REFERENCES

1. Wyns C, De Geyter C, Calhaz-Jorge C, Kupka MS, Motrenko T, Smeenk J, Bergh C, Tandler-Schneider A, Rugescu IA, Vidakovic S, Goossens V. ART in Europe, 2017: results generated from European registries by ESHRE. *Hum Reprod Open* 2021; **3**: 1–17.
2. Berntsen S, Söderström-Anttila V, Wennerholm U-B, Laivuori H, Loft A, Oldereid NB, Bente Romundstad L, Bergh C, Pinborg A. The health of children conceived by ART: "the chicken or the egg?" *Hum Reprod Update* 2019; **25**: 137–158.
3. Pinborg A. Short- and long-term outcomes in children born after assisted reproductive technology. *BJOG* 2019; **126**: 145–148.
4. Bergh C, Wennerholm UB. Long-term health of children conceived after assisted reproductive technology. *Ups J Med Sci* 2020; **125**: 152–157.
5. Ernstad EG, Wennerholm U-B, Khatibi A, Petzold M, Bergh C. Neonatal and maternal outcome after frozen embryo transfer: Increased risks in programmed cycles. *Am J Obstet Gynecol* 2019; **221**: 126.e1–18.
6. Maheshwari A, Pandey S, Raja EA, Shetty A, Hamilton M, Bhattacharya S. Is frozen embryo transfer better for mothers and babies? Can cumulative meta-analysis provide a definitive answer? *Hum Reprod Update* 2018; **24**: 35–58.
7. Pinborg A, Loft A, Aaris Henningsen AK, Rasmussen S, Andersen AN. Infant outcome of 957 singletons born after frozen embryo replacement: The Danish National Cohort Study 1995–2006. *Fertil Steril* 2010; **94**: 1320–1327.
8. Sandin S, Nygren KG, Iliadou A, Hultman CM, Reichenberg A. Autism and mental retardation among offspring born after in vitro fertilization. *JAMA* 2013; **310**: 75–84.
9. Husen SC, Koning IV, Go ATJI, Groenenberg IAL, Willemsen SP, Rousian M, Steegers-Theunissen RPM. IVF with or without ICSI and the impact on human embryonic brain development: the Rotterdam Periconceptional Cohort. *Hum Reprod* 2021; **36**: 596–604.
10. Yin L, Xu Y, Li H, Ling C, Wai Choy K, Xia F, Deng X. Effect of assisted reproductive technology on fetal brain development assessed by prenatal ultrasonography. *J Perinat Med* 2015; **43**: 103–109.
11. Robinson HP. Sonar Measurement of Fetal Crown-Rump Length as Means of Assessing Maturity in First Trimester of Pregnancy. *Br Med J* 1973; **4**: 28–31.
12. Salomon LJ, Alfirevic Z, Da Silva Costa F, Deter RL, Figueras F, Ghi T, Glanc P, Khalil A, Lee W, Napolitano R, Papageorghiou A, Sotiradis A, Stirnemann J, Toi A, Yeo G. ISUOG Practice Guidelines: ultrasound assessment of fetal biometry and growth. *Ultrasound Obstet Gynecol* 2019; **53**: 715–723.
13. Figueras F, Meler E, Iraola A, Eixarch E, Coll O, Figueras J, Francis A, Gratacos E, Gardosi J. Customized birthweight standards for a Spanish population. *Eur J Obstet Gynecol Reprod Biol* 2008; **136**: 20–24.
14. Gestational Hypertension and Preeclampsia: ACOG Practice Bulletin, Number 222. *Obstet Gynecol* 2020; **135**: e237–260.
15. National Diabetes Data Group. Guide to diagnosis and classification of diabetes mellitus and other categories of glucose intolerance. *Diabetes Care* 1997; **20**: 1039–1057.
16. Bricker D, Squires J, Mounts L, Potter L, Nickel R, Twombly E, Farrell J. *Ages & Stages Questionnaires: A Parent-Completed Child-Monitoring System - Spanish Version*, 2nd edn. Paul H Brookes Publishing Co.: Baltimore, MD, USA, 1999.
17. World Medical Association. Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects. *JAMA* 2013; **310**: 2191–2194.
18. Cuevas Saiz I, Carme Pons Gatell M, Vargas MC, Delgado Mendive A, Rives Enedáguila N, Moragas Solanes M, Carrasco Canal B, Teruel López J, Busquets Bonet A, Hurtado de Mendoza Acosta MV. The Embryology Interest Group: updating ASEBIR's morphological scoring system for early embryos, morulae and blastocysts. *Med Reprod y Embriol Clínica* 2018; **5**: 42–54.
19. Gardner DK, Lane M, Stevens J, Schlenker T, Schoolcraft WB. Blastocyst score affects implantation and pregnancy outcome: Towards a single blastocyst transfer. *Fertil Steril* 2000; **73**: 1155–1158.
20. Kuwayama M. Highly efficient vitrification for cryopreservation of human oocytes and embryos: The Cryotop method. *Theriogenology* 2007; **67**: 73–80.
21. Paladini D, Malinger G, Birnbaum R, Monteagudo A, Pilu G, Salomon LJ, Timor-Tritsch IE. ISUOG Practice Guidelines (updated): sonographic examination of the fetal central nervous system. Part 2: performance of targeted neurosonography. *Ultrasound Obstet Gynecol* 2021; **57**: 661–671.
22. Egaña-Ugrinovic G, Sanz-Cortes M, Figueras F, Bargalló N, Gratacós E. Differences in cortical development assessed by fetal MRI in late-onset intrauterine growth restriction. *Am J Obstet Gynecol* 2013; **209**: 126.e1–8.
23. Alonso I, Borenstein M, Grant G, Narbona I, Azumendi G. Depth of brain fissures in normal fetuses by prenatal ultrasound between 19 and 30 weeks of gestation. *Ultrasound Obstet Gynecol* 2010; **36**: 693–699.
24. Hahner N, Puerto B, Perez-Cruz M, Policiano C, Monterde E, Crispi F, Gratacos E, Eixarch E. Altered cortical development in fetuses with isolated nonsevere ventriculomegaly assessed by neurosonography. *Prenat Diagn* 2018; **38**: 365–375.
25. Paules C, Miranda J, Policiano C, Crovetto F, Youssef L, Hahner N, Nakaki A, Crispi F, Gratacós E, Eixarch E. Fetal neurosonography detects differences in cortical development and corpus callosum in late-onset small fetuses. *Ultrasound Obstet Gynecol* 2021; **58**: 42–47.
26. Pistorius LR, Stoutenbeek P, Groenendaal F, De Vries L, Manten G, Mulder E, Visser G. Grade and symmetry of normal fetal cortical development: A longitudinal two- and three-dimensional ultrasound study. *Ultrasound Obstet Gynecol* 2010; **36**: 700–708.
27. Sarmiento Camposa JA, Squires J, Ponte J. Universal developmental screening: Preliminary studies in Galicia, Spain. *Early Child Dev Care* 2011; **181**: 475–485.
28. Lui JH, Hansen DV, Kriegstein AR. Development and evolution of the human neocortex. *Cell* 2011; **146**: 18–36.
29. Quarello E, Stirnemann J, Ville Y, Guibaud L. Assessment of fetal Sylvian fissure operculization between 22 and 32 weeks: A subjective approach. *Ultrasound Obstet Gynecol* 2008; **32**: 44–49.
30. Husen SC, Koning IV, Go ATJI, Van Graafeiland AW, Willemsen SP, Groenenberg IAL, Steegers-Theunissen RPM. Three-dimensional ultrasound imaging of fetal brain fissures in the growth restricted fetus. *PLoS One* 2019; **14**: 1–14.
31. Basso A, Youssef L, Nakaki A, Paules C, Miranda J, Casu G, Salazar L, Gratacos E, Eixarch E, Crispi F, Crovetto F. Fetal neurosonography at 31–35 weeks reveals altered cortical development in pre-eclampsia with and without small-for-gestational-age fetus. *Ultrasound Obstet Gynecol* 2022; **59**: 737–746.
32. Lee FT, Seed M, Sun L, Marini D. Fetal brain issues in congenital heart disease. *Transl Pediatr* 2021; **10**: 2182–2196.
33. Hahner N, Benkarim OM, Aertsen M, Perez-Cruz M, Piella G, Sanroma G, Bargallo N, Deprest J, Gonzalez Ballester MA, Gratacos E, Eixarch E. Global and regional changes in cortical development assessed by MRI in fetuses with isolated nonsevere ventriculomegaly correlate with neonatal neurobehavior. *Am J Neuroradiol* 2019; **40**: 1567–1574.
34. Nieuwenhuys R. The insular cortex. A review. *Prog Brain Res* 2012; **195**: 123–163.
35. Pollmann S. Working memory dependence of spatial contextual cueing for visual search. *Br J Psychol* 2019; **110**: 372–380.
36. Leech R, Sharp DJ. The role of the posterior cingulate cortex in cognition and disease. *Brain* 2014; **137**: 12–32.
37. Michelet T, Badets A. The anterior midcingulate cortex might be a neuronal substrate for the ideomotor mechanism. *Exp Brain Res* 2021; **239**: 2345–2355.
38. Hadland KA, Rushworth MFS, Gaffan D, Passingham RE. The effect of cingulate lesions on social behaviour and emotion. *Neuropsychologia* 2003; **41**: 919–931.
39. Lockwood PL. The anatomy of empathy: Vicarious experience and disorders of social cognition. *Behav Brain Res* 2016; **311**: 255–266.
40. Chiu PH, Kayali MA, Kishida KT, Tomlin D, Klinger LG, Klinger MR, Montague PR. Self Responses along Cingulate Cortex Reveal Quantitative Neural Phenotype for High-Functioning Autism. *Neuron* 2008; **57**: 463–473.
41. Rubia K, Alegría AA, Brinson H. Brain abnormalities in attention-deficit hyperactivity disorder: a review. *Rev Neurol* 2014; **58** (Suppl 1): S3–16.
42. Middelburg KJ, Heineman MJ, Bos AF, Hadders-Algra M. Neuromotor, cognitive, language and behavioural outcome in children born following IVF or ICSI - A systematic review. *Hum Reprod Update* 2008; **14**: 219–231.
43. Bay B, Mortensen EL, Kesmodel US. Assisted reproduction and child neurodevelopmental outcomes: A systematic review. *Fertil Steril* 2013; **100**: 844–853.
44. Hart R, Norman RJ. The longer-term health outcomes for children born as a result of IVF treatment. Part II-mental health and development outcomes. *Hum Reprod Update* 2013; **19**: 244–250.
45. Liu L, Gao J, He X, Cai Y, Wang L, Fan X. Association between assisted reproductive technology and the risk of autism spectrum disorders in the offspring: A meta-analysis. *Sci Rep* 2017; **7**: 1–8.
46. Rumbold AR, Moore VM, Whitrow MJ, Oswald TK, Moran LJ, Fernandez RC, Barnhart KT, Davies MJ. The impact of specific fertility treatments on cognitive development in childhood and adolescence: A systematic review. *Hum Reprod* 2017; **32**: 1489–1507.
47. Rönö K, Rissanen E, Bergh C, Wennerholm UB, Opdahl S, Romundstad LB, Henningsen AKA, Spangmose AL, Pinborg A, Gissler M, Tiitinen A. The neurodevelopmental morbidity of children born after assisted reproductive technology: a Nordic register study from the Committee of Nordic Assisted Reproductive Technology and Safety group. *Fertil Steril* 2022; **117**: 1026–1037.
48. Kissin DM, Zhang Y, Boulet SL, Fountain C, Bearman P, Schieve L, Yeargin-Allsopp M, Jamieson DJ. Association of assisted reproductive technology

(ART) treatment and parental infertility diagnosis with autism in ART-conceived children. *Hum Reprod* 2015; **30**: 454–465.
49. Drenth Olivares M, Kuiper DB, Haadsma ML, Heineman KR, Heineman MJ, Hadders-Algra M. IVF procedures are not, but subfertility is associated with neurological condition of 9-year-old offspring. *Early Hum Dev* 2019; **129**: 38–44.
50. Carson C, Kelly Y, Kurinczuk JJ, Sacker A, Redshaw M, Quigley MA. Effect of pregnancy planning and fertility treatment on cognitive outcomes in children at ages 3 and 5: Longitudinal cohort study. *BMJ* 2011; **343**: 7820.
51. Hvidtjørn D, Grove J, Schendel D, Særke C, Schieve LA, Uldall P, Ernst E, Jacobsson B, Thorsen P. Multiplicity and early gestational age contribute to an increased risk of cerebral palsy from assisted conception: A population-based cohort study. *Hum Reprod* 2010; **25**: 2115–2123.
52. Källén AJB, Finnström OO, Lindam AP, Nilsson EME, Nygren KG, Otterblad Olausson PM. Is there an increased risk for drug treated attention deficit/hyperactivity disorder in children born after in vitro fertilization? *Eur J Paediatr Neurol* 2011; **15**: 247–253.
53. Agarwal P, Loh SKE, Lim SB, Sriram B, Daniel ML, Yeo SH, Heng D. Two-year neurodevelopmental outcome in children conceived by intracytoplasmic sperm injection: Prospective cohort study. *BJOG* 2005; **112**: 1376–1383.
54. Spangmose AL, Malchau SS, Schmidt L, Vassard D, Rasmussen S, Loft A, Forman J, Pinborg A. Academic performance in adolescents born after ART - A nationwide registry-based cohort study. *Hum Reprod* 2017; **32**: 447–456.
55. Källén B, Finnström O, Nygren KG, Olausson PO. In vitro fertilization in Sweden: Child morbidity including cancer risk. *Fertil Steril* 2005; **84**: 605–610.
56. Zhou CL, Xu GF, Yang Q, Wang HH, Guo MX, Xiong YM, Guo XY, Hou M, Jin LY, Sheng JZ, He L, Jin L, Huang HF. Diminished verbal ability among children conceived through ART with exposure to high serum estradiol in utero. *J Assist Reprod Genet* 2020; **37**: 1931–1938.
57. Chen X, Kong L, Piltonen TT, Gissler M, Lavebratt C. Association of polycystic ovary syndrome or anovulatory infertility with offspring psychiatric and mild neurodevelopmental disorders: a Finnish population-based cohort study. *Hum Reprod* 2020; **35**: 2336–2347.
58. Mainigi M, Rosenzweig JM, Lei J, Mensah V, Thomaier L, Talbot CC, Olalere D, Ord T, Rozzah R, Johnston MV, Burd I. Peri-Implantation Hormonal Milieu: Elucidating Mechanisms of Adverse Neurodevelopmental Outcomes. *Reprod Sci* 2016; **23**: 785–794.
59. Gore AC, Martien KM, Gagnidze K, Pfaff D. Implications of prenatal steroid perturbations for neurodevelopment, behavior, and autism. *Endocr Rev* 2014; **35**: 961–991.
60. Lester BM, Marsit CJ. Epigenetic mechanisms in the placenta related to infant neurodevelopment. *Epigenomics* 2018; **10**: 321–333.
61. Li H, Liu L, Dang M, Zhang W, Liu J. Increased susceptibility of mice obtained from in vitro fertilization to global cerebral ischemia-reperfusion injury: possible role of hydrogen sulphide and its biosynthetic enzymes. *Int J Neurosci* 2020; **130**: 533–540.
62. McQuillen PS, Miller SP. Congenital heart disease and brain development. *Ann N Y Acad Sci* 2010; **1184**: 68–86.
63. Terho AM, Pelkonen S, Opdahl S, Romundstad LB, Bergh C, Wennerholm UB, Henningsen AA, Pinborg A, Gissler M, Tiitinen A. High birth weight and large-for-gestational-age in singletons born after frozen compared to fresh embryo transfer, by gestational week: a Nordic register study from the CoNARTaS group. *Hum Reprod* 2021; **36**: 1083–1092.
64. Valenzuela-Alcaraz B, Serafini A, Sepulveda-Martínez A, Casals G, Rodríguez-López M, Garcia-Otero L, Cruz-Lemini M, Bijnens B, Sitges M, Balasch J, Gratacós E, Crispi F. Postnatal persistence of fetal cardiovascular remodelling associated with assisted reproductive technologies: a cohort study. *BJOG* 2019; **126**: 291–298.
65. Boutet ML, Casals G, Valenzuela-Alcaraz B, García-Otero L, Crovetto F, Cívico MS, Borrás A, Manau D, Gratacós E, Crispi F. Cardiac remodeling in fetuses conceived by ARTs: fresh versus frozen embryo transfer. *Hum Reprod* 2021; **36**: 2697–2708.
66. Sites CK, Wilson D, Barsky M, Bernson D, Bernstein IM, Boulet S, Zhang Y. Embryo cryopreservation and preeclampsia risk. *Fertil Steril* 2017; **108**: 784–790.
67. Rizzo G, Pietrolucci ME, Mappa I, Bitsadze V, Khizroeva J, Makatsariya A, D'Antonio F. Fetal Cardiac Remodeling Is Affected by the Type of Embryo Transfer in Pregnancies Conceived by in vitro Fertilization: A Prospective Cohort Study. *Fetal Diagn Ther* 2020; **47**: 772–778.
68. Spangmose AL, Malchau SS, Henningsen AA, Forman JL, Rasmussen S, Loft A, Schmidt L, Pinborg A. Academic performance in adolescents aged 15–16 years born after frozen embryo transfer compared with fresh embryo transfer: a nationwide registry-based cohort study. *BJOG* 2019; **126**: 261–269.
69. Balayla J, Sheehy O, Fraser WD, Séguin JR, Trasler J, Monnier P, MacLeod AA, Simard MN, Muckle G, Bérard A. Neurodevelopmental outcomes after assisted reproductive technologies. *Obstet Gynecol* 2017; **129**: 265–272.

SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:

Figure S1 Distribution of cortical grading scores of main sulci in fetuses of pregnancies conceived spontaneously or using assisted reproductive technology with fresh or frozen embryo transfer, excluding cases of transfer in the blastocyst stage.

Table S1 Distribution of crude Ages and Stages Questionnaire *Z*-scores below −2 SD from the spontaneously conceived study population mean

Table S2 Pearson's correlations between each Ages & Stages Questionnaire domain score and sulci depth in entire study population of pregnancies conceived spontaneously or using assisted reproductive technology (ART) with fresh or frozen embryo transfer, and in only those conceived by ART

Tables S3 and S4 Baseline, fertility and perinatal characteristics (Table S3) and fetal neurosonographic assessment (Table S4) of pregnancies conceived spontaneously or using assisted reproductive technologies with fresh or frozen embryo transfer, excluding cases of transfer in the blastocyst stage