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Assisted Reproductive Technologies and imprinting disorders: Results of a study from a French congenital malformations registry

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ABSTRACT:

Introduction: Assisted Reproductive Technologies (ART) is increasingly used to help infertile couples to have children around the world. A number of studies have been published reporting an increased risk of major malformations in children born following ART, especially an increased incidence of epigenetic diseases (ED). This study aimed to assess the incidence of epigenetic diseases with affected imprinting genes in infants or children from pregnancies obtained through IVF/ICSI compared to infants or children from pregnancies obtained spontaneously.

Patient data: This is a monocentric retrospective epidemiological study based on data from a French congenital malformations registry called REMERA (*Registre des Malformations en Rhône-Alpes*) which exhaustively monitors all pregnancies in Rhone-Alpes region, whatever their nature of onset (spontaneous pregnancies or pregnancies from ART). This registry collects all malformations, except minor malformations (EUROCAT), and all poly-malformative syndromes concerning all fetuses and children born alive or not, from 20 weeks of pregnancy (or 22 weeks of amenorrhea) and all medical termination of pregnancy whatever the term. Inclusion criteria are all diagnoses of epigenetic diseases (ED) related to parental imprinting recorded in the period January 2006 to December 2015.

Methods: For each year, the total number of births (including stillbirths) was collected from the annual activity reports of the registry. The exhaustive number of cases of epigenetic diseases was known in the registry. Were collected the number of births resulting from ART pregnancies in the study population. This incidence of ED was compared between births from spontaneous pregnancies and those obtained through ART (IVF/ICSI) with a generalized linear model (GLM: binomial regression).

Results: In total, 46 cases of epigenetic diseases were analyzed on the REMERA registry files from 2006 to 2015. 4 cases from the 46 analyzed cases were from pregnancies induced by ART. ART was a risk factor for epigenetic disease (OR = 2.9 [1.06–8.22] (p = .039)). In ART-pregnancies there were 2 diagnoses: Beckwith-Wiedemann syndrome (BWS) (3 cases out of 4) and Silver-Russell syndrome (SRS) (1 out of 4).

Discussion: Infants and children obtained through IVF/ICSI appear to be related to a higher risk of epigenetic diseases compared to naturally conceived children. The perspectives of this study are to raise awareness about the creation of registries of congenital malformations and genetic and epigenetic syndromes with systematic and strict reports of all the cases on all the French territory and thus to widen this study with a bigger cohort.

1. Introduction

Assisted Reproductive Technologies (ART) is increasingly used to help infertile couples to have children around the world (Kushnir et al., 2017) (Gianaroli et al., 2012). More than 5 million children were born thanks to these assisted reproductive therapies (Cedars, 2013).

Since the 80's, in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI), embryo culture, and embryo cryopreservation have

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Table 1
4 imprinting disorders and their association with ART. Epigenetic disorders in imprinting syndromes take place essentially in imprinted loci on 2 chromosomes the 11 and the 15. The imprinted loci involved in BWS and SRS are IGF2-H19 and KCNQ1 of the chromosomal region 11p15.5. These epigenetic anomalies lead to opposed phenotypes (Eggermann et al., 2016). Epigenetic disorders on imprinted loci SNRPN of the chromosome 15q11–13PWS are involved in PWS and AS, which induce the repression of UBE3A gene (Fauque et al., 2008).

	Chromosome region 11p15–5		Chromosome region 15q11–13	
	Beckwith-Wiedemann	Silver-Russell	Prader-Willi	Angelman
Loci	**IGF2-H19 and KCNQ1**		**SNRPN**	
Prevalence (Orphanet)	1-5/10000	1-9/1 000 000	1-9/100 000	**1–9/100 000**
Imprinting disorders	- hypermethylation of H19 - hypomethylation of KCNQ1OT1(- LIT1) (Choufani et al., 2013)	- paternal hypomethylation of H19 (Bliek et al., 2006)	- deletion on 15q11-q13 - maternal uniparental disomy (mUPD) of chromosome 15 - imprinting center (IC) defects (microdeletion of PWS-IC, primary epimutation) - unbalanced translocations - (Yoon Cho et al., 2013)	- deletion in chromosome 15q11-q13 (70%) - point mutation (10%) - paternal uniparental disomy (2%) - imprinting defect (< 5%) (Wheeler et al., 2017)
Phenotype	- placentomegaly - macrosomia - organomegaly - exomphalos - renal dysplasia - hypoglycemia - (Weksberg et al., 2005)	- intrauterine and postnatal growth restriction - small for gestational age (2–3 s.d. below the mean) - relative macrocephaly - face/body asymmetry - (Wakeling et al., 2016)	- hypotonia - mental disability - psychomotor delay - hypogonadism - hyperphagia, obesity - (Cheon, 2016)	- severe mental disability - communication impairments - movement disorders - microcephalia (Williams et al., 2010)

been rapidly developed (Magli et al., 2008). Thus, ART also involve the manipulations of oocytes and sperm in the laboratory.

Since then, the quick advances of ART raised the question of the short- and long-term consequences for the health of these ART-children (Chen and Heilbronn, 2017).

A number of meta-analysis have been published reporting an increased risk of major malformations in children born following ART when compared with naturally conceived children (Basatemur and Sutcliffe, 2008).

Concerns have also been raised regarding the risk of rare imprinting disorders after using ART, with an increased incidence of epigenetic diseases (Feuer et al., 2013) with many reported cases of Beckwith-Wiedemann' and other syndromes in children conceived through IVF/ICSI (Maher et al., 2003) (Maher, 2005) (Sutcliffe et al., 2006) (Manipalviratn et al., 2009) (Smallwood and Kelsey, 2012) (Shufaro and Laufer, 2013) (Table 1).

Indeed, these rare diseases are related to modifications of a family of genes called imprinted genes involved in embryo and fetal development (Arnaud and Feil, 2005).

Disruption of the essential epigenetic marks in imprinted genes is the common element in these imprinted pathologies (Fauque et al., 2008).

Epigenetics mentions heritable changes in gene expression – active versus inactive genes – that do not involve changes to the underlying DNA sequence (Iliadou et al., 2011).

Epigenetic mechanisms govern early and late development, regulating gene expression in diverse cell types of the organism (Shufaro and Laufer, 2013).

One of the major types of epigenetic markers that control gene expression is DNA methylation at CpG dinucleotides and is the most commonly studied epigenetic process in relation to fertility treatment and imprinting diseases (Lazaraviciute et al., 2015).

Thus, epigenetic activity is closely linked with critical steps in development occurring by the time of conception (Hackett and Surani, 2013), including regulation and parental mono-allelic gene expression (Iliadou et al., 2011).

Even if ART are considered as safe, recent studies suggest that there may be links between ART and increased risks for epigenetic anomalies with modifications of DNA methylation for example leading potentially to imprinting syndromes (Fauque et al., 2008) (Manipalviratn et al., 2009) (Smallwood and Kelsey, 2012) (Shufaro and Laufer, 2013). However, this link remains unclear and difficult to assess (Shufaro and Laufer, 2013).

Using data from a French congenital malformations registry, this study aimed to assess the incidence of epigenetic diseases with affected imprinting genes in infants or children from pregnancies obtained through IVF/ICSI compared to infants or children from pregnancies obtained spontaneously.

2. Material and methods

This is a monocentric retrospective epidemiological study based on data from a French congenital malformations registry called REMERA (*Registre des Malformations en Rhône-Alpes*) which exhaustively monitors all pregnancies in Rhone-Alpes region, whatever their nature of onset (spontaneous pregnancies or pregnancies from ART).

This registry collects all malformations, except minor malformations (EUROCAT) and all polymalformative syndromes related or not to chromosomal aberrations. It concerns all fetuses and children born alive or not, from 20 weeks of pregnancy (or 22 weeks after last menstrual period) and all termination of pregnancies whatever the term.

Each validated case benefited from a verification of the sources, inclusion criteria (temporal and geographical), confirmation of the diagnosis (eg. karyotype result, fetopathology report, complementary examination results), classification of the type of malformation, a negative duplicate search and a coding check.

Inclusion criteria are all diagnoses of epigenetic diseases (ED) related to parental imprinting. For each case included in the period January 2006 to December 2015. The REMERA file was used to extract the following data: diagnosis, newborn or fetus vital state, moment when the diagnosis was made, the term, the sex ratio, the size, newborn or fetus weight, the type of ART technique performed to obtain pregnancy, and maternal age.

For each year, the total number of births (including stillbirths) was collected from the annual activity reports of the registry (https://www.remera.fr/?page_id=56). The exhaustive number of cases of epigenetic diseases was known in the register. Were collected the number of births resulting from ART pregnancies in the study population.

The incidence of ED and the 95% confidence intervals were calculated using counts of ED and the total number of births from spontaneous pregnancies and pregnancies obtained through ART. This incidence of ED was compared between births from spontaneous