Université de Montréal

Développement cognitif, moteur et verbal à l'âge de 2 ans suite à la procréation médicalement assistée: trouvailles de l'étude de cohorte, prospective et longitudinale « 3D »

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Infant cognitive, motor and language development at 2 years of age following conception through assisted reproductive technologies (ART) relative to natural conception: findings from the prospective, longitudinal, cohort "3D-Study"

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Résumé

Objectifs : L'impact a long terme des techniques de procréation médicalement assistée (PMA) en ce qui concerne le développement neurologique des enfants demeure source de controverse. Cette étude vise à évaluer et comparer le développement cognitif, moteur, et verbal des enfants âgés de 2 ans issus des techniques de PMA par rapport à ceux issus par une conception naturelle.

Méthodes: L'étude de cohorte prospective « 3D » a été menée entre 2010-2012. 2,366 femmes enceinte on été recrutées, dont 278 ont utilisé la PMA: stimulation ovarienne (OS), insémination intra-utérine (IUI), fertilisation in-vitro (IVF), injection intra-cytoplasmique du sperme (ICSI) ou maturation in-vitro (IVM). La conception naturelle a été définie comme une grossesse spontanée. Le développement cognitif, moteur et verbal a été comparé entre les groupes de PMA vs. conception naturelle a l'aide des outils standardisés et validés suivants : "The Bayley Scales of Infant and Toddler Development, 3rd ed. (BSID-III)" ainsi que des "MacArthur-Bates Communicative Development Inventories". Des modèles de régression linéaire ajustés évaluant l'impact des techniques de PMA sur les issus du neurodéveloppement ont été utilisés, tenant compte de la conception naturelle comme groupe de référence.

Résultats: Un total de 175 enfants dans le groupe PMA (62,9%) et 1.345 enfants dans le groupe de conception naturelle (64,4%) ont subi une évaluation neurodéveloppementale à 24 mois de vie. En ajustant pour les variables potentiellement confondantes, la PMA n'a eu aucun effet statistiquement significatif sur les scores de l'échelle cognitive BSID-III [B1 (SE) = -1,60 (0,9), p = 0,08], de l'échelle motrice [B1 (SE) = -1,33 (1,0), p = 0,18] ou dans les scores linguistiques du MacArthur-Bates [B1 (SE) = -0,28 (2.1), p = 0,89]. Aucune différence significative n'a été observée en comparant les techniques de PMA individuelles ou la conception sous-fertile, ni lorsqu'on compare les techniques in-vivo ou in-vitro (p>0,05).

Conclusion : Dans cette étude de cohorte prospective, les enfants nés suite a la PMA semblent avoir un développement cognitif, moteur et verbal similaire aux enfants nés après la conception naturelle à l'âge de 2 ans. Ces résultats pourraient être utiles dans le counseling clinique des patients utilisant des techniques de PMA.

Mots clés: neurodéveloppement, procréation assistée, développement cognitif, développement moteur, développement verbal.

Abstract

Objective: Whether assisted reproductive techniques (ART) have an impact on infants' long-term neurodevelopmental outcomes remains controversial. In this study, we compared infants' cognitive, motor, and language development at 2 years of age following ART relative to natural conception.

Methods: The prospective cohort "3D-Study" was carried out from 2010-2012. 2,366 pregnant women were recruited, of which 278 conceived with ART: ovarian stimulation (OS), intrauterine sperm insemination (IUI), in-vitro fertilization (IVF), intra-cytoplasmic sperm injection (ICSI) or in-vitro maturation (IVM). Natural conception was defined as the unassisted establishment of pregnancy. Cognitive, motor, and language neurodevelopmental outcomes were compared between ART and natural conception groups at 24 months using "The Bayley Scales of Infant and Toddler Development, 3rd ed. (BSID-III)" and the "MacArthur-Bates Communicative Development Inventories". Adjusted linear regression models evaluated the effect of ART on neurodevelopmental outcomes, using natural conception as reference.

Results: 175 infants in the ART group (62.9%) and 1,345 infants in the natural conception group (64.4%) underwent neurodevelopmental assessment at 24 months of age. After adjusting for relevant confounders, infants born after ART showed no difference in BSID-III cognitive scores [B_1(SE) = -1.60(0.9), p=0.08], composite motor scores [B_1(SE) = -1.33(1.0), p=0.18] or MacArthur-Bates language scores [B_1(SE) = -0.28(2.1), p=0.89]. No statistically significant difference was observed when comparing independent ART techniques or subfertile conceptions, nor comparing in-vivo or in-vitro techniques (p>0.05).

Conclusion: In this prospective cohort, infants born after ART had no significant differences in cognitive, motor, and language development relative to infants born following natural conception at 2 years of age. These findings may be useful in the clinical counseling of patients undergoing ART.

Keywords: infant neurodevelopment, assisted reproduction, cognition, motor skills, language development

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Keywords and Abbreviations

ART – Assisted reproductive technology/techniques

ASRM – American Society of Reproductive Medicine

BAS – British Ability Scales

Bayley III – Bayley Scales of Infant and Toddler Development (3rd Ed.)

CIHR – Canadian Institute of Health Research

CNS – Central nervous system

eSET – Elective single embryo transfer

ESHRE - European Society for Human Reproduction and Embryology

EC – Expressive Communication Scale (Bayley-III, Language scale)

GAC – General Adaptive Composite (Bayley-III, Adaptive behaviour)

ICSI – Intra-cytoplasmic sperm injection

IRNPQEO – Integrated Research Network in Perinatology of Quebec and Ontario

IUI – Intra-uterine insemination

IVF – In-vitro Fertilization

IVM – In-vitro Maturation

LBW – Low birthweight

NIH – National Institute of Health

OI – Ovulation induction

OS – Ovarian stimulation

RC – Receptive Communication Scale (Bayley-III, Language scale)

SE – Socio-Emotional Scale (Bayley-III)

SES – Socioeconomic status

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Background

Infertility in the Public Health context

Whereas research efforts in human reproduction have traditionally focused on the biological causes of infertility, its public health dimensions have received relatively little attention. Because the global focus of public health policies and programs has justifiably been on containing population growth and providing affordable, safe, and effective family planning services, the inability to procreate has not traditionally been a priority in many developing countries [1]. However, in developed nations like the U.S. and Canada, overall rates of infertility are now between 10-20% and on the rise despite public health prevention strategies and increased access to fertility treatments [1, 2]. This apparent paradox is rooted in the social determinants of health, which play a particularly important role in reproductive outcomes [3]. What some attribute to positive changes in social paradigms, such as the reduction in gender-based inequalities and the rise of the feminist movement [4, 5], women are increasingly likely to take on more active roles in society during their reproductive years, thereby delaying childbearing to later in life. This new reality has several identifiable causes: the lengthening of the period of education, the more frequent entry of women into the labour market, the uncertainties of this market, and the availability of effective and reliable contraceptive methods which prevent early unplanned pregnancies [3]. This trend is unlikely to be reversed in the near future [3], and given that female fertility declines as a function of age, couples are increasingly resorting to assisted reproduction in order to build families [6]. Similarly, despite medical advances in the understanding of disease, other factors, which play a role in infertility, like obesity, metabolic syndrome, diabetes, and polycystic ovarian syndrome (PCOS), have all seen either an increase in their incidence, or a plateau at best. This too contributes to a greater use of assisted reproductive techniques.

In Canada, reports indicate that the number of ART cycles performed increased by around 50% from 2001 to 2006 [7]. This number is expected to further increase as provinces begin to provide coverage and public funding for ART [8]. Indeed, in order to meet the changing conditions of society and tackle the decreasing birth rates in Europe and the U.S. [9, 10], which now stands at an average of 1.6 children per family, many

governments and public health systems are facilitating access of infecund couples to fertility treatments [7].

Consequences of a changing paradigm – A call for action

In light of the increasing proportion of live births achieved with the help of assisted reproductive technologies (ART), which now stands at 1.4 - 5% in North America [11], it is imperative to understand and evaluate the biological, psychological, and social impact that the rise in ART treatments may bear on society, the afflicted couple and the ensuing children.

Infertility treatments and long-term infant outcomes

Following the development of in-vitro fertilization (IVF) in the 1970's and the discovery of its potential advantages and clinical applicability for the treatment of infertility amongst affected couples, over five million pregnancies have been achieved worldwide by assisted reproductive technologies (ART) [12]. Though no strict definition currently exists, "ART" is an all-encompassing term, which refers to all treatments, or procedures that include the in-vitro handling of both human oocytes and sperm or of embryos for the purposes of establishing a pregnancy [13, 14]. Clinically, the treatment of infertility and provision of assisted reproduction is carried through the utilization of one or more of the following methods: intrauterine sperm insemination (IUI), ovarian stimulation (OS), ovulation induction (OI), as well as reproductive techniques whereby both oocyte and sperm are handled in-vitro, such as IVF (in-vitro fertilization) and ICSI (intra-cytoplasmic sperm injection). Gamete and embryo cryopreservation, in-vitro-maturation (IVM) as well as oocyte and embryo donation are frequently utilized in this context as well [13, 14].

In Canada, reports indicate that the number of ART cycles performed increased by around 50% from 2001 to 2006. In 2010, the Quebec government began to cover infertility treatments, including in-vitro fertilization cycles and intracytoplasmic sperm injections, through the Quebec Program for Assisted Reproduction. Despite recent changes in the province of Quebec, this number was expected to further increase as provinces began to provide coverage and public funding for IVF [7, 8]. However, the

program changed on November 10, 2015 and is currently governed by Bill 20. This bill: preserves RAMQ coverage for infertility consultations, preserves RAMQ coverage for up to 9 cycles of intrauterine insemination, removes RAMQ coverage for IVF except in cases of cancer, mandates single embryo transfer for ALL women age 36 and younger, mandates transfer of no more than 2 embryos in women age 37 or older, removes RAMQ coverage for the purchase of donor sperm, and for eligible patients (no prior children, vasectomy or tubal ligation) replaced RAMQ coverage for fertility treatments with a tax credit (up to a maximum of $20,000) based on family income

Nevertheless, despite changes in policy, given the increasingly prevalent treatment of infertility, it is imperative that healthcare professionals become aware of both desired and undesired consequences such treatments may infer on a couple, as well as the ensuing children [15]. Given the unprecedented rise in ART-mediated pregnancies worldwide, numerous studies have since sought to establish the maternal, obstetrical, and neonatal outcomes following such methods of conception [16-18]. There have been a number of follow-up studies of children born from ART addressing their neurodevelopmental status, which have been reviewed exhaustively [12, 19] as practitioners have looked for answers and information for counselling expectant couples. Evidence on the long-term effects of infertility treatments remains equivocal, with the main criticisms of existing studies being that they frequently do not account for birth plurality and/or chorionicity if twins are even included [20], have small sample sizes [21], are rarely able to characterize the infertility treatment protocols except broadly, rely on clinic-based populations, have inadequate selection of comparison children[21], or fail to account for known determinants of growth and development [19]. Indeed, although the short-term perinatal outcomes in children born after assisted reproduction have been thoroughly investigated, inconsistency concerning long-term development persists [12, 17, 18, 22, 23]. In particular, neurodevelopmental outcomes as a function of the type of ART used is lacking [24].

Neurodevelopmental disorders – Definition, criteria and diagnosis

Neurodevelopmental adverse outcomes and disorders lack a strict standard definition. Over time, it has become clear that neurodevelopment relates to both physical,

psychological, and social development, and that overlap between the developments of each facet of an infant is as complex as it is interdependent. Neurodevelopmental disorders (NDDs) develop over time and are associated with a wide variation of mental, emotional, behavioral, and physical features. Commonly known NDDs include autism spectrum disorders, cerebral palsy, attention deficit/hyperactivity disorder (ADHD), communication, speech, and language disorders, and genetic disorders such as fragile X syndrome (FXS) and Down syndrome. These various disorders, at symptom level, seem to share similar behavioral symptoms and diagnostic criteria; however, in the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV), diagnostic criteria preclude the comorbid diagnosis of multiple disorders such as autism and ADHD. This is a significant limitation in the diagnostic criteria, as symptoms frequently overlap and best practice treatment suggestions may differ depending on one's presentation.

This issue has been recognized in the recent revisions of the DSM-IV and is reflected in the proposed changes of the DSM-V. A new cluster of NDDs is proposed, which includes six categories: Intellectual Developmental Disorders, Communication Disorders, Autism Spectrum Disorders, Attention Deficit/Hyperactivity Disorder, Learning Disorders, and Motor Disorders. This new cluster has eight main features, highlighting the characteristics of deficits/ delays in "maturationally-influenced" psychological features, cognitive impairment, genetic influences, and overlap amongst the NDDs [25]. The recognition of the prevalence of comorbidities in this cluster is important, especially for school psychologists, in order to gain a more complete and comprehensive insight into a child's array of capabilities and deficits without being limited by the possibilities of exclusion due to outdated diagnostic criteria. Especially since these disorders often overlap, differential diagnosis is necessary to provide appropriate services. For the purposes of research standardization, neurodevelopmental outcomes is a composite term and typically refers to cognitive, neurologic, and/or sensory outcomes, where neurodevelopmental impairment has been defined as the presence of one or more of the following: 1) Cognitive delay based on scores on standardized cognitive tests that are 2 standard deviations (SD) below the mean. As an example, this would correspond to score of 70 or below on the Mental Developmental Index of the Bayley Scales of Infant Development. 2) Moderate to severe cerebral palsy (CP) defined

as a score of ≥2 on the Gross Motor Function Classification System (GMFCS), Hearing deficit/loss requiring amplification, or 3) Severe visual impairment with visual acuity of 20/200 or less in the better-seeing eye with best conventional correction (definition of legal blindness). In addition, behavioral, psychological, and functional outcomes are increasingly being recognized as important long-term neurodevelopmental outcomes and will be discussed within this review.

Neurodevelopment and embryogenesis

In order to understand the adverse neurodevelopment of infants, it is imperative to first summarize the normal embryological development of the brain and central nervous system (CNS). The development of the brain is an orchestrated, tightly regulated, and genetically encoded process with clear influence from the environment, including mode of conception [12]. Any deviation from this program early in life can result in a critical insult and an ensuing neurodevelopmental disorder. Indeed, depending on the specific timing of said insult, it might lead to a distinct pathology later in life [26]. Because these pre-defined regulatory process are amenable to intrinsic as well as external influences, there are many causes of neurodevelopmental disorders, which can range from psychosocial deprivation, genetic and metabolic diseases, immune disorders, infectious diseases, nutritional factors, physical trauma, and toxic and environmental factors. Some neurodevelopmental disorders—such as autism and other pervasive developmental disorders—are considered multifactorial syndromes. Indeed, vulnerable periods during the development of the nervous system are sensitive to environmental insults because they are dependent on the temporal and regional emergence of critical developmental processes (i.e., proliferation, migration, differentiation, synaptogenesis, myelination, and apoptosis). Evidence from numerous sources demonstrates that neural development extends from the embryonic period through adolescence [27].

The normal development of infants is clinically described through expected landmarks achieved as a function of age. Table 1 depicts the non-motor landmarks as a function of age for the cognitive, language, emotional and social domains, as described by Zeanah et al. [28]. The last column, denoted in bold, describes the expected attainments by 18 to 36 months of age, the age group which this project evaluated. These

landmarks are theorized to be the result of adequate central nervous system development, which begins in-utero.

Physiologically, neurodevelopment begins in the early prenatal stage with a complex neurological development that begins with proliferation of radial glia and neurons. These continue to develop in the postnatal years. This process is not complete until almost 3 years of age. Migration of neurons, which occurs from the 2nd to the 6th month of gestation, and again within the cerebellum post-natally, is a very important and complex process. Synapse formation, which occurs essentially in the last trimester as well as in the first 2 years of life, is critical to ongoing functioning and development. Myelination is an important process that begins in the second half of gestation and goes on to adolescence, with different systems myelinating at different times, thus becoming amenable to physiological insult at different stages in life. Indeed, it is suspected that abnormalities in maturation of the central nervous system may underlie neurodevelopmental disorders [29].

Risk factors for infertility

Infertility is a complex disorder with significant medical, psychosocial, and economic aspects. Aside from genetic and hereditary factors, which are family-dependent, a number of lifestyle factors have been associated with subfertility, and these include: advanced maternal age [30], smoking [31], obesity, the presence of metabolic syndrome and other medical co-morbidities like diabetes, and cardiovascular disease, alcohol intake [32], at-risk sexual behavior, and physical activity [33].

Known Effects of ART techniques

While ART techniques have allowed millions of women with subfertility diagnoses to achieve pregnancy, they have also been associated with adverse obstetrical outcomes. The precise reasons for this increase in adverse outcomes are not clear, but potential candidates include: maternal and paternal characteristics, underlying medical conditions associated with subfertility and infertility, sperm factors, the use of fertility medications, laboratory conditions during embryo culture, culture medium, cryopreservation and thawing, prenatal genetic diagnosis (if performed), differences in

obstetrical management, increased proportion of multiple gestations and vanishing twins, or a combination of these factors[16]. The main complications described include: higher risks of congenital anomalies, early pregnancy loss, spontaneous abortion, ectopic pregnancy, multiple pregnancy, rates of preterm delivery, low birthweight, as well as hypertension, diabetes, and delivery via cesarean section [16].

Biological plausibility of ART leading to adverse developmental outcomes

Although insemination to a great extent resembles spontaneous conception, there are several biologically as well as non-biologically plausible reasons for increased vigilance regarding the mental development of children conceived after ART or OI. These procedures involve medical hyperstimulation, handling, and culture of gametes and early embryos at a particularly vulnerable period of development [34, 35]. Studies imply that ART may affect the epigenetic control in early embryogenesis [36, 37], and ART has been associated with an increased risk of imprinting disorders in both experimental animal studies and epidemiological human studies [38, 39]. Thus, medical ovarian hyperstimulation could impact fetal neurodevelopment [38], and the use of different culture media used for IVF affects the phenotype of the offspring by significantly altering physical and biochemical parameters, such as birth weight [40], blood pressure, fasting glucose, pubertal gonadotropin levels [41], growth factors, and blood lipids [42]. Moreover, the altered selection of the fertilizing spermatozoa may be of importance, especially in the case of ICSI, in which a single spermatozoon is introduced into the oocyte by micro-insemination.

However, possible neurodevelopmental deficits in children born after ART or OI may originate from several other factors rather than the treatments. The procedures are major contributors to multiple gestations, which are at risk of preterm delivery, low birth weight, and small for gestational age [43]—three important risk factors for neurodevelopmental deficits [44-46]. Even when restricted to singletons, however, children born after ART and OI still have a higher rate of preterm delivery and reduced fetal growth [47, 48]. Further, differences in neurodevelopment could be due to the underlying subfertility [48, 49] or known or unknown conditions in the parents. Thus, men with low sperm quality are more likely to have chromosomal abnormalities that they

may pass on to the offspring [50], and as a result ICSI children may be at increased risk for delayed neurodevelopment. Finally, a number of predictors of neurodevelopment (e.g., parental education, socioeconomic status) may differ between infertile couples and couples with no problems conceiving spontaneously [51-53]. These risk factors are elucidated on Table 2.

The state of current knowledge

In order to elucidate the current literature on the topic of neurodevelopmental outcomes following ART, a systematic search was undertaken on PubMed, EMBASE, MEDLINE and Scopus databases using the following keywords: Infertility; Reproductive Techniques; Assisted, Fertilization; In-vitro, Insemination, Artificial; Cognition, Pediatric; Motor Skills; Language; Neurodevelopment, Pediatric; Bayley Scale of Infant and Toddler Development and MacArthur-Bates Communicative Development Inventories. The BOOLEAN search code can be found as a supplement at the end of this document. No date or language restriction was considered and retrieved articles were evaluated for additional bibliographic references using the snowball method. Of an initial search retrieving 763 articles, 695 were excluded with reasons, and 68 were evaluated in full as they addressed the topic of this review. The research flowchart appears in the supplement to this document as well. The findings of this review are herein subdivided according to separate neurodevelopmental domains, which will be addressed in the study, namely, cognitive, motor, and language outcomes after assisted reproduction.

Cognitive development following ART

Cognitive development focuses on a child's development in terms of information processing, conceptual resources, and perceptual skills. Since 1998, when Bowen et al. found a significantly lower mental development index (MDI) scores in ICSI children at the age of 13 months compared to spontaneously conceived children, much attention has been drawn to this topic [54]. Though this study has been widely criticised because of the small sample size it used, as well as the inclusion of premature children, multiple pregnancies and children born after cryopreserved embryos, it paved the way for numerous other studies that have not consistently found differences in cognitive development related to the mode of conception. For example, a study by Place et al.

concluded that the group differences between ICSI and spontaneous conception disappeared when the model was adjusted for levels of parental education [55]. Yet, in a prospective follow-up study by Knoester et al., though the authors failed to account for underlying infertility factors, cognitive development among ICSI singletons was lower than among IVF and naturally conceived singletons [56]. On the other hand, Cederblad et al. [57] found no differences in cognition or intelligence as a function of the mode of conception, as did Leulens et al. [58], who in a continuation of two large-scale, multicentre studies on the development of 5-year-old ICSI children, found comparable cognitive development until age 8. In a separate study, Leslie et al. describe similar findings [59].

In a prospective, case-controlled, matched follow-up study by Winter et al. the cognitive abilities and motor skills of 5- to 6-year-old singletons born after PGD (n = 47) were assessed in comparison with 49 ICSI and 48 spontaneously conceived children. The overall cognitive development of PGD singletons did not differ from controls [P = 0.647, $\eta(2) = 0.006$; 95% confidence interval (CI) (0, 0.043)]. The partial IQ scores for Verbal and Performance intelligence revealed similar results. Analysis of motor development based on the total score as well as subscales did indicate a significant difference between the three conception groups [P = 0.033, $\eta(2) = 0.050$, 95% CI (0, 0.124)]. Post hoc analysis indicated that the significant difference was situated between performances of ICSI and SC children. Balance capacities [P = 0.004, $\eta(2) = 0.079$, 95% CI (0.025, 0.163)] and its post hoc analysis yielded equivalent results. Motor capacities of PGD singletons, however, did not differ from any of the two other conception groups.

The study by Knoester et al. [56] follow-up of singletons conceived by ICSI (n = 83) showed lower IQ scores than IVF singletons (n = 83) (adjusted mean difference IQ: 3.6 [95% confidence interval (CI) −0.8, 8.0]). However, after categorizing IQ outcomes (<85, 85–115, >115), no significant difference in the distribution of IQ was found. Singletons conceived by ICSI (n = 86) achieved lower IQ scores than NC singletons (n = 85); the adjusted mean difference varied between 5 and 7 points (5.6 [95% CI 0.9, 10.3]; 7.1 [95% CI 1.7, 12.5]) depending on the covariates included in the model. Adjustment for prematurity did not change the results. Percentages in IQ categories <85, 85–115, and

>115 were 12%, 64%, and 24% for ICSI and 6%, 54%, and 40% for NC, respectively. In the study by Leunens et al. ICSI children's IQs were significantly higher than those of SC children [ICSI 112, CI 95% (105–118), SD 14.8; SC 107, CI 95% (101–113), SD 13.6, $P = 0.001$, Cohen's $d = 0.35$]. However, it should be noted that these IQ scores are still situated within the same SD, thus resulting in similar IQ ranges as shown in the confidence intervals indicated in the test manual, relevant to clinical practice, with Cohen's d showing that this is a small effect ($d < 0.50$)[52].

Despite these conflicting results, two large systematic reviews of over 80 studies have addressed this topic, and both conclude that "there is sufficient data to support that there is no difference in development and mental health between IVF and spontaneously conceived children" [12, 19] and though only a minority of studies showed significantly lower scores on language tests among IVF children compared with spontaneously conceived children [60], "most studies showed no associations with cognitive […] development" [12]. In general, the longer-term cognitive health of children born from ART treatments appears reassuring, and is very similar to that of naturally conceived children; however, further studies are required to explore any association with depression, and its causality in more detail. The higher incidence of multiple births, preterm births, and low birthweight infants following IVF and ICSI must be considered separately from the impact of the technique itself. Also, mothers of IVF children are generally older than mothers who give birth without medical intervention, and attempts to match natural conception mothers for maternal age have presented difficulties, as has matching for birth order of the target child and number of children in the family.

Motor development following ART

Motor development refers to changes in children's ability to control their body's movements, from infants' first spontaneous waving and kicking movements to the adaptive control of reaching, locomotion, and complex sport skills. Motor development can be divided into two sections: gross motor development and fine motor development. Gross motor development involves the development of the large muscles in the child's body. These muscles allow a child to sit, stand, walk and run, among other activities. Fine motor development involves the small muscles of the body, especially in the hand. These

are concerned with grasping small objects, and pointing, among others [61]. In a population-based study, the children conceived with ICSI, compared with those born naturally from subfertile couples, did appear to be particularly prone to delays in gross motor development (e.g., sitting without support at 9 months and walking without support at 16 months [20]. On the other hand, Leunens et al. conducted a long-term follow-up of children's motor skills after ICSI, at 8 and 10 years respectively, and found no significant differences relative to naturally conceived children [58, 62]. In a study by Koivurova et al. which compared a Finnish IVF cohort to a matched cohort of naturally conceived children found that though the growth of IVF children was behind that of control children during the first 3 years of life, their psychomotor development was similar [63]. When a cohort of twins alone was considered, no differences were elucidated either [64]. In order to further clarify whether the techniques themselves increased the risk of poor motor development, a prospective study by Bonduelle et al. found no differences in motor development between ICSI and IVF children, with similar rates of motor disability as the naturally conceived population [65]. Sutcliffe et al. conducted a prospective study on 208 ICSI and 211 SC singletons at the age of 17 months, who were assessed all by one single examiner with an identical protocol for both groups. No difference in neurodevelopmental outcome was found between ICSI and SC differences regarding the psychomotor development of children [66]. All in all, relative to cognitive development, this review finds larger consensus regarding the safety of ART techniques in this regard in the literature. Nevertheless, prospective evidence of motor skills at 24 months of age evaluated with gold-standard testing, is lacking.

Language/verbal development following ART

A population-based study from Denmark found no effect of fertility or treatment on attention skills, but comparing the infertility treatment with the subfertile group, it found that that the infertility-treated group overall had a slight delay in achieving language milestones [20]. Similarly, a prospective population based cohort study from the Millennium Cohort in England, children born after assisted reproduction performed consistently better in verbal ability tests (3.8 (−0.2 to 7.9) at age 3 and 3.5 (0.2 to 6.8) at age 5) using the British Ability Scales (BAS II), which suggests that on average these

children are three to four months ahead; this difference did not completely disappear with adjustment for confounders. On the other hand, children born after infertility treatment had lower mean scores in non-verbal tests (−1.2 (−4.1 to 1.6) after assisted reproduction and −1.5 (−3.5 to 0.4) after induced ovulation) and in spatial ability tests (−2.7 (−6.9 to 1.6) after assisted reproduction), though the differences were not clinically or statistically significant [67]. Interestingly, in a study by Pinborg et al. women were asked to subjectively rate their infant's speech development on a 5-point scale from 'much better' to 'much worse' compared with children at the same age level[68]. The IVF/ICSI twin mothers were more likely to assess their children's speech development better than other children at the same age compared with the control twin mothers, but less likely compared with the IVF/ICSI singleton mothers (Table IV). The differences in speech development remained after adjustment for birthweight.

Table 1. Non-motor landmarks as a function of age for the cognitive, language, emotional and social domains [28]				
Domain	**First 2 months**	**2 to 7 months**	**7 to 18 months**	**18 to 36 months**
Cognitive	Cross-modal fluency allows translation of perceptual experiences across different modalities; remarkable ability to detect invariant aspects of various perceptual experiences; habituation, operant and classical conditioning present prenatally	Enhanced habituation. classical conditioning, and operant conditioning	Differentiation of means and ends; object permanence; inter-subjectivity makes it possible for infants to share thoughts feelings and desires with others and to be aware of subjective experiences; visual memory predicts later intelligence; enhanced participation	**Symbolic representation as reflected in true symbolic play; recognition of gender differences; ability to entertain imaginings that are different from reality for first time**
Language	Crying major means of communication; occasional cooing sounds begin after several weeks	Cooing becomes responsive; bilabial "raspberry" sounds; consonant vocalizations appear and progress to polysyllabic babbling (e.g.”gagagaga" or "lalalala1a")	Intentional communication appears and gestural communication dominates; understanding of a word as an agreed-upon symbol to designate an object; may imitate or spontaneously produce speech sounds or words without comprehension then gradually begin to express word sound correctly across contexts	**Blossoming of expressive language leads to 2 and then 3-word combinations; expressive vocabulary grows from an average of 50 words at 18 months to 500 at 36 months; receptive language begins to decontextualize so that words themselves become meaningful without other cues**
Emotional	Distress, contentment, and interest are discrete emotions detectable at birth	Distress differentiates into sadness. disgust and anger; contentment differentiates into joy and contentment; interest differentiates into interest and surprise	Emotional expressions of smiling. pouting, and anger begin to be used instrumentally to help infants obtain desired goals; affective sharing in which caregivers match infant positive affect through another sensory modality, may be observed. Infants may be relatively impervious to frustration at this time; social referencing to caregivers to resolve emotional uncertainty observed	**"Moral" emotions appear: Embarrassment, empathy, and envy after 18 months, and guilt, pride, and shame after 24 months**
Social	Physical attributes of the baby draw adults into involvement and interaction	Enhanced interest and ability to engage adults in synchronous and reciprocal social interchanges; play periods alternate with timeouts; affective mismatches during interactions stimulate coping capacities of the infant	Preferred attachments to a small number of care-giving adults develops; stranger wariness and separation protests appears; social referencing to resolve uncertainty	**Enhanced capacity for expressing needs; conflicting agendas of others leads to increased negotiations with caregivers; increased interest in peer-relatedness; begins interactive play; concerns with personal possessions and sensitive to being included or excluded**

Adverse neurodevelopmental outcomes and ART

Adverse infant neurodevelopmental outcomes affect up to 8.5% to 16% of the population [69, 70], and can be classified into numerous categories depending on the suspected aetiology and common characteristic leading to the development of perceived deficits. Globally, and for the purposes of standardization, each outcome may be categorized into one of the following domains, which have been validated with the Bayley Scale of Infant and Toddler Development [71-73]: (1) cognitive development, including educational achievement; (2) behavioural and socio-emotional development, including coping and temperament; (3) psychomotor development; and (4) language acquisition. Examples of important risk factors associated with adverse neurodevelopmental outcomes are domain-dependent, and appear in the following table (Table 2):

Table 2. Risk factors associated with adverse neurodevelopmental outcomes

Developmental Outcome	Cognitive [74-76]	Language [77-79]	Motor [74, 80]	Socio-emotional [81, 82]
Risk Factors	Prematurity, Low birthweight, maternal education, maternal ethnicity, postpartum depression, smoking, alcohol and toxic exposure during pregnancy, maternal obesity, and gestational diabetes.	Family history of delayed speech acquisition, parental education, low SES, prematurity, mental retardation, gestational diabetes, fetal alcohol syndrome, congenital malformations.	Prematurity, low birthweight, low APGAR score, congenital anomalies, multiplicity, low SES, neonatal disease, teratogen exposure during pregnancy	Maternal attachment, parental maternal illness, low SES, postpartum depression

The largest systematic review which addressed this topic, analyzed over 80 studies describing long-term outcomes following ART, and determined that relative to natural conception, infants born following ART showed no deficits in psychomotor development, but concluded that only a few quality studies investigated cognitive or behavioural development, and that therefore more specific data was required to determine the impact of ART on these outcomes [12]. Some of the limitations described in this extensive review include the methodology used to assess neurodevelopmental outcomes which was largely heterogeneous, ranging from self-reported data, to data collected by objective testers using validated methods [12, 83]. In addition, age ranges

amongst the infants studied had a wide scope. Such finding is particularly important because more reliable measures of neurodevelopmental outcomes (e.g., intelligence) and more specific cognitive functions can be obtained at older ages, and because the pattern of cognitive deficits may change as the child grows older: early cognitive deficits may not reflect long-term influences on cognitive development, whereas deficits in more complex cognitive functions may only be detectable in later childhood or adolescence.

Direct comparisons with other studies of children born after ART and cognitive development are difficult because researchers have used a wide range of cognitive measures and have sampled different groups of children, and there is great variation in methodological quality [83]. One of the major limitations of previous studies is the impossibility to analyze results from sub-cohorts of children born after specific form of fertility treatment. Given the availability of such data in the "3D-study", we sought to answer this question within a Canadian population using this data. More precisely, this study seeks to answer the call from the NIH which states that "continued research is needed to overcome lingering data gaps in light of the equivocal literature for many neurodevelopmental disabilities relative to ART, increasing utilization of services and changes in the clinical management of infecund couples such as the adoption of natural cycles or in-vitro maturation treatment options. […] cohorts with longitudinal assessment of the multifaceted nature of neurodevelopment across critical and sensitive windows is paramount for the development of empirically based guidance for clinical and population health." [84].

Objective

From this large-scale question, this focused literature review seeks to understand the impact of assisted reproduction on the neurodevelopmental outcomes of children and asks: "Do children conceived after assisted reproduction have similar neurodevelopmental outcomes as children conceived through natural conception at 24 months of age?" Using standardized and validated tools designed to appraise cognitive, language, and motor skills (Bayley Scale of Infant and Toddler Development, 3rd edition and MacArthur Bates Communicative Development Inventories), and using a prospectively followed cohort from a well-designed longitudinal research study ("3D-

Study"), the objective of the present study is to measure cognitive and neurodevelopmental outcomes at 2 years of age following conception through type-specific ART, relative to natural conception.

Specific Aims

I. To describe the ART-type utilization trend in Quebec and establish baseline medical and socio-demographic differences between an ART and a non-ART cohort.

II. To quantify cognitive, motor and language development of two-year old infants prospectively followed since conception using the Bayley-III scale of infant and toddler development and MacArthur Bates Communicative Development Inventories.

a. To stratify the results according to ART vs. non-ART conception.

b. To stratify the ART cohort according to type of ART used and describe type-specific and age-matched risks relative to the non-ART cohort using each one of the Bayley-III scales and MacArthur Bates Communicative Development Inventories, as a continuous variable:

i. Cognitive
ii. Language
iii. Motor

c. To adjust the analysis for potential confounders within a breadth of conception and pregnancy-related data available.

The PICOS Model

	Population	Infants of 2 years of age
	Intervention	Assisted reproductive techniques
	Comparison	Natural Conception
	Outcome	Cognitive, language, motor skills as measured by the Bayley-III scale and the MacArthur Bates Inventories.
	Study Design	Analysis from a prospective, longitudinal cohort study.

Research Protocol

Data source

Integrated Research Network in Perinatology of Quebec and Eastern Ontario

The 3D study is a collaborative effort from the Integrated Research Network in Perinatology of Quebec and Eastern Ontario (IRNPQEO). IRNPQEO links five universities and their affiliated teaching obstetrics/pediatrics hospitals: four in Quebec and one in Ontario. The mission of this multi-institutional network and its transdisciplinary research programme is to serve as a catalyst: 1) To enhance the quality and impact of perinatal research in Quebec and in Canada; 2) To train the next generation of researchers in an environment that reflects the Canadian Institute of Health Research (CIHR) four pillars; and 3) To create an innovative regional/provincial clinical research model ensuring evidence-based care. The IRNPQEO is a coalition of several established and emerging teams and individual researchers, many of whom are national and international leaders. IRNPQEO researchers share the major premise that pregnancy is the '*foundation period*' for future health and development. The coalition brings to focus a complementary expertise in genetics and teratology, obstetrics, neonatology and pediatrics, developmental psychology and neurology, nutrition, sociology, anthropology, knowledge transfer, health administration and health pedagogy. Through this transdisciplinary collaboration, its aim is to address critical knowledge gaps in perinatal health as well as to synthesize and to transfer essential knowledge and recommendations to health and social policy makers. Important knowledge gaps concerning the long-term impact of various adverse exposures (environmental or genetic) during pregnancy on the health of future generations are due to the lack of prospective clinical research transcending obstetrics (pregnancy) into the neonatal and pediatric years. To address this concern, the network conducted the prospective, longitudinal 3D cohort study.

Assisted Reproductive Technologies (ART) Cohort Study

A proportion of the births in the 3D cohort stem from assisted reproductive techniques, which include ovarian stimulation (OS), intrauterine sperm insemination (IUI), in-vitro fertilization (IVF), intra-cytoplasmic sperm injection (ICSI) or in-vitro

maturation (IVM). On the other hand, natural conception was defined as the unassisted establishment of pregnancy. As mentioned previously, the general 3D-study cohort is used to identify patients for etiological studies of adverse birth outcomes, and one of the main pillars for study addresses the long-term consequences of ART.

It is known that the number of ART births has more than doubled over a decade in North America [85]. Poor perinatal health observed following ART is classically attributed to higher-order multiple birth rates. However, more recent studies have demonstrated an increased risk of morbidity and mortality among ART singletons [38, 86]. Because infertility is multifactorial in origin, it remains unclear if this increase is due to the underlying infertility disorder and/or to the treatments used [87-90]. Recent data suggests that the underlying effect is not attributed to the couple's subfertility [91]. Furthermore, there is increasing evidence that genetic factors as well as technical factors (hormonal stimulation and culture media) can impair epigenetic processes controlling implantation, placentation, organ formation and fetal growth. There are reports in humans of imprinting disorders associated with methylation abnormalities in key imprinted genes amongst ART-conceived children [92]. Little is known concerning ART-associated long-term child health. Some small studies have evaluated the relationship between ART and neurodevelopmental outcomes [52, 93-97], but results have been inconsistent [98, 99]. Equivocal results could be explained by methodological differences and lack of longitudinal studies. The IRNPQEO ART study provides a unique opportunity for longitudinal follow-up, beginning in the pre-pregnancy period to infancy to understand the ART associated epigenetic changes and long-term infant health.

3D Study

The "3D" study, which stands for "*Découvrir, Développer, Devenir*" (Discover, Develop, Become) is a cohort of paired triads of mother-father and child, which were prospectively followed over the gestational period and up until 24 months post-partum. A total number of 8 primary study points were planned, and health data including medical history, as well as paternal and maternal-fetal variables during pregnancy were obtained, according to the following timeline (Figure 1, incomplete list of variables):

In addition, a bio-bank consisting of biological samples from the mother, father and child were collected at different times during the study follow-up.

The 3D study built a Core Pregnancy Cohort that is now used to create and identify specific sub-groups for analysis and study. Indeed, under the core cohort, a number of projects and sub-analyses were originally planned:

- Project 1: Assisted Reproductive Technologies (ART) Cohort Study
- Project 2: Determinants and consequences of intrauterine growth restriction
- Project 3: Effect of bacterial vaginosis, vitamin D status and stress on preterm birth
- Project 4: Birth Defects Study: The role of Copy Number Variants in fetal anomalies

The general Core Pregnancy Cohort is used to identify patients for etiological studies of adverse birth outcomes (preterm, IUGR, birth defects) and for the study of intrauterine factors on long-term infant neurodevelopment, metabolic and cardiovascular health. This core cohort also includes a group of ART pregnancies, which can be studied against comparator subjects derived from the core cohort. Detailed infant follow-up is conducted on all live births belonging to the cohort.

The IRNPQEO core pregnancy cohort originally aimed to recruit 3,500 trios comprised of mother, father and child from May 2010 until May 2012 at the beginning of pregnancy. This study aimed to collect rich information on environmental, nutritional, genetic and psychosocial exposures during pregnancy. Pregnant women meeting inclusion criteria bearing a singleton fetus were recruited in the 10 collaborating centres during the 1st trimester (8-13 $^{6/7}$ weeks) of pregnancy. Approximately 80% of women in these centres registered for prenatal care in the first trimester. Patients were recruited at the time of hospital visits for routine 1st trimester ultrasound testing. An over-recruitment of 10% was originally planned to allow for drop-outs and loss to follow-up up to delivery. All recruited women and their partner (if consented) underwent a prenatal data collection by an interviewer, and data was complemented with medical records. Patients were then seen at mid ($20^{0/7}$- $23^{6/7}$ weeks) and late (32-34 $^{6/7}$ weeks) gestation and at delivery. Questionnaire data was collected, and biological samples taken and

stored for the entire cohort. Maternal blood and urine samples were collected at each prenatal visit and blood was also collected at delivery. Vaginal secretions and maternal were also collected. Infant cord blood, umbilical cord segment, placenta samples and meconium were collected at delivery, in addition to maternal hair and baby's hair. Paternal blood was collected for isolation of DNA for family-based genetic studies at visit 1, 2 or 3 according to availability. Postnatal follow up took place at 3, 12 and 24 months and included questionnaire data collection from the mother and the father. Maternal milk was obtained at the 3 months visit, and the baby underwent a visual acuity test. Anthropometric measurements were obtained at 3, 12 and 24 months. At 24 months, urine and blood were collected from the toddler, blood pressure was recorded, and a neurodevelopmental test (Bayley III, MacArthur-Bates) was done. If a participant was unwilling to complete a postnatal study visit at the hospital, a home visit was offered. Should no blood sample have been obtained from the mother, father or child during their participation, they were offered to provide a saliva sample once the child was 2 years of age. This core cohort will provide a rich resource for studying early etiologic factors of pregnancy complications (e.g. gestational diabetes, gestational hypertension) and birth outcomes (IUGR, PTB), both in the proposed IRNPQEO studies and in future proposals. All information was collected by professionals trained for the collection of data in this study.

3D-ART Study

By enrolling in the "3D-study", patients agreed to fill 20 sequential questionnaires regarding pertinent socio-demographic, health and pregnancy characteristics in the prenatal period, and 16 questionnaires in the first two years following birth, regarding maternal and infant health in the post-natal period. In addition, patients agreed to provide relevant biological samples as well as authorized the investigators to access medical records in order to complement health data, thus providing a full clinical picture with each gestation as the unit of analysis, and each infant as the unit of analysis in the post-natal period. Finally, patients granted the right for their infants to undergo complementary testing throughout the research period, which finalized with cognitive and neurodevelopmental testing using the Bayley-III scale at 2

years post-partum. Patients upon enrolment gave the consent for the utilization of all study-related data. Patients were prospectively followed at regular intervals from known conception (in the ART cohort) or the first day of known gestation (the natural conception cohort) throughout pregnancy until 2 years following birth. Maternal as well as paternal and feto-neonatal and infant data were collected.

Assessment Tool

The Bayley Scales of Infant Development, 3rd edition

The Bayley Scales of Infant Development (BSID), originally published in 1969, is a standard series of measurements originally developed by psychologist Nancy Bayley used primarily to assess the motor (fine and gross), language (receptive and expressive), and cognitive development of infants and toddlers, ages 0-3. The BSID tool consists of a series of developmental play tasks and takes between 45 - 60 minutes to administer. Raw scores of successfully completed items are then converted to scale scores and to composite scores. These scores are used to determine the child's performance compared with norms taken from typically developing children of their age (in months). Completed by the parent or caregiver, this questionnaire establishes the range of behaviors that the child can currently achieve and enables comparison with age norms. As of 2005 and up to the present time, the BSID is in its third edition (BSID-III).

More specifically, the BSID-III is composed of five scales designed for children aged from one to 42 months: 1) The cognitive scale, which assesses cognitive processing like memory, exploration and manipulation and sensorimotor development; 2) The language scale, this consists of the receptive (RC) and expressive (EC) communications subscales. The RC regroups items on preverbal behaviours, vocabulary development and verbal comprehension. The EC assesses preverbal communication, vocabulary and morpho-syntactic development; 3) The motor scale, which is divided into the (FM) and gross (GM) motor subtests and evaluates quality of movement, sensory integration perceptual-motor integration, prehension and other basic milestones; 4) The social-emotional scale, which, assesses emotional and social functioning as well as sensory processing; 5) The adaptive and behavior scale estimates the attainment of practical skills necessary for a child to function independently and meet environmental demands.

For each scale, a total raw score is generated and converted to a composite score, which is age-standardized with a mean score of 100 (SD = 15). The BSID-III is used to describe the current developmental functioning of infants and toddlers and to assist in diagnosis and treatment planning for infants with developmental delays or disabilities. It is frequently used in research to describe the developmental status of children with particular medical conditions and developmental disabilities and it is administered by examiners who are experienced clinicians specifically trained in BSID-III test procedures.

Specifications of BSID-III [100, 101]

- **Time to Administer**: The test is given on an individual basis and takes 30-90 minutes to complete depending on the age of the child.
- **Scoring**: Raw scores of successfully completed items are converted to subtest scaled scores and to composite standard scores. These scores are used to determine the child's performance compared with normative group of typically developing children of their age (in months). Global measure of all cognitive development: language, motor.
- **Psychometric Properties**: The Bayley Scales have been shown to have high reliability and validity.
- **Standardization Sample [101]**: Ages 16 days to 42 months 15 days
    - Cognitive, motor, and language scales - Sample n = 1,700
    - Social-Emotional Scale - n = 456
    - Adaptive Behavior Scale - n = 1,350
- **Stratification [101]**: Normed per United States sample collected from January to October 2004, with stratification by: age, sex, race/ethnicity, parent education level, geographic region. Norms were established using samples that did not include disabled, premature, and other at-risk children. Corrected scores are sometimes used to evaluate these groups, but their use remains controversial. The Bayley has relatively poor predictive value to later IQ scores, unless the

scores are very low. It is considered a good screening device for identifying children in need of early intervention. Scores do not represent IQs.

- **Validity Studies**: Validity studies with clinical groups
- **Comparison studies with major tests:**
    - Average Reliability [101]:
        - Adaptive Behaviour .97
        - Social-Emotional .90
        - Motor .92
        - Language .93
        - Cognitive .91

In our study, we used the Bayley-III cognitive and motor scales to assess neurodevelopment. We used the McArthur Bates Communicative Development Inventory (MCDI) to assess language development.

Neurodevelopmental Outcomes Measured by the Bayley-III [30][101]

Cognitive (Average reliability = 0.91)

The Cognitive Scale is comprised of 91 items that assess sensorimotor development, exploration and manipulation, object relatedness, concept formation and memory. The expectations or "norms" are that by 6 months, the infant plays with single object, by 9 months relational acts emerge, and by 2 years symbolic play takes place.

Motor (Average reliability = 0.92)[101]

The Motor Scale is composed of two sub-scales: fine and gross motor subtests. The Gross Motor subtest is comprised of 72 items, which seek to test movements of the limb and torso, static positioning, dynamic motion (including locomotion and coordination), balance and motor planning. The Fine Motor subtest is comprised of 66 items, which test prehension, perceptual-motor integration, motor planning and speed, visual tracking, reaching, object grasping, object manipulation, functional hand skills, and responses to tactile information.

MacArthur-Bates Communicative Development Inventories (MCDI) [102]

In order to evaluate verbal and language development, we used the toddler short-form of "MacArthur-Bates Communicative Development Inventories"[103], a norm-referenced parent questionnaire that captures important information about infant's developing abilities. Specifically, we used a 100-word vocabulary production checklist and a question about early word combinations. The short-form MCDI is designed for children between 16 and 30 months and is highly correlated with the original checklist, which contains more than 600-words [102, 103]. Correlations of the short form range from 0.80 to 0.97 as a function of age, with increasing Pearson correlation scores in toddlers than infants, respectively [103]. The English MCDI toddler short-form was normalized on a sample of Americans who spoke English as their primary language, and its reliability, content and concurrent validity have been established[102, 103]. A short-form has been developed for French-speaking children in Québec using the approach described by Fenson et al [103]; however, the normalization of French-short is underway (unpublished data).

IRB-Ethics Approval

The institutional ethics review board has approved the conduction of this study as part of the larger "3D Study".

Study Population

Inclusion criteria

The "3D-Study" enrolled: 1) pregnant women between 8 $^{0/7}$-13 $^{6/7}$ completed weeks and 2) planning delivery in a "3D-Study" associated hospital, 3) undergoing neurodevelopment at age 2 years.

Exclusion criteria

Exclusion criteria included: 1) women <18 years of age; 2) illegal intravenous drug users; 3) inability to communicate in English or French; 4) severe illnesses/life threatening conditions, and 5) multiple pregnancies, which includes twins or higher

order multiples, as well as mothers whose previous pregnancies had been enrolled in the study.

Study Objective

Using standardized and validated tools designed to appraise cognitive, language, and motor development (Bayley Scale of Infant and Toddler Development, 3rd edition, MacArthur-Bates Communicative Development Inventory), and using a prospectively followed cohort from a well-designed longitudinal research study ("3D-Study"), the objective of the present study is to measure cognitive and neurodevelopmental outcomes at 2 years of age following conception through type-specific ART, relative to natural conception.

Specific Aims

I. To describe the ART-type utilization trend in Quebec and establish baseline medical and socio-demographic differences between an ART and a non-ART cohort.

II. To quantify cognitive neurodevelopment of two-year old infants prospectively followed since conception using the Bayley-III scale of infant and toddler development and the MacArthur-Bates Communicative Development Inventory.

a. To stratify the results according to ART vs. non-ART conception.
b. To stratify the ART cohort according to type of ART used and describe type-specific and age-matched risks relative to the non-ART cohort using each one of the Bayley-III scales, as a continuous variable:
    i. Cognitive
    ii. Language
    iii. Motor
c. To adjust the analysis for potential confounders within a breadth of conception and pregnancy-related data available.

Research Question

"Do **infants at 2 years of age**, following pregnancies achieved with the use of **assisted reproductive techniques**, exhibit differences in **cognitive, language, and motor development as measured by the Bayley-III scale and the MacArthur-Bates Communicative Development Inventory** relative to infants born following **natural conception**"?

Hypothesis

H_0**:** When taking into account all potential risk factors for delayed cognition, infants born from ART-conceived pregnancies have no significant differences regarding neurodevelopmental outcomes relative to infants born from natural conception.

H_A*:* Even after controlling for known risks factors for cognitive development, infants born from ART-conceived pregnancies, particularly ICSI, demonstrate significant differences regarding neurodevelopmental outcomes relative to infants born from natural conception.

Power calculation

Although a total number of 278 pregnancies were established with assisted reproductive technologies, only 175, or 62.9%, underwent neurodevelopmental testing. Based on the proportion of infants having undergone the assessments in the study (ART, n=175; natural conception, n=1,345), a post-hoc power calculation was conducted to determine whether a minimal clinically significant difference in BSID-III scores could be detected. Using previously reported mean and variance BSID-III cognitive scores at 24 months of age, we used a 2-sided type-I error (α) of 5% (with 95% confidence) and obtained 98.57% power to detect a 5-point difference between groups, which was considered to be the minimal clinically significant threshold.

Sample Size

- Full Cohort:
    - 2,456 participants giving initial consent, including ART and non-ART
    - 2,366 participants having completed initial visit

- 2,185 participants having completed the prenatal portion of the study
- 278 participants conceived with ART
  - OS (n=53)
  - IUI (n=79)
  - IVF (n=32)
  - ICSI (n=105)
  - IVM (n=9)

Study Design

Prospective cohort study with the exposed group defined by ART-use or infertile and no ART-use (subfertile group, defined as the natural conception after 6 months of trying), and a non-exposed group defined by natural conception. All groups had follow-up since pregnancy until 2 years post-partum for the parents, and 2 years of age for the baby. Following application of both inclusion and exclusion criteria, focus was primarily placed on the following questionnaires:

1) **1-A** = Baseline Questionnaire - Interview (between 8 0/7 and 13 6/7 weeks)
2) **ART** = Description of ART indication and method
3) **5-A =** Labor and Delivery questionnaire
4) **8-A** = Maternal interview at 24 months post-partum
5) **8-B** = Bayley-III measuring scale reports and MacArthur-Bates

We sought the following variables for analysis:

Socio-Demographic Data

- Maternal and Paternal age
- Medical History
  - Asthma, Diabetes, Thyroid disease, Major depression—past and present, Hypertension, Dyslipidemia, Cardiovascular disease Seizures, Anemia, Sexually transmitted infection
- Maternal Ethnicity
  - Caucasian, Black, Latin American, Asian, Other
- Marital Status and Level of Maternal Education
- Income
  - Less than 40,000 CAD, 40,000-80,000 CAD, more than 80,000
- Alcohol and Smoking
  - Before and during pregnancy
- Obstetrical history
  - Gravidity, parity

Infertility Characteristics

- Infertility history: more than 6 months, more than 12 months
- Mean time to conception (months)
- Infertility diagnosis—female factors
  - Endometriosis, Tubal factor, PCOS/anovulation, Diminished ovarian reserve, Uterine malformation, Single woman, Unexplained infertility Other female factor
- Infertility diagnosis—male factors
  - Oligozoospermia, Teratospermia, Azoospermia, Ejaculatory dysfunction

Pregnancy and Neonatal Characteristics (Questionnaire 5-A = Question 8, ART)

- Fetal sex
- IQR Birth weight (g)
- IQR Fetal presentation
  - Cephalic, Breech, Other, Unknown
- Mode of delivery
  - Vaginal, Cesarean, Vacuum, Forceps
- NICU admission
- 5-min Apgar score
- Presence of congenital anomalies
- Presence of Gestational Diabetes
- Presence of Hypertensive disorder of pregnancy
- Presence of PE/Eclampsia

Statistical Analysis

We carried out our analysis in four steps. First, we divided our cohort according to the mode of conception into two main groups: ART vs. spontaneous conception. The ART cohort was subdivided into 5 groups according to the specific mode of assisted conception: OS, IUI, IVF, ICSI, and IVM. As previously defined, spontaneous conceptions were subdivided into two groups according to the self-reported time to pregnancy: less than or equal to 6 months (fertile spontaneous conceptions) or greater than 6 months (subfertile conceptions).

We then described each subgroup according to their baseline demographic and gestational characteristics. We used Chi-Square and one-way ANOVA to test for statistical differences between groups. These include parental age, ethnicity, level of maternal education, living status, family income, lifestyle (smoking, alcohol and drug use), gravidity, and maternal medical-comorbidities (Table 1). We included descriptors

of infertility diagnoses for patients undergoing ART and those defined as subfertile (Table 2). Subsequently, we described obstetrical outcomes including fetal gender, gestational age at delivery, mode of delivery, and 5-min APGAR scores, amongst others (Table 3).

We then described the cohort of those mothers whose infants underwent neurodevelopmental testing, and evaluated the BSID-III (cognitive and motor) and MCDI (language) scores for each mode of conception, using Chi-Square and ANOVA statistical testing to determine within-group variability.

Finally, we applied linear regression models to evaluate both the crude and adjusted effects of ART on scale scores, using the natural conception group (time to pregnancy <6 months) as the referent group. Estimates for individual ART techniques were calculated, as were estimates for grouped modes of conception: in-vivo (OS and IUI) and in-vitro (IVF, ICSI and IVM). Analyses were adjusted for parental age (years), family income (CAN$), maternal ethnicity (Caucasian vs. not), maternal education (level), marital status (married vs. not), maternal history of depression (yes/no), maternal smoking intake and alcohol consumption during pregnancy (yes/no), antidepressant use (yes/no) and folic acid intake during pregnancy (yes/no). We adjusted for maternal depression status and antidepressant use, as maternal stress and poor mental health have been associated to both higher rates of infertility and poor infant neurodevelopmental outcomes[104, 105].

We expressed our linear regression coefficients in two ways, namely B_1 (beta coefficient) and β' (standardized beta coefficient). Whereas B_1 represents the change in the score units of each scale for ART conception relative to a natural conception, β' represents that change as a factor of standard deviation units. Sensitivity analyses were carried out to evaluate the robustness of the model adjusting for thyroid disease, breastfeeding status, as well as removing single women and same sex couples from our model. Point plots are provided for graphic representation of these scale scores. We sought and received approval from the institutional ethics review board (ERB) at the CHU Sainte-Justine Center (acting as the central ERB) in Montreal, Quebec. All analyses were conducted using SAS 9.3 (Carey, NC).

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Neurodevelopmental Outcomes After Assisted Reproductive Technologies

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Abbreviations
ART – Assisted reproductive technology/techniques
CI – Confidence Interval
IVF – In-vitro Fertilization
IVM – In-vitro Maturation

Précis:

At 2 years of age, children born after assisted reproductive technologies have similar cognitive, motor, and language development compared to children born after natural conception.

Abstract

Objective: To compare children's cognitive, motor, and language development at 2 years of age after assisted reproductive technologies (ART) relative to natural conception.

Methods: The 3D-Study (2010-2012) is a prospective cohort study, which sought to improve the understanding between perinatal events, obstetric outcomes, and child development. A total of 2,366 pregnant women were recruited, of which 278 conceived with ART: ovarian stimulation, intrauterine sperm insemination, in-vitro fertilization (IVF), intra-cytoplasmic sperm injection or in-vitro maturation (IVM). Natural conception was defined as the unassisted establishment of pregnancy. Cognitive, motor, and language neurodevelopmental outcomes were compared between ART and natural conception groups at 24 months using The Bayley Scales of Infant and Toddler Development, 3rd ed. and the MacArthur-Bates Communicative Development Inventories. Adjusted linear regression models evaluated the effect of ART on neurodevelopmental outcomes, using natural conception as reference.

Results: A total of 175 children in the ART group (62.9%) and 1,345 children in the natural conception group (64.4%) underwent neurodevelopmental assessment at 24 months postpartum. After adjusting for relevant confounders, children born after ART showed no difference in Bayley scales' cognitive scores [B_1(SE)=-1.60(0.9), 95% CI:-3.36–0.16], composite motor scores [B_1(SE)=-1.33(1.0), 95% CI:-3.29–0.63] or MacArthur-Bates language scores [B_1(SE)=-0.28(2.1), 95% CI:-4.39–3.83]. No difference was observed when independent ART techniques were compared nor when comparing in-vivo (ovarian stimulation or intrauterine insemination) or in-vitro (IVF, intra-cytoplasmic sperm injection or IVM) techniques ($p>0.05$).

Conclusion: Children born after ART had similar cognitive, motor, and language development as children born after natural conception at 2 years of age. These findings may be useful in the clinical counseling of patients undergoing ART.

Abstract word count: 298

Introduction

Technological advances and changing social paradigms have led to the increased use of assisted reproductive technologies (ART) for the purposes of procreation[7]. The main techniques to treat infertility include: ovarian stimulation and intrauterine sperm insemination, as well as techniques whereby oocytes and sperm are handled in-vitro, as in-vitro fertilization (IVF), intra-cytoplasmic sperm injection and in-vitro maturation (IVM)[12, 16, 22]. Herein, we refer to ART as any of the aforementioned infertility treatments leading to conception outside natural coitus.

In Canada, reports indicate that the use of fertility treatments increased by 50% over the last decade[7, 8]. While the short-term perinatal outcomes after ART are well established, long-term neurodevelopmental outcomes, including cognitive, motor, and language development, are still source of controversy[12, 54, 60, 65, 106].

A review from the National Institutes of Health recognized that "lingering data gaps [exist] in the equivocal literature for many neurodevelopmental disabilities relative to ART" and that "[…] cohorts with longitudinal assessment […] of neurodevelopment […] are paramount for the development of empirically-based guidance […]"[107]. Similarly, the largest systematic review of over 80 studies addressing long-term neurodevelopment after ART concluded that additional data was required to determine the true impact of fertility treatments on these outcomes[12].

In our study, we tested the hypothesis that neurodevelopment at two years is related to mode of conception. As such, using standardized and validated tools, the

objective of this study was to compare children's cognitive, motor and language development at 2 years of age after ART relative to natural conception.

Materials and Methods

We analysed data from the 3D-Study (Découvrir, Développer, Devenir), a prospective, longitudinal cohort, carried out from 2010-2012 by the Integrated Research Network in Perinatology of Quebec and Eastern Ontario in Canada[108, 109]. The 3D-Study recruited 2,366 women in their first trimester of pregnancy and their respective births across 9 sites in the province of Quebec, and gathered extensive data on the mother-father-child triad from conception until 2 years post-partum.At 2 years post-partum, children underwent cognitive, motor, and language testing using the Bayley Scales of Infant and Toddler Development, 3rd ed. and the MacArthur-Bates Communicative Development Our primary objective was to compare the neurodevelopment in children born with the help of fertility treatments (exposed) relative to those born off pregnancies conceived naturally (controls). Our secondary objective was to describe baseline medical and socio-demographic differences between an ART and a non-ART cohort in Quebec.

The 3D-Study enrolled: 1) pregnant women between 8 $^{0/7}$-13 $^{6/7}$ completed weeks and 2) planning delivery in a 3D-Study associated hospital. Exclusion criteria included: 1) women <18 years of age; 2) illegal intravenous drug users; 3) non-English or French speakers; 4) severe illnesses/life-threatening conditions, and 5) multiple pregnancies, which includes twins or higher order multiples, and mothers whose previous pregnancies had been enrolled in the study.

The Bayley Scales of Infant and Toddler Development, 3rd ed. is a validated and standardized developmental assessment for infants aged 1 to 42 months including 5 independent scales (Cognitive, Motor (fine and gross), Language, Adaptive Function, and Socio-emotional)[101]. In our study, we used the Cognitive scale, which assesses cognitive processes like memory, exploration, manipulation and sensorimotor development, as well as the Motor scale, which is divided into the fine motor (FM) and gross motor (GM) subtests and evaluates quality of movement, sensory integration, perceptual-motor integration, prehension and other milestones. Each scale consists of a series of developmental play tasks. Scale-specific raw scores of completed items are then converted to scaled scores and to composite scores as a function of age. For the Fine and Gross Motor subtests, only scaled scores are available. The scaled and composite scores are then compared with normalized scores taken from typically developing children of similar age. Mean is set at 10 and 100 with a standard deviation of 3 and 15 for the scaled scores (fine and gross motor) and the composite score (cognitive, motor), respectively. The Bayley scales (3rd ed.) have established test-retest reliability, internal consistency as well as convergent and divergent validity[101]. In our study, trained individuals who were blinded to the exposure administered the tool.

In order to evaluate language development, we used the toddler short-form of MacArthur-Bates Communicative Development Inventories[103], a norm-referenced parent questionnaire that captures important information about infant's developing abilities. Specifically, we used a 100-word vocabulary production checklist and a question about early word combinations, which can be reported on a 100 point scale.[102, 103]. The English MacArthur-Bates toddler short-form has established

reliability, as well as content and concurrent validity [102, 103]. A French-version of the short-form has been adapted for French-speaking children in Québec using the approach described by Fenson et al[103].

Based on the proportion of children having undergone the assessments (ART, n=175; natural conception, n=1,345), a power calculation was conducted to determine whether a minimal clinically significant difference in the Bayley scales (3rd ed.) scores could be detected. Using previously reported mean and variance composite cognitive scores at 24 months of age, we used a 2-sided type-I error (α) of 5% and obtained 98.57% power to detect a 5-point difference between groups [110].

We carried out our analysis in four steps. First, we described each subgroup according to their baseline demographic and gestational characteristics (Table 1). We included descriptors of infertility diagnoses for patients undergoing ART and those defined as subfertile (Table 2). Subsequently, we described obstetrical outcomes in the ART vs. natural conception group (Table 3).

We then evaluated the Bayley scales (3rd ed.) (cognitive and motor) and MacArthur-Bates (language) scores for each mode of conception, using Chi-Square and ANOVA statistical testing to determine within-group variability. Finally, we applied linear regression models to evaluate both the crude and adjusted effects of ART on scale scores, using the natural conception group as reference. Estimates for individual ART techniques were calculated, as were estimates for grouped modes of conception: in-vivo (ovarian stimulation and intrauterine insemination) and in-vitro (IVF, intra-cytoplasmic sperm injection and IVM). Analyses were adjusted for parental age (years), family

income ($CAD), maternal ethnicity (Caucasian vs. not), maternal education (level), marital status (married vs. not), maternal history of depression (yes/no), maternal smoking intake, alcohol consumption during pregnancy (yes/no), antidepressant use (yes/no) and folic acid intake during pregnancy (yes/no). Sensitivity analyses were carried out to evaluate the robustness of the model adjusting for thyroid disease, breastfeeding status, as well as removing single women and same sex couples from our model. In accordance to a provincial policy of elective single embryo transfer during the study period, the vast majority of patients undergoing embryo transfer (IVF, intra-cytoplasmic sperm injection, IVM) in our study received a single embryo per cycle. An exemption was made if the patient was older than 35 years of age and had prior cycle failures, in which case the transfer of 2 embryos was considered.We sought and received approval from the institutional ethics review board (ERB) at the CHU Sainte-Justine Center (acting as the central ERB) in Montreal, Quebec. All analyses were conducted using SAS 9.3 (Carey, NC).

Results

Our final cohort consisted of 2,366 women carrying singleton pregnancies. We compared 278 pregnancies after ART to 2,088 pregnancies after natural conception. The ART cohort was comprised of the following techniques: ovarian stimulation (n=53), intrauterine insemination (n=79), IVF (n=32), intra-cytoplasmic sperm injection (n=105) and IVM (n=9). The spontaneous conception cohort was comprised of subfertile patients (n=490) and patients achieving natural conception <6 months (n=1,598). Patients undergoing ART were more likely to be older, more educated, of lower parity, and with higher rates of thyroid disease. The later finding may be due to more intense screening

in the ART group, as well as to underlying thyroid dysfunction leading to infertility. On the other hand, mothers in the natural conception group were more likely to be Caucasian, multiparous, and with higher rates of caffeine, smoking and alcohol consumption before and during pregnancy (Table 1).

In Table 2, infertility characteristics were compared between patients undergoing ART and those identified as being subfertile, who conceived after 6 months of trying. Patients having undergone ART had longer time to conception and higher rates of underlying infertility diagnoses in both females and males ($p<0.0001$).

Table 3 presents obstetrical and neonatal outcomes between both groups. Babies born after ART were more likely to be of lower birthweight (3,279g, IQR = 697 vs. 3,356g, IQR = 1,034), more likely to be born via caesarean delivery (36.5% vs. 25.1%) and to be admitted to the neonatal intensive care unit (7.7% vs. 3.9%). While statistical differences were noted in the gestational age at birth, these are unlikely to be of clinical significance (38.4 weeks, IQR = 2.0 vs. 38.8 weeks, IQR = 2.0, $p=0.006$).

A total of 175/278 children in the ART group (62.9%) and 1,345/2,088 in the natural conception group (64.4%) underwent neurodevelopmental assessments at 24 months. No significant differences were observed in cognitive (composite mean score ± SD: 98.5 ± 11.2 vs. 100.1 ± 11.4, $p=0.08$), fine motor (scaled mean score 11.4 ± 2.3 vs. 11.6 ± 2.7, $p=0.41$), gross motor (scaled mean score 8.8 ± 2.0 vs. 8.9 ± 2.3, $p=0.37$), or language scores (53.9 ± 23.6 vs. 55.6 ± 24.4, $p=0.50$) (Table 4). Finally, Table 5 showcases the linear regression models. After adjusting for relevant confounders, children born after ART showed no difference in Bayley scales (3rd ed.)cognitive

composite scores [B_1(SE)=-1.60(0.9), β'=-0.045, p=0.08], composite motor scores [B_1(SE)=-1.33(1.0), β'=-0.036, p=0.18] or MacArthur-Bates language scores [B_1(SE)=-0.28(2.1), β'=-0.003, p=0.89], relative to natural conception. No significant differences were observed when comparing in-vivo vs. in-vitro techniques separately (p>0.05), nor when comparing independent techniques individually. However, our study was not powered to compare the latter (Appendix 2, available online http://links.lww.com/xxx). Sensitivity analyses showed no differences in the model estimates when adjusting for thyroid disease, breastfeeding rates, nor when removing single women or same sex couples from the model.

Relative to subjects lost to follow-up in the ART cohort, mothers of children that underwent testing were more likely to be Caucasian and of higher income. Among the natural conception cohort, mothers of children that underwent testing were more likely to be Caucasian, older, of higher education and income, and of lower parity (Appendix 3, available online at http://links.lww.com/xxx).

Discussion

Creating families through assisted reproduction raises a number of concerns about potentially adverse consequences for child development [12, 17, 18, 22, 23]. However, these concerns stem from largely retrospective studies with small sample sizes and heterogeneous methodologies [19]. By specifying the infertility treatments used, accounting for predictors of development, and using standardized testing, our prospective study overcomes some of these limitations, and provides re-assuring results

in that children born after ART appear to have similar cognitive, motor, and language skills than children born after natural conception at 2 years of age.

The recent Upstate KIDS Study sought to assess the same question in this report, notably, the association between the mode of conception and children's development[106]. According to its results, children's development at age 3 appears independent on mode of conception[106]. While the prospective nature of the KIDS study is a major strength, a number of its limitations are addressed by our study. While the KIDS study recruited newborns, the 3D-Study recruited mothers during first trimester, allowing us to prospectively gather prenatal data that may have impacted neurodevelopment, such as antidepressant, folic acid, alcohol and smoking exposure. Secondly, their study used the Age and Stage Questionnaires to assess neurodevelopment. Unlike the Bayley scales (3rd ed.), which are administered by a third-party blinded to the exposure, the Age and Stage Questionnaires requires parental administration, which may introduce confirmatory bias [111]. Third, while the 3D-Study required a prospective, 2-step verification of exposure including ovarian stimulation and intrauterine insemination, the KIDS study could not verify the validity of the exposure because there is no registry in the U.S.[106]. Nevertheless, the replication of similar findings in both studies despite the use of different methodologies is encouraging, and may serve to re-assure patients undergoing ART.

Each facet of neurodevelopment after ART has been studied previously. To date, two large systematic reviews of over 80 studies addressed cognitive development after ART, concluding that: "there is sufficient data to support […] no difference in

development […] between IVF and spontaneously conceived children"[12, 19] and that "most studies showed no associations with cognitive […] development"[12]. Because we cannot preclude that differences in cognition may appear later in life, a follow-up of children from prospective studies such as this one may be necessary.

Similarly, prospective evidence of motor skills at 24 months of age evaluated with standardized testing is lacking in the literature. Though some studies do point to delays in motor development between 16-18 months[20] , our findings concur with the majority of the literature that motor development is not affected by the mode of conception.

Most of the controversy seems to be found in the language development after ART [60, 83] [20]. As evidenced by the lack of consensus, there is a call for prospective evaluation of children's language skills after ART, as we have done in our study, where we find no significant difference in MacArthur-Bates scores at 24 months of age.

The strengths of the present study include: the use of a prospective cohort of pregnant women with up to 3 years of follow-up., the use of standardized tools administered by professionals blinded to exposure, and the analysis of a number of ART techniques. In addition, we adjusted for a vast array of pertinent confounders, including maternal depression, which is notably lacking in the literature [112]. Likewise, our study uses North American data, which may enhance external validity amongst Canadian and U.S. centers. Finally, we conducted sensitivity analyses, which confirmed the robustness of our model.

On the other hand, a number of limitations are worth mentioning. Though this study was powered to estimate the impact of ART as an overall category, it was not powered to detect a difference among individual techniques. Likewise, we considered the main ART technique as exposure, and could not account for the type of cycle (natural vs. stimulated) used Furthermore, though loss to follow-up rates were moderate in each group, a post-hoc power calculation reveals adequate power to answer the study question. Moreover, given the study design, we were not able to untangle the effects of the underlying infertility from the ART technique used, as this is an example of confounding by indication. Finally, the children in our study population were young, and in certain cases, developmental characteristics may have a limited predictive value for long-term development.

All in all, the findings hereby presented may be useful in the clinical counseling of patients undergoing ART. Future prospective studies with long-term follow-up, powered to study individual ART techniques, as well as evaluation of behavioral outcomes (such as attention deficit/hyperactivity and autism-like behaviors) are necessary.

Table 4. Baseline patient characteristics

Variable	ART (n = 278)	No ART (n = 2,088)	*p-value*
Maternal age, y (mean ± SD)	34.9 (4.5)	31.6 (4.5)	<0.001
Paternal age, y (mean ± SD)	36.3 (6.1)	33.4 (5.8)	<0.001
Maternal Ethnicity, n (%)			0.0151
Caucasian	202 (72.7)	1,681 (80.5)	
Black	23 (8.3)	135 (6.5)	
Latin American	17 (6.1)	101 (4.8)	
Asian	25 (9.0)	100 (4.8)	
Other	11 (4.0)	71 (3.4)	
Maternal Education, n (%)			
Post-secondary	267 (96.0)	1,927 (90.0)	0.0011
Household income, n (%)			0.4886
< 40,000	48 (17.2)	352 (16.9)	
40-000-80,000	80 (28.8)	634 (30.4)	
>80,000	131 (47.1)	1,003 (48.0)	
Refused to disclose	19 (6.8)	99 (4.7)	
Mother living alone, n (%)	10 (3.6)	123 (5.9)	0.8120
Pre-pregnancy weight, kg (mean ± SD) ǂ	65.4 (14.9)	65.1 (21.5)	0.6300
Gravidity, n (%)			<0.001
1	132 (47.5)	728 (34.9)	
2	85 (30.6)	676 (32.4)	
>2	61 (21.9)	684 (32.8)	
Medical Comorbidities, n (%)			
Asthma	41 (14.8)	344 (16.5)	0.4637
Diabetes	3 (1.1)	18 (0.9)	0.7170
Thyroid Disease	45 (16.2)	162 (7.8)	<0.001
Major Depression – *Past*	20 (7.2)	156 (7.5)	0.8687
Major Depression – *Present*	2 (0.7)	19 (0.9)	0.7503
Hypertension	6 (2.2)	52 (2.5)	0.7365
Dyslipidemia	8 (2.9)	69 (3.3)	0.7063
Cardiovascular disease	6 (2.2)	19 (0.91)	0.0558
Seizures	10 (3.6)	27 (1.3)	0.0036
Anemia	47 (16.9)	343 (16.4)	0.8397
Sexually transmitted infection	27 (9.7)	235 (11.3)	0.4413
Folic Acid intake, n (%)	170 (61.2)	1,070 (51.3)	0.0019
Maternal caffeine intake, n (%)			
During pregnancy	40 (14.4)	405 (19.4)	0.0447
Maternal smoking, n (%)			
Before pregnancy	38 (13.7)	405 (19.4)	0.0215
During pregnancy	9 (3.2)	106 (5.1)	0.1804
Maternal alcohol consumption, n (%)			
Before pregnancy	157 (64.3)	1,418 (79.1)	<0.001
During pregnancy	3 (1.1)	60 (2.9)	0.0808

Legend = Gravidity: total lifetime number of confirmed pregnancies including the current one.
SD: standard deviation. Values are rounded up.

Table 5. Infertility characteristics

Variable	ART (n = 278)	Subfertile conception (n = 490)	*p-value*
Infertility for > 6 months, n (%)	247 (88.9)	490 (100.0)	<0.0001
Infertility for > 12 months, n (%)	225 (80.9)	253 (51.6)	<0.0001
Time to conception, months			
mean (SD)	29.1 (28.0)	11.3 (13.3)	<0.0001
median	24.0	7.0	
Infertility diagnosis – Female factors[ǂ]			
Endometriosis	23 (8.2)	9 (1.8)	<0.0001
Tubal factor	28 (10.1)	3 (0.6)	<0.0001
PCOS + Anovulation	85 (30.6)	30 (6.1)	<0.0001
Diminished ovarian reserve	28 (10.1)	7 (1.4)	<0.0001
Uterine malformation	1 (0.4)	2 (0.4)	<0.0001
Single woman	7 (3.3)	-	-
Same sex couple	6 (2.8)	-	-
Unexplained infertility	39 (17.1)	2 (0.4)	<0.0001
Other female factor	23 (8.3)	10 (2.0)	<0.0001
Unknown	37 (13.3)	8 (1.63)	<0.0001
Infertility diagnosis – Male factors [£]			
Oligozoospermia	42 (15.1)	4 (0.7)	<0.0001
Teratospermia	48 (18.0)	3 (0.5)	<0.0001
Azoospermia	18 (6.5)	0 (0.0)	-
Ejaculatory dysfunction	2 (0.7)	0 (0.0)	-

Legend = PCOS: Polycystic ovarian syndrome; Subfertile conception: natural conception after 6 months or longer of having tried to conceive.

[ǂ] = Values are rounded up. Percentages may not add up to 100% as multiple infertility diagnoses in the same patient may exist.

Table 6. Obstetrical and Neonatal outcomes

Variable	ART (n = 278)	No ART (n = 2,088)	*p-value*
Fetal sex, n (%)			
Male	129 (49.1)	987 (50.4)	0.8025
Gestational age, w (mean ± SD)	38.4 (2.8)	38.8 (2.1)	0.0056
Birthweight, g (mean ± SD)	3,279.0 (638.9)	3,356.9 (525.8)	0.0304
Fetal presentation, n (%) Ω			0.2334
Cephalic	243 (92.4)	1,823 (92.8)	
Breech	13 (4.9)	99 (5.0)	
Other	0 (0.0)	15 (0.7)	
Unknown	7 (2.7)	31 (1.5)	
Mode of delivery, n (%)			0.0005
Vaginal	138 (52.5)	1,275 (64.9)	
Caesarean	96 (36.5)	494 (25.1)	
Vacuum	14 (5.3)	109 (5.5)	
Forceps	15 (5.7)	88 (4.5)	
NICU admission, n (%)	20 (7.7)	75 (3.9)	0.0042
5-min APGAR score, median	9	9	0.8800
Congenital anomalies, n (%)	10 (3.8)	60 (3.1)	0.5246

Legend = NICU: Neonatal intensive care unit.
SD: standard deviation. Values are rounded up.

Table 7. ANOVA – Bayley Scales of Infant Development 3rd ed. and MacArthur-Bates Communicative Development Inventories Scale Scores at 24 months of age

Variable	ART (n = 175)	No ART (n = 1,345)	*p-value*
Cognitive Composite Score, mean (SD)	98.5 (11.2)	100.1 (11.4)	0.0788
Motor Composite Score, mean (SD)	100.8 (9.8)	101.8 (12.2)	0.2821
Fine Motor – Scaled Score, mean (SD)	11.4 (2.3)	11.6 (2.7)	0.4136
Gross Motor – Scaled Score, mean (SD)	8.8 (2.0)	8.9 (2.3)	0.3720
MacArthur-Bates Scale Score, mean (SD)	31.8 (32.4)	31.3 (32.3)	0.7927

Legend = SD: standard deviation
MacArthur-Bates Scale Score: out of 100

Table 8. Adjusted linear regressions*

	All ART			In-vivo (OS + IUI)			In-vitro (IVF + ICSI + IVM)		
Variable	B_1 (SE)	β'	*p-value*	B_1 (SE)	β'	*p-value*	B_1 (SE)	β'	*p-value*
Cognitive Composite Score	-1.60 (0.9)	-0.045	0.0799	-2.22 (1.2)	-0.048	0.0650	-0.60 (1.3)	-0.013	0.6404
Motor Composite Score	-1.33 (1.0)	-0.036	0.1864	-1.64 (1.3)	-0.033	0.2220	-1.04 (1.4)	-0.020	0.4674
Fine Motor – Scaled Score	-0.24 (0.2)	-0.030	0.2641	-0.26 (0.3)	-0.024	0.3714	-0.22 (0.3)	-0.020	0.4765
Gross Motor – Scaled Score	-0.19 (0.2)	-0.026	0.3305	-0.26 (0.3)	-0.028	0.3001	-0.12 (0.3)	-0.013	0.6422
MacArthur-Bates Scale Score	-0.28 (2.1)	-0.003	0.8936	2.18 (2.8)	0.016	0.4480	-2.88 (2.8)	-0.021	0.3205

*Adjusted for maternal age, paternal age, maternal education, income, ethnicity, marital status, smoking intake, alcohol consumption during pregnancy, history of depression, antidepressant use and folic acid intake.

ART= assisted reproductive technology; SE = standard error.

β' = standardized beta coefficient. Whereas B_1 represents the change in the score units of each scale for ART conception relative to a natural conception, β' represents that change as a factor of standard deviation units. Both are interchangeable.

Non-ART/Natural conception as reference.

Table 9. Supplemental file. Adjusted Linear Regressions for specific ART techniques*

Variable	OS (n = 36)	IUI (n = 55)	IVF (n = 21)	ICSI (n = 56)	IVM (n = 7)	Subfertile conception (n = 330)	Natural conception (n = 1,015)
Cognitive Composite Score - B_1 (SE)	-0.11 (0.1)	-1.44 (1.5)	-0.16 (2.5)	-0.86 (1.6)	0.85 (4.2)	0.20 (0.7)	
p-value	0.1447	0.3465	0.9463	0.5783	0.8406	0.7759	
Motor Composite Score - B_1 (SE)	-2.82 (2.1)	-0.90 (1.7)	-2.59 (2.7)	-0.70 (1.7)	2.24 (4.5)	-1.35 (0.8)	
p-value	0.1733	0.5919	0.3299	0.6802	0.6213	0.0906	
Fine Motor – Scaled Score - B_1 (SE)	-0.70 (0.5)	0.01 (0.4)	-0.17 (0.6)	-0.37 (0.4)	0.89 (1.0)	-0.29 (0.2)	Reference
p-value	0.1233	0.9813	0.7769	0.3197	0.3698	0.0962	
Gross Motor – Scaled Score - B_1 (SE)	-0.20 (0.4)	-0.30 (0.3)	-0.68 (0.5)	0.14 (0.3)	-0.18 (0.9)	-0.15 (0.1)	
p-value	0.6183	0.3465	0.1707	0.6741	0.8272	0.3014	
MacArthur-Bates Scale Score - B_1 (SE)	-0.12 (4.5)	3.67 (3.7)	9.61 (6.3)	-6.11 (3.3)	10.35 (10.5)	1.04 (1.7)	
p-value	0.9774	0.3178	0.4062	0.0667	0.3257	0.5394	

Legend = OS: Ovarian stimulation alone; IUI: Intrauterine insemination (natural and stimulated cycles combined); IVF: In-vitro fertilization (natural and stimulated cycles combined); ICSI: Intracytoplasmic sperm injection (natural and stimulated cycles combined); IVM: In-vitro maturation (natural and stimulated cycles combined); subfertile conception: natural conception after 6 months or longer of having tried to conceive.

SE: standard error. Values are rounded up.

*Adjusted for maternal age, paternal age, income, maternal education, ethnicity, marital status, smoking intake, alcohol consumption, history of depression, antidepressant use and folic acid intake.

Non-ART/Natural conception as reference.

Table 10. Baseline patient characteristics amongst tested vs. lost to follow-up according to exposure

	Tested (n = 1,520)			Lost to follow-up (n = 846)		
Variable	ART (n = 175)	No ART (n = 1,345)	*p-value*	ART (n = 103)	No ART (n = 743)	*p-value*
Maternal age, y; mean (SD)	34.5 (4.5)	31.8 (4.3)	<0.0001	34.6 (4.9)	31.2 (4.8)	<0.0001
Paternal age, y; mean (SD)	36.4 (5.7)	33.5 (5.5)	<0.0001	36.2 (6.9)	33.3 (6.2)	<0.0001
Maternal Ethnicity, n (%)			0.3693			0.0220
Caucasian	137 (78.3)	1,129 (83.9)		65 (63.1)	552 (74.3)	
Black	12 (6.9)	66 (4.9)		11 (10.9)	69 (9.3)	
Latin American	11 (6.3)	56 (4.2)		6 (5.9)	45 (6.1)	
Asian	10 (5.7)	55 (4.1)		15 (14.6)	45 (6.1)	
Other	5 (2.9)	39 (2.9)		6 (5.8)	32 (4.3)	
Maternal Education, n (%)						
Post-secondary	168 (96.0)	1,239 (92.8)	0.1154	99 (96.1)	625 (84.8)	0.0018
Household income, n (%)			0.0040			0.5606
< 40,000	24 (13.7)	177 (13.1)		24 (23.3)	175 (23.6)	
40-000-80,000	44 (25.1)	395 (29.4)		36 (34.9)	239 (32.2)	
>80,000	92 (52.6)	731 (54.4)		39 (37.9)	272 (36.6)	
Refused to disclose	15 (8.6)	42 (3.1)		4 (3.9)	57 (7.7)	
Mother living alone, n (%)	8 (4.6)	55 (4.1)	0.7634	2 (1.9)	68 (9.2)	0.0128
Pre-pregnancy weight, kg; mean (SD)	65.4 (14.4)	65.1 (24.4)	0.8958	65.5 (15.6)	65.0 (14.9)	0.7801
Gravidity, n (%)			0.0519			<0.0001
1	82 (46.9)	516 (38.4)		50 (48.4)	212 (28.5)	
2	54 (30.9)	428 (31.8)		31 (30.1)	248 (33.4)	
>2	39 (22.3)	401 (29.8)		22 (21.4)	283 (38.1)	
Medical Comorbidities, n (%)						
Asthma	26 (14.9)	215 (16.0)	0.7008	15 (14.6)	129 (17.3)	0.4787
Diabetes	1 (0.6)	10 (0.7)	0.8006	2 (1.9)	8 (1.1)	0.4465
Thyroid Disease	28 (16.0)	107 (7.9)	0.0004	17 (16.5)	55 (7.4)	0.0019
Major Depression – *Past*	12 (6.9)	91 (6.8)	0.9639	8 (7.8)	65 (8.8)	0.7396
Major Depression – *Present*	0 (0.0)	11 (0.8)	0.2299	2 (1.9)	8 (1.1)	0.4465
Hypertension	2 (1.1)	28 (2.1)	0.4009	4 (3.9)	24 (3.2)	0.7283
Dyslipidemia	6 (3.4)	43 (3.2)	0.8704	2 (1.9)	26 (3.5)	0.4076
Cardiovascular disease	2 (1.1)	15 (1.1)	0.9739	4 (3.9)	4 (0.5)	0.0010
Seizures	8 (4.6)	16 (1.2)	0.0007	2 (1.9)	11 (1.5)	0.7213
Anemia	33 (18.9)	208 (15.5)	0.2478	14 (13.6)	135 (18.2)	0.2531
Sexually transmitted infection	23 (13.1)	159 (11.8)	0.6125	4 (3.9)	76 (10.2)	0.0392

Legend = Gravidity: total lifetime number of confirmed pregnancies including the current one.
SD: standard deviation. Values are rounded up.

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Appendice

Le 15 mars 2016

Bonjour Dr Jacques Balayla,

Notre comité d'éthique de la recherche a pris connaissance de votre protocole intitulé « Infant Neurodevelopment at 2 Years of Age following Conception through Assisted Reproductive Technologies (ART) relative to Natural Conception : Findings from the Prospective, Longitudinal, Cohort 3D-Study ».

Votre projet de recherche n'a pas besoin d'être soumis à l'évaluation éthique formelle de notre comité puisqu'après vérification, nous constatons que les objectifs et hypothèses énoncés font partie de ceux du projet IRNPQEO, notamment le volet « Assisted Reproductive Techonologies (ART) Cohort Study ». Le projet IRNPQEO fait déjà l'objet d'une approbation éthique de notre comité. Une approbation supplémentaire n'est donc pas requise.

En vous souhaitant la bonne poursuite de vos travaux de recherche,

Me Geneviève Cardinal, présidente
Comité d'éthique de la recherche
GC/nd

CC : W. Fraser
A. Bérard

Appendix

BOOLEAN search code (code was broken down into subparts for actual search):

a. **(((((((("infertility"[MeSH Terms] OR "infertility"[All Fields]) OR ("reproductive techniques, assisted"[MeSH Terms] OR ("reproductive"[All Fields] AND "techniques"[All Fields] AND "assisted"[All Fields]) OR "assisted reproductive techniques"[All Fields] OR ("reproductive"[All Fields] AND "techniques"[All Fields] AND "assisted"[All Fields]) OR "reproductive techniques, assisted"[All Fields])) OR (("fertilisation"[All Fields] OR "fertilization"[MeSH Terms] OR "fertilization"[All Fields]) AND ("in-vitro techniques"[MeSH Terms] OR ("vitro"[All Fields] AND "techniques"[All Fields]) OR "in-vitro techniques"[All Fields] OR "vitro"[All Fields] OR "in-vitro"[All Fields]))) OR ("insemination, artificial"[MeSH Terms] OR ("insemination"[All Fields] AND "artificial"[All Fields]) OR "artificial insemination"[All Fields] OR ("insemination"[All Fields] AND "artificial"[All Fields]) OR "insemination, artificial"[All Fields])) AND (("cognition"[MeSH Terms] OR "cognition"[All Fields]) AND ("pediatrics"[MeSH Terms] OR "pediatrics"[All Fields] OR "pediatric"[All Fields]))) OR ("motor skills"[MeSH Terms] OR ("motor"[All Fields] AND "skills"[All Fields]) OR "motor skills"[All Fields])) OR ("programming languages"[MeSH Terms] OR ("programming"[All Fields] AND "languages"[All Fields]) OR "programming languages"[All Fields] OR "language"[All Fields] OR "language"[MeSH Terms])) OR (Neurodevelopment[All Fields] AND ("pediatrics"[MeSH Terms] OR "pediatrics"[All Fields] OR "pediatric"[All Fields]))) OR (Bayley[All Fields] AND ("weights and measures"[MeSH Terms] OR ("weights"[All Fields] AND "measures"[All Fields]) OR "weights and measures"[All Fields] OR "scale"[All Fields]) AND ("infant"[MeSH Terms] OR "infant"[All Fields]) AND Toddler[All Fields] AND ("growth and development"[Subheading] OR ("growth"[All Fields] AND "development"[All Fields]) OR "growth and development"[All Fields] OR "development"[All Fields]))) OR (MacArthur-Bates[All Fields] AND ("communication"[MeSH Terms] OR "communication"[All Fields] OR "communicative"[All Fields]) AND ("growth and development"[Subheading] OR ("growth"[All Fields] AND "development"[All Fields]) OR "growth and development"[All Fields] OR "development"[All Fields]) AND ("personality inventory"[MeSH Terms] OR ("personality"[All Fields] AND "inventory"[All Fields]) OR "personality inventory"[All Fields] OR "inventories"[All Fields] OR "equipment and supplies"[MeSH Terms] OR ("equipment"[All Fields] AND "supplies"[All Fields]) OR "equipment and supplies"[All Fields]))**

Original Research

Neurodevelopmental Outcomes After Assisted Reproductive Technologies

*Jacques Balayla, MD, Odile Sheehy, MSc, William D. Fraser, MD, Jean R. Séguin, PhD, Jacquetta Trasler, MD, PhD, Patricia Monnier, MD, PhD, Andrea A. MacLeod, MSc, PhD, Marie-Noëlle Simard, PhD, Gina Muckle, PhD, and Anick Bérard, PhD, on Behalf of the 3D-Study Research Group From the Integrated Research Network in Perinatology of Quebec and Eastern Ontario**

OBJECTIVE: To compare children's cognitive, motor, and language development at 2 years of age after assisted reproductive technologies (ARTs) relative to natural conception.

METHODS: The 3D-Study (2010–2012) is a prospective cohort study, which sought to improve the understanding among perinatal events, obstetric outcomes, and child development. A total of 2,366 pregnant women were recruited, of whom 278 conceived with ART: ovarian stimulation, intrauterine sperm insemination, in vitro fertilization, intracytoplasmic sperm injection, or in vitro maturation. Natural conception was defined as the unassisted establishment of pregnancy. Cognitive, motor, and language neurodevelopmental outcomes were compared between ART and natural conception groups at 24 months using the Bayley Scales of Infant and Toddler Development, 3rd edition, and the MacArthur-Bates Communicative Development Inventories. Adjusted linear regression models evaluated the effect of ART on neurodevelopmental outcomes using natural conception as a reference.

RESULTS: A total of 175 children in the ART group (62.9%) and 1,345 children in the natural conception group (64.4%) underwent neurodevelopmental assessment at 24 months postpartum. After adjusting for relevant confounders, children born after ART showed no difference in Bayley scales' cognitive scores (B_1 [standard error]=−1.60 [0.9], 95% confidence interval [CI] −3.36 to 0.16), composite motor scores (B_1 [standard error]=−1.33 [1.0], 95% CI −3.29 to 0.63), or MacArthur-Bates language scores (B_1 [standard error]=−0.28 [2.1], 95% CI −4.39 to 3.83). No difference was observed when independent ART techniques were compared nor when comparing in vivo (ovarian stimulation or intrauterine insemination) or in vitro (in vitro fertilization, intracytoplasmic sperm injection, or in vitro maturation) techniques ($P>.05$).

CONCLUSION: Children born after ART had similar cognitive, motor, and language development as children born after natural conception at 2 years of age. These findings may be useful in the clinical counseling of patients undergoing ART.

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**For a list of investigators from the 3D-Study (Découvrir, Développer, Devenir) from the Integrated Research Network in Perinatology of Quebec and Eastern Ontario, see Appendix 1 online at http://links.lww.com/AOG/A911.*

From the Departments of Obstetrics and Gynecology and Psychiatry and the School of Rehabilitation and the Faculty of Pharmacy, University of Montreal, the Research Unit on Medications and Pregnancy, Research Center, CHU Sainte-Justine, the Departments of Pediatrics, Human Genetics, and Pharmacology & Therapeutics, McGill University, Montreal Children's Hospital and Research Institute of the McGill University Health Centre, the Department of Obstetrics and Gynecology, Research Institute of the McGill University Health Center, and École d'orthophonie et d'audiologie, Université de Montréal, Montréal, the Department of Obstetrics and Gynecology, Université de Sherbrooke, Centre de Recherche du CHUS, Sherbrooke, and the School of Psychology, Laval University, and the Population Health and Optimal Health Practices Research Unit, CHU de Québec-Université Laval Research Centre, Québec City, Québec, Canada.

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Each author has indicated that he or she has met the journal's requirements for authorship.

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Technologic advances and changing social paradigms have led to the increased use of assisted reproductive technologies (ARTs) for the purposes of procreation.[1] The main techniques to treat infertility include: ovarian stimulation and intrauterine sperm insemination as well as techniques whereby oocytes and sperm are handled in vitro, like in vitro fertilization (IVF), intracytoplasmic sperm injection, and in vitro maturation.[2–4] We refer to ART as any of the aforementioned infertility treatments leading to conception outside natural coitus.

In Canada, reports indicate that the use of fertility treatments increased by 50% over the past decade.[1,5] Although the short-term perinatal outcomes after ART are well established, long-term neurodevelopmental outcomes, including cognitive, motor, and language development, are still a source of controversy.[3,6–9]

A review from the National Institutes of Health recognized that "lingering data gaps [exist] in the equivocal literature for many neurodevelopmental disabilities relative to ART" and that "…cohorts with longitudinal assessment…of neurodevelopment…are paramount for the development of empirically-based guidance…."[10] Similarly, the largest systematic review of more than 80 studies addressing long-term neurodevelopment after ART concluded that additional data were required to determine the true effect of fertility treatments on these outcomes.[3]

In our study, we tested the hypothesis that neurodevelopment at 2 years is related to mode of conception. As such, using standardized and validated tools, the objective of this study was to compare children's cognitive, motor, and language development at 2 years of age after ART relative to natural conception.

MATERIALS AND METHODS

We analyzed data from the 3D-Study (Découvrir, Développer, Devenir), a prospective, longitudinal cohort carried out from 2010 to 2012 by the Integrated Research Network in Perinatology of Quebec and Eastern Ontario in Canada.[11,12] The 3D-Study recruited 2,366 women in their first trimester of pregnancy and their respective births across nine sites in the province of Quebec and gathered extensive data on the mother–father–child triad from conception until 2 years postpartum. At 2 years postpartum, children underwent cognitive, motor, and language testing using the Bayley Scales of Infant and Toddler Development, 3rd edition, and the MacArthur-Bates Communicative Development Our primary objective was to compare the neurodevelopment in children born with the help of fertility treatments (exposed) relative to those born off pregnancies conceived naturally (controls). Our secondary objective was to describe baseline medical and sociodemographic differences between an ART and a non-ART cohort in Quebec.

The 3D-Study enrolled: 1) pregnant women between 8 0/7 and 13 6/7 completed weeks of gestation and 2) planning delivery in a 3D-Study–associated hospital. Exclusion criteria included: 1) women younger than 18 years of age, 2) illegal intravenous drug users, 3) non-English or French speakers, 4) severe illnesses or life-threatening conditions, and 5) multiple pregnancies, which includes twins or higher order multiples and mothers whose previous pregnancies had been enrolled in the study.

The Bayley Scales of Infant and Toddler Development, 3rd edition, is a validated and standardized developmental assessment for children aged 1–42 months that includes five independent scales (cognitive, motor [fine and gross], language, adaptive function, and socioemotional).[13] In our study, we used the cognitive scale, which assesses cognitive processes like memory, exploration, manipulation, and sensorimotor development as well as the motor scale, which is divided into the fine motor and gross motor subtests and evaluates quality of movement, sensory integration, perceptual–motor integration, prehension, and other milestones. Each scale consists of a series of developmental play tasks. Scale-specific raw scores of completed items are then converted to scaled scores and to composite scores as a function of age. For the fine and gross motor subtests, only scaled scores are available. The scaled and composite scores are then compared with normalized scores taken from typically developing children of similar age. Mean is set at 10 and 100 with a standard deviation of 3 and 15 for the scaled scores (fine and gross motor) and the composite score (cognitive, motor), respectively. The Bayley scales (3rd edition) have established test–retest reliability, internal consistency as well as convergent and divergent validity.[13] In our study, trained individuals who were blinded to the exposure administered the tool.

To evaluate language development, we used the toddler short form of MacArthur-Bates Communicative Development Inventories,[14] a norm-referenced parent questionnaire that captures important information about a child's developing abilities. Specifically, we used a 100-word vocabulary production checklist and a question about early word combinations, which can be reported on a 100-point scale.[14,15] The English MacArthur-Bates toddler short form has established

reliability as well as content and concurrent validity.[14,15] A French version of the short form has been adapted for French-speaking children in Québec using the approach described by Fenson et al.[14]

Based on the proportion of children having undergone the assessments (ART, n=175; natural conception, n=1,345), a power calculation was conducted to determine whether a minimal clinically significant difference in the Bayley scales (3rd edition) scores could be detected. Using previously reported mean and variance composite cognitive scores at 24 months of age, we used a two-sided type I error (α) of 5% and obtained 98.57% power to detect a 5-point difference between groups.[16]

We carried out our analysis in four steps. First, we described each subgroup according to their baseline demographic and gestational characteristics (Table 1). We included descriptors of infertility diagnoses for

Table 1. Baseline Patient Characteristics According to Mode of Conception

Variable	ART (n=278)	No ART (n=2,088)	*P*
Maternal age (y)	34.9±4.5	31.6±4.5	<.001
Paternal age (y)	36.3±6.1	33.4±5.8	<.001
Maternal ethnicity			.015
Caucasian	202 (72.7)	1,681 (80.5)	
Black	23 (8.3)	135 (6.5)	
Latin American	17 (6.1)	101 (4.8)	
Asian	25 (9.0)	100 (4.8)	
Other	11 (4.0)	71 (3.4)	
Maternal education			
Postsecondary	267 (96.0)	1,927 (90.0)	.001
Household income (Canadian dollars)			.489
Less than 40,000	48 (17.2)	352 (16.9)	
40,000–80,000	80 (28.8)	634 (30.4)	
Greater than 80,000	131 (47.1)	1,003 (48.0)	
Refused to disclose	19 (6.8)	99 (4.7)	
Mother living alone	10 (3.6)	123 (5.9)	.812
Prepregnancy weight (kg)*	65.4±14.9	65.1±21.5	.630
Gravidity			<.001
1	132 (47.5)	728 (34.9)	
2	85 (30.6)	676 (32.4)	
Greater than 2	61 (21.9)	684 (32.8)	
Medical comorbidities			
Asthma	41 (14.8)	344 (16.5)	.464
Diabetes	3 (1.1)	18 (0.9)	.717
Thyroid disease	45 (16.2)	162 (7.8)	<.001
Major depression—past	20 (7.2)	156 (7.5)	.869
Major depression—present	2 (0.7)	19 (0.9)	.750
Hypertension	6 (2.2)	52 (2.5)	.736
Dyslipidemia	8 (2.9)	69 (3.3)	.706
Cardiovascular disease	6 (2.2)	19 (0.91)	.056
Seizures	10 (3.6)	27 (1.3)	.004
Anemia	47 (16.9)	343 (16.4)	.840
Sexually transmitted infection	27 (9.7)	235 (11.3)	.441
Folic acid intake	170 (61.2)	1,070 (51.3)	.002
Maternal caffeine intake			
During pregnancy	40 (14.4)	405 (19.4)	.045
Maternal smoking			
Before pregnancy	38 (13.7)	405 (19.4)	.022
During pregnancy	9 (3.2)	106 (5.1)	.180
Maternal alcohol consumption			
Before pregnancy	157 (64.3)	1,418 (79.1)	<.001
During pregnancy	3 (1.1)	60 (2.9)	.081

ART, assisted reproductive technologies.
Data are mean±standard deviation or n (%) unless otherwise specified.
Gravidity is the total lifetime number of confirmed pregnancies including the current pregnancy.
* Values are rounded up.
Statistical tests used: χ^2, analysis of variance, *t* test.

patients undergoing ART and those defined as subfertile (Table 2). Subsequently, we described obstetric outcomes in the ART compared with natural conception group (Table 3).

We then evaluated the Bayley scales (3rd edition) (cognitive and motor) and MacArthur-Bates (language) scores for each mode of conception using χ^2 and analysis of variance statistical testing to determine within-group variability. Finally, we applied linear regression models to evaluate both the crude and adjusted effects of ART on scale scores using the natural conception group as a reference. Estimates for individual ART techniques were calculated as were estimates for grouped modes of conception: in vivo (ovarian stimulation and intrauterine insemination) and in vitro (IVF, intracytoplasmic sperm injection, and in vitro maturation). Analyses were adjusted for parental age (years), family income (Canadian dollars), maternal ethnicity (Caucasian compared with not), maternal education (level), marital status (married compared with not), maternal history of depression (yes or no), maternal smoking intake, alcohol consumption during pregnancy (yes or no), antidepressant use (yes or no), and folic acid intake during pregnancy (yes or no). Sensitivity analyses were carried out to evaluate the robustness of the model adjusting for thyroid disease, breastfeeding status as well as removing single women and same-sex couples from our model. In accordance with a provincial policy of elective single embryo transfer during the study period, the vast majority of patients undergoing embryo transfer (IVF, intracytoplasmic sperm injection, in vitro maturation) in our study received a single embryo per cycle. An exemption was made if the patient was older than 35 years of age and had prior cycle failures, in which case the transfer of two embryos was considered. We sought and received approval from the institutional ethics review board at the CHU Sainte-Justine Center (acting as the central ethics review board) in Montreal, Quebec. All analyses were conducted using SAS 9.3.

RESULTS

Our final cohort consisted of 2,366 women carrying singleton pregnancies. We compared 278 pregnancies after ART with 2,088 pregnancies after natural conception. The ART cohort was comprised of the following techniques: stimulation (n=53), intrauterine insemination (n=79), IVF (n=32), intracytoplasmic sperm injection (n=105), and in vitro maturation (n=9). The spontaneous conception cohort was comprised of subfertile patients (n=490) and patients

Table 2. Infertility Characteristics Among Assisted Reproductive Technologies Compared With Subfertile Conceptions

Variable	ART (n=278)	Subfertile Conception (n=490)	*P*
Infertility for more than 6 mo	247 (88.9)	490 (100.0)	<.001
Infertility for more than 12 mo	225 (80.9)	253 (51.6)	<.001
Time to conception (mo)	29.1±28.0	11.3±13.3	<.001
Median	24.0	7.0	
Infertility diagnosis—female factors*			
Endometriosis	23 (8.2)	9 (1.8)	<.001
Tubal factor	28 (10.1)	3 (0.6)	<.001
PCOS+anovulation	85 (30.6)	30 (6.1)	<.001
Diminished ovarian reserve	28 (10.1)	7 (1.4)	<.001
Uterine malformation	1 (0.4)	2 (0.4)	<.001
Single woman	7 (3.3)	—	—
Same-sex couple	6 (2.8)	—	—
Unexplained infertility	39 (17.1)	2 (0.4)	<.001
Other female factor	23 (8.3)	10 (2.0)	<.001
Unknown	37 (13.3)	8 (1.63)	<.001
Infertility diagnosis—male factors*			
Oligozoospermia	42 (15.1)	4 (0.7)	<.001
Teratospermia	48 (18.0)	3 (0.5)	<.001
Azoospermia	18 (6.5)	0 (0.0)	—
Ejaculatory dysfunction	2 (0.7)	0 (0.0)	—

ART, assisted reproductive technologies; PCOS, polycystic ovary syndrome.
Data are n (%) or mean±standard deviation unless otherwise specified.
Subfertile conception is natural conception after 6 months or longer of having tried to conceive.
Statistical tests used: χ^2, analysis of variance, *t* test.
* Values are rounded up. Percentages may not add up to 100%, because multiple infertility diagnoses may exist in the same patient.

Table 3. Obstetric and Neonatal Outcomes According to Mode of Conception

Variable	ART (n=278)	No ART (n=2,088)	*P*
Fetal sex			
Male	129 (49.1)	987 (50.4)	.803
Gestational age (wk)	38.4±2.8	38.8±2.1	.006
IQR	2.0	2.0	
Birth weight (g)	3,279.0±638.9	3,356.9±525.8	.030
IQR	697	1,034	
Fetal presentation*			.233
Cephalic	243 (92.4)	1,823 (92.8)	
Breech	13 (4.9)	99 (5.0)	
Other	0 (0.0)	15 (0.7)	
Unknown	7 (2.7)	31 (1.5)	
Mode of delivery			.000
Vaginal	138 (52.5)	1,275 (64.9)	
Cesarean	96 (36.5)	494 (25.1)	
Vacuum	14 (5.3)	109 (5.5)	
Forceps	15 (5.7)	88 (4.5)	
NICU admission	20 (7.7)	75 (3.9)	.004
5-min Apgar score	9 (3–10)	9 (5–10)	.880
Congenital anomalies	10 (3.8)	60 (3.1)	.525

ART, assisted reproductive technologies; IQR, interquartile range; NICU, neonatal intensive care unit.
Data are n (%), mean±standard deviation, or median (range) unless otherwise specified.
Statistical tests used: χ^2, analysis of variance, *t* test.
* Values are rounded up.

achieving natural conception at less than 6 months (n=1,598). Patients undergoing ART were more likely to be older, more educated, of lower parity, and with higher rates of thyroid disease. The later finding may be the result of more intense screening in the ART group as well as underlying thyroid dysfunction leading to infertility. On the other hand, mothers in the natural conception group were more likely to be Caucasian, multiparous, and with higher rates of caffeine, smoking, and alcohol consumption before and during pregnancy (Table 1).

In Table 2, infertility characteristics were compared between patients undergoing ART and those identified as being subfertile, who conceived after 6 months of trying. Patients having undergone ART had a longer time to conception and higher rates of underlying infertility diagnoses in both females and males ($P<.001$).

Table 3 presents obstetric and neonatal outcomes between both groups. Neonates born after ART were more likely to be of lower birth weight (3,279 g [interquartile range 697] compared with 3,356 g [interquartile range] 1,034), more likely to be born by cesarean delivery (36.5% compared with 25.1%), and to be admitted to the neonatal intensive care unit (7.7% compared with 3.9%). Although statistical differences were noted in the gestational age at birth, these are unlikely to be of clinical significance (38.4 weeks of gestation [interquartile range 2.0] compared with 38.8 weeks of gestation [interquartile range 2.0], $P=.006$).

A total of 175 of 278 children in the ART group (62.9%) and 1,345 of 2,088 in the natural conception group (64.4%) underwent neurodevelopmental assessments at 24 months. No significant differences were observed in cognitive (composite mean score±standard deviation: 98.5±11.2 compared with 100.1±11.4, $P=.08$), fine motor (scaled mean score 11.4±2.3 compared with 11.6±2.7, $P=.41$), gross

Table 4. Analysis of Variance—Bayley Scales of Infant and Toddler Development, 3rd Edition, and MacArthur-Bates Communicative Development Inventories Scale Scores at 24 Months of Age

Variable	ART (n=175)	No ART (n=1,345)	*P*
Cognitive composite score	98.5±11.2	100.1±11.4	.079
Score range	65–130	55–145	
Motor composite score	100.8±9.8	101.8±12.2	.282
Score range	82–142	61–164	
Fine motor—scaled score	11.4±2.3	11.6±2.7	.414
Score range	7–18	3–19	
Gross motor—scaled score	8.8±2.0	8.9±2.3	.372
Score range	4–16	1–19	
MacArthur-Bates Scale score	53.9±23.6	55.6±24.4	.507
Score range	1–100	5–100	

ART, assisted reproductive technologies.
Data are mean±standard deviation or minimum–maximum unless otherwise specified.
MacArthur-Bates Scale Score: out of 100. In the present study, 8% of the sample responses were based on the English version of the inventory and the remaining children's responses were based on the French version. The mean scores and standard deviations were similar for both language groups.
Statistical test used: *t* test.

Table 5. Adjusted Linear Regressions of Neurodevelopmental Scores Among All Assisted Reproductive Technologies, In Vivo, and In Vitro Conceptions Relative to Natural Conception*

	All ART				In Vivo (OS+IUI)			
Variable	B_1 (SE)	95% CI	β′	*P*	B_1 (SE)	95% CI	β′	*P*
Cognitive composite score	−1.60 (0.9)	−3.36 to 0.16	−0.045	.080	−2.22 (1.2)	−4.57 to 0.13	−0.048	.065
Motor composite score	−1.33 (1.0)	−3.29 to 0.63	−0.036	.186	−1.64 (1.3)	−4.18 to 0.90	−0.033	.222
Fine motor—scaled score	−0.24 (0.2)	−0.68 to 0.15	−0.030	.264	−0.26 (0.3)	−0.84 to 0.32	−0.024	.371
Gross motor—scaled score	−0.19 (0.2)	−0.58 to 0.20	−0.026	.331	−0.26 (0.3)	−0.84 to 0.32	−0.028	.300
MacArthur-Bates Scale Score	−0.28 (2.1)	−4.39 to 3.83	−0.003	.894	2.18 (2.8)	−3.30 to 7.66	0.016	.448

ART, assisted reproductive technology; OS, ovarian stimulation; IUI, intrauterine insemination; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; IVM, in vitro maturation; B_1, linear regression β coefficient; SE, standard error; CI, confidence interval; β′, standardized linear regression β coefficient.

Whereas B_1 represents the change in the score units of each scale for ART conception relative to a natural conception, β′ represents that change as a factor of standard deviation units. Both are interchangeable.

Non-ART and natural conception as a reference.

Statistical test used: adjusted linear regression.

* Adjusted for maternal age, paternal age, maternal education, income, ethnicity, marital status, smoking intake, alcohol consumption during pregnancy, history of depression, antidepressant use, and folic acid intake.

motor (scaled mean score 8.8±2.0 compared with 8.9±2.3, *P*=.37), or language scores (53.9±23.6 compared with 55.6±24.4, *P*=.50) (Table 4). Finally, Table 5 showcases the linear regression models. After adjusting for relevant confounders, children born after ART showed no difference in Bayley scales (3rd edition) cognitive composite scores (B_1 [standard error]=−1.60 [0.9], β′=−0.045, *P*=.08), composite motor scores (B_1 [standard error]=−1.33 [1.0], β′=−0.036, *P*=.18), or MacArthur-Bates language scores (B_1 [standard error]=−0.28 [2.1], β′=−0.003, *P*=.89) relative to natural conception. No significant differences were observed when comparing in vivo and in vitro techniques separately (*P*>.05) nor when comparing independent techniques individually. However, our study was not powered to compare the latter (Appendix 2, available online at http://links.lww.com/AOG/A911). Sensitivity analyses showed no differences in the model estimates when adjusting for thyroid disease, breastfeeding rates nor when removing single women or same-sex couples from the model.

Relative to participants lost to follow-up in the ART cohort, mothers of children who underwent testing were more likely to be Caucasian and of higher income. Among the natural conception cohort, mothers of children who underwent testing were more likely to be Caucasian, older, of higher education and income, and of lower parity (Appendix 3, available online at http://links.lww.com/AOG/A911).

DISCUSSION

Creating families through ART raises a number of concerns about potentially adverse consequences for child development.[2,3,17–19] However, these concerns stem from largely retrospective studies with small sample sizes and heterogeneous methodologies.[20] By specifying the infertility treatments used, accounting for predictors of development, and using standardized testing, our prospective study overcomes some of these limitations and provides reassuring results in that children born after ART appear to have similar cognitive, motor, and language skills than children born after natural conception at 2 years of age.

The recent Upstate KIDS Study sought to assess the same question in this report, notably, the association between the mode of conception and children's development.[9] According to its results, children's development at age 3 years appears independent on mode of conception.[9] Although the prospective nature of the KIDS study is a major strength, a number of its limitations are addressed by our study. Whereas the KIDS study recruited newborns, the 3D-Study recruited mothers during the first trimester, allowing us to prospectively gather data on prenatal factors that may have affected neurodevelopment such as antidepressant, folic acid, alcohol, and smoking exposure. Second, their study used the Age and Stage Questionnaires to assess neurodevelopment. Unlike the Bayley scales (3rd edition), which are administered by a third party blinded to the exposure, the Age and Stage Questionnaires require parental administration, which may introduce confirmatory bias.[21] Third, although the 3D-Study required a prospective, two-step verification of exposure including ovarian stimulation and intrauterine insemination, the KIDS study could not verify the validity of the

In Vitro (IVF+ICSI+IVM)			
B_1 (SE)	95% CI	β′	*P*
−0.60 (1.3)	−3.14 to 1.94	−0.013	.640
−1.04 (1.4)	−3.78 to 1.70	−0.020	.467
−0.22 (0.3)	−0.80 to 0.36	−0.020	.476
−0.12 (0.3)	−0.70 to 0.47	−0.013	.642
−2.88 (2.8)	−8.36 to 2.60	−0.021	.320

exposure because there is no registry in the United States.[9] Nevertheless, the replication of similar findings in both studies despite the use of different methodologies is encouraging and may serve to reassure patients undergoing ART.

Each facet of neurodevelopment after ART has been studied previously. To date, two large systematic reviews of more than 80 studies addressed cognitive development after ART, concluding that, "there is sufficient data to support…no difference in development…between IVF and spontaneously conceived children"[3,20] and that "most studies showed no associations with cognitive…development."[3] Because we cannot preclude that differences in cognition may appear later in life, a follow-up of children from prospective studies such as this one may be necessary.

Similarly, prospective evidence of motor skills at 24 months of age evaluated with standardized testing is lacking in the literature. Although some studies do point to delays in motor development between 16 and 18 months,[22] our findings concur with the majority of the literature that motor development is not affected by the mode of conception.

Most of the controversy seems to be found in language development after ART.[7,22,23] As evidenced by the lack of consensus, there is a call for prospective evaluation of children's language skills after ART as we have done in our study, in which we find no significant difference in MacArthur-Bates scores at 24 months of age.

The strengths of the present study include: the use of a prospective cohort of pregnant women with up to 3 years of follow-up, the use of standardized tools administered by professionals blinded to exposure, and the analysis of a number of ART techniques. In addition, we adjusted for a vast array of pertinent confounders, including maternal depression, which is notably lacking in the literature.[24] Likewise, our study uses North American data, which may enhance external validity amongst Canadian and U.S. centers. Finally, we conducted sensitivity analyses, which confirmed the robustness of our model.

On the other hand, a number of limitations are worth mentioning. Although this study was powered to estimate the effect of ART as an overall category, it was not powered to detect a difference among individual techniques. Likewise, we considered the main ART technique as exposure and could not account for the type of cycle (natural compared with stimulated) used. Furthermore, although loss to follow-up rates were moderate in each group, a post hoc power calculation reveals adequate power to answer the study question. Moreover, given the study design, we were not able to untangle the effects of the underlying infertility from the ART technique used, because this is an example of confounding by indication. Finally, the children in our study population were young, and in certain cases, developmental characteristics may have a limited predictive value for long-term development.

All in all, the findings hereby presented may be useful in the clinical counseling of patients undergoing ART. Future prospective studies with long-term follow-up, powered to study individual ART techniques as well as evaluation of behavioral outcomes (such as attention deficit or hyperactivity and autism-like behaviors), are necessary.

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