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Donation of Gametes and Risk of Preeclampsia

Anna Blazquez Ventura



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PhDThesis

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Facultat de Medicina i Ciències de la Salut

Departament d' Obstetricia i Ginecologia, Pediatria, Radiologia i
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Donation of Gametes and Risk of Preeclampsia

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Donation of gametes and Risk of Preeclampsia

ABBREVIATION LIST

ART:	assisted reproductive technology
BMI:	body mass index
CV:	cardiovascular
DD:	double donation of gametes
DET:	double embryo transfer
ET:	embryo transfer
IVF:	in vitro fertilization
FP:	women with female partner
GH:	gestational hypertension
GnRHa:	agonist of the Gonadotropin-releasing Hormone
HLA:	Human Leukocyte Antigen
ICSI:	intracytoplasmic sperm injection
KIR:	killer immunoglobulin receptor
MP:	women with male partner
OD:	oocyte donation
PE:	preeclampsia
sEng:	soluble endoglin
SET:	single embryo transfer
sFlt-1:	soluble fms-like tyrosine kinase 1
SW:	single Women
T-reg:	regulatory T cells
TGF-β:	transforming growth factor- β
uNK:	uterine Natural Killer cell
VEGF:	vascular endothelial growth factor
PGE:	prostaglandin E
PIGF:	placental growth factor

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INTRODUCTION

PREECLAMPSIA

Epidemiology

Preeclampsia (PE) is a pregnancy hypertensive disorder which involves multiple maternal organs, but characterized by the dysfunction of one, the placenta⁽¹⁾. It complicates between 2 and 8% of all pregnancies, and while its mortality in developed countries is decreasing, it is still one of the main causes of maternal death all over the world, compounding 16% of pregnant women deaths⁽²⁾. In the last decades, the incidence of PE has been increasing in developed countries, because its risk factors, such as hypertension, maternal age, diabetes mellitus, obesity, and multiple births have also increased⁽³⁾.

Definition

Pre-eclampsia is classically defined as new-onset hypertension (arterial tension >140/90 mmHg in at least 2 determinations taken at least 6h apart) associated with proteinuria (>300 mg protein in a 24h urine), diagnosed at or after 20 weeks of pregnancy⁽⁴⁾. Although proteinuria is present in the majority of cases, most current guidelines do not require this sign in the presence of other organ-end signs or symptoms. For instance, The American College of Obstetricians and Gynecologists guidelines defines PE in the absence of proteinuria as hypertension with any of the

followings: new-onset thrombocytopenia, impaired liver function, renal insufficiency, pulmonary edema, or visual or cerebral disturbances ⁽⁵⁾.

Classification

PE is classified in two subclasses: early-onset pre-eclampsia (< 34 weeks of gestation), and late-onset (> 34 weeks of gestation). In the early-onset syndrome, it is believed that the cause is an intrinsic placental dysfunction, triggered by an immune maladaptation of the mother to the fetus ⁽⁶⁾. On the other hand, in the late-onset PE, the origin must be found in a hidden cardiovascular dysfunction of the mother that, due to the pregnancy increase in hemodynamic demands, leads to placental dysfunction ⁽⁷⁾. The first one, with an immunological trigger, is also called “placental PE”, and the late-onset, with a vascular origin, is termed “maternal PE” ⁽¹⁾. This division is relevant as the early-onset PE is more severe, with fetal growth restriction and adverse maternal and neonatal consequences ^(8,9). On the other hand, despite late-onset PE being milder, it is a large contributor to adverse perinatal outcomes due to the fact that it is 5-10 times more frequent than early-onset PE.

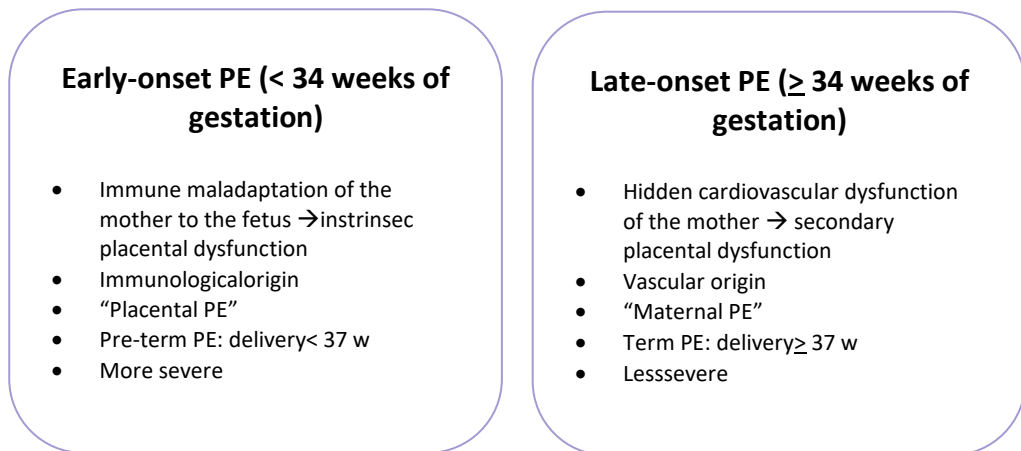


Figure 1: classification of PE.

Pathogenesis

Many theories have been proposed to describe PE pathogenesis, and we cannot select one as the unique explanation for the disorder. The pathogenesis of the syndrome in the early stages of pregnancy could be different from the one that develops in the last stages; moreover, sometimes more than one physiopathology line is needed to justify all the findings in PE.

The common point in all theories is a dysfunctional placental vascularization. Classically, the progression of the disease has been explained with an impaired initial trophoblastic invasion of the decidua, which in turn leads to defective spiral artery remodeling. This alteration leads to the uteroplacental hypoperfusion. In a second stage, there is a release of antiangiogenic factors from the placenta, such as soluble fms-like tyrosine kinase 1 (sFlt-1), soluble endoglin (sEng). The first one, sFlt-1, binds

and inhibits the vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), both with angiogenic action, while sEng does the same but with transforming growth factor- β (TGF- β). The imbalance between these antiangiogenic factors with the angiogenic ones, triggers a systemic maternal inflammatory response and endothelial dysfunction responsible of the features of PE (1,2,6,10,11).

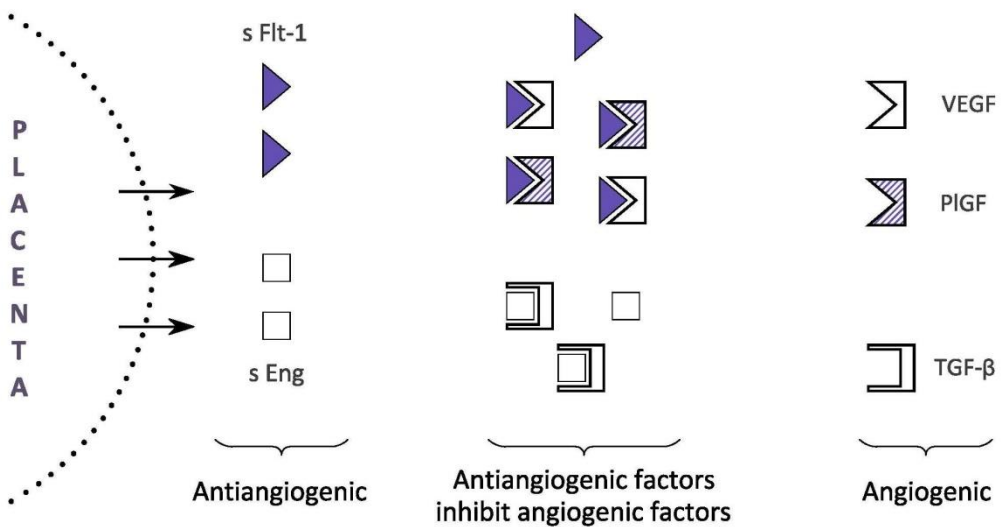


Figure 2: current understanding of angiogenic and antiangiogenic factors in PE.
Adapted from Enkhmaa et al, *J Women's Health* 2016.

The controversy appears in the previous step of all this process, in the hypothesis of the cause of the altered trophoblast invasion. And even more, theories diverge on whether if it is the dysfunctional placenta what causes the systemic vascular symptomatology, or if it is a maternal impaired cardiovascular function what causes an hypoperfusion of the placenta and, secondarily, its dysfunction.

The immunological theory

The immunological theory of PE pathogenesis postulates that the vascular dysfunction is the result of a maladaptation of the mother to fetal agents, and rests on the observation that the trophoblastic cells invading the decidua during early pregnancy express class I HLA-C antigens (both maternal and paternal), which are polymorphic, unlike the non-classical HLA-G and HLA-E that are also expressed in trophoblastic cells. The HLA-C is a strong ligand for the killer immunoglobulin receptor (KIR) which is present on the surface of the uterine natural killer cells (uNK). The KIR receptor also has a wide variability, as more than 350 different KIR genotypes have been described, although they can be classified in 2 basic haplotypes: KIR A and KIR B. KIR A is inhibiting and KIR B is activating, depending on their up-regulation or down-regulation of the uNK function. Despite there are three different combinations (KIR AA, KIR AB and KIR BB), if they have at least one haplotype of KIR B they act as activating, so in the practice we still divide them in two groups⁽¹²⁾. At the same time, HLA-C is also divided into two subtypes: HLA-C1 and HLA-C2. In the trophoblastic cell, then, that express one paternal and one maternal allotype of HLA-C, we can find three combinations: HLA-C1/C1, HLA-C1/C2 and HLA-C2/C2.

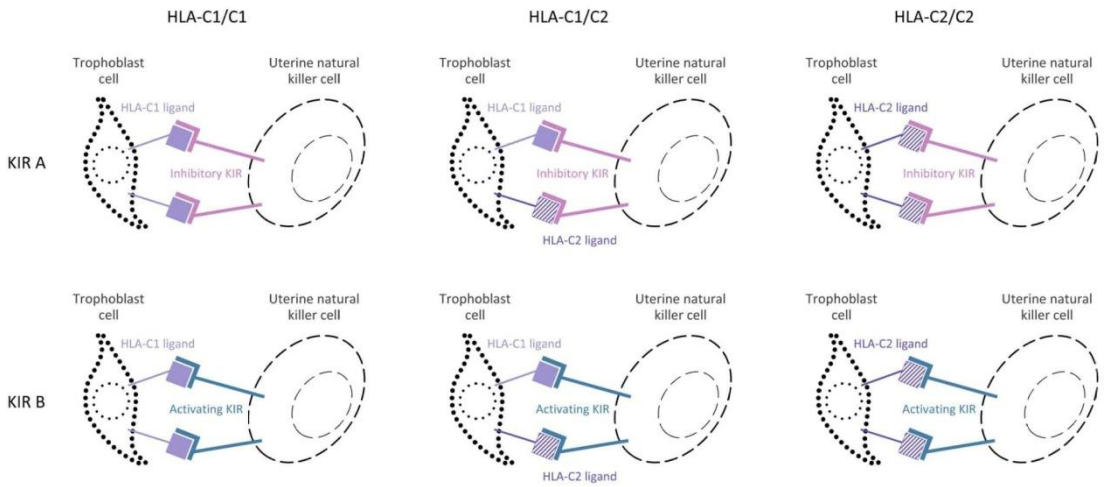


Figure 3: possible combinations of trophoblastic HLA-C and maternal KIRs.
Adapted from Morin, Fert &Steril 2017.

The mode of action of uNK differs from that of systemic NK cells, as uNK modulate proangiogenic and endothelial factors (PIGF, VEGF, TGF- β) which in turn stimulate decidual neovascularization, promoting changes in the spiral arteries to supply proper blood flow to the fetus.

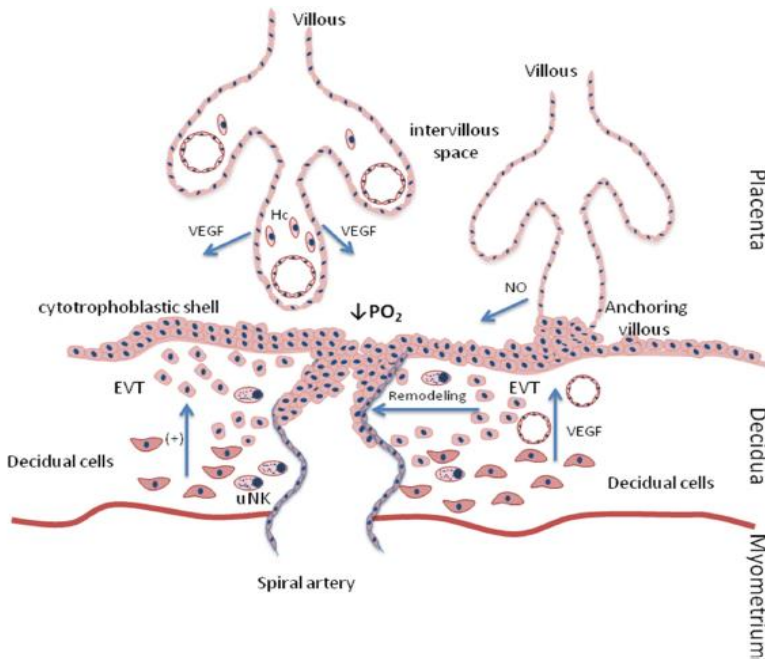


Figure 4: Placental angiogenesis during early pregnancy. From Maliqueo, *Frontiers in Physiology*, 2016.

Some maternal KIR genotypes (especially the AA genotype) combined with certain trophoblastic HLA-C allotypes (particularly HLA-C2, when the fetus has more C2 genes than the mother or when fetal C2 is inherited paternally) can favor a dysfunction of uNK, which is in turn associated with an altered maternal blood supply to the placenta, ultimately inducing disorders like PE and fetal growth restriction^(13,14).

This rationale supports the placental origin for early-onset PE, characterized by small placenta with histological findings of hypoperfusion⁽¹⁵⁾, and associated with fetal growth restriction^(16,17). But in the cases of late-onset PE, the most common manifestation are proper of an hypertensive disorder, placental lesions of

hypoperfusion are less frequent or even absent ⁽¹⁵⁾, and usually fetuses have normal weight for gestational age ^(18,19).

The cardiovascular theory

Several studies have shown increased cardiovascular risk factors in women after pre-eclamptic pregnancies ^(20–22), but many others found that risk factors are present before gestation and can predict PE. Furthermore, these factors are strongly associated with future cardiovascular risk, more than PE itself ^(23,24). Pregnancy is a state where the hemodynamic demands on the cardiovascular system are increased, as well as other metabolic functions, as for example healthy pregnancies have certain degree of insulin resistance. All these physiological changes can be excessive for pregnancies with pre-gestational cardiovascular risk factors, which have a predisposing endothelial dysfunction, and could lead secondary to PE ⁽²⁵⁾.

Risk factors

Several risk factors have been identified for PE. Most of them are present before the pregnancy and are used by healthcare professionals to assess the risk of each patient at the beginning of the pregnancy, in order to schedule appropriate obstetrical follow up and prophylactic measures.

Not all the factors have the same significance in the risk for developing PE. The following list ranks them by the relative risk that confer, from higher to lower:

1. History of PE in a previous pregnancy: having suffered PE in a previous gestation confers a sevenfold risk for PE ^(26,27).
2. Underlying medical condition:
 - a. Autoimmune disease such as systemic lupus erythematosus, or especially antiphospholipid syndrome, increases six times the risk for PE ^(28,29).
 - b. Diabetes mellitus (DM) before pregnancy nearly quadruples the risk for PE ⁽³⁰⁾.
 - c. Pre-existing hypertension doubles the risk for developing PE ⁽²⁸⁾.
 - d. Renal disease⁽²⁸⁾.
3. Obesity: A pre-pregnancy Body Mass Index of >35 increases more than three times the risk for PE ^(28,31).
4. Parity: nulliparity gives almost a threefold risk for developing PE ⁽²⁸⁾.
5. Multiple pregnancy: twin pregnancies almost triples the risk for PE compared with singleton pregnancies ⁽³²⁾.

6. Family history of PE, hypertension and diabetes mellitus (in mother or sisters) increases almost three times the risk for PE ^(33,34).
7. Advanced maternal age: pregnant women above 35 years, and especially above 40 years, have twice the risk to develop PE ⁽³⁵⁾.
8. Change in partner: changing the partner confers a higher risk to develop PE ^(36,37).
9. Race: African-American pregnant women have higher risk than Caucasian women⁽³⁸⁾.
10. Conception mode: assisted reproductive technology (ART) is associated with more risk to develop PE compared to natural gestations. The use of donated gametes confers an additional risk to ART ^(39,40).

Noticeably, smoking has been found to act as a protective factor for PE ⁽⁴¹⁾.

ASSISTED REPRODUCTIVE TECHNOLOGY

Since the first baby born by in vitro fertilization in 1978, ART has undergone many changes, especially due to the advances in medicine and the steadily increase in the number of patients demanding fertility treatments. The number of fertility clinics has increased, making ART more accessible to people all over the world, despite important differences among regions⁽⁴²⁾. Technology allows the culture of the embryo until blastocyst stage, the use of donated gametes, embryo cryopreservation and preimplantation genetic diagnosis, but outcomes of babies conceived by ART are still a matter of study, as in many cases they are more adverse than in naturally conceived babies.

Assisted Reproduction Technology and PE

ART is associated with a higher risk for PE compared to naturally conceived pregnancies. While this association has been proved in several occasions, the reason for it remains unexplained. The risk factors of the patients who needed fertility treatments, the causes of the infertility itself, and the hormone therapy that they received, are all plausible causes for the increased risk for PE in ART patients. To complicate matter further, ART is also associated with an increased rate for multiple gestation⁽⁴²⁾.

One study comparing singleton pregnancies conceived by ART (in vitro fertilization, intracytoplasmic sperm injection and frozen embryo replacement) or spontaneous conception, showed that, even after adjustment for maternal age and parity, the risk of ART pregnancies and PE remained higher ⁽⁴³⁾. Different theories have been proposed to explain the relationship between ART and PE. One theory is based on the infertility of these patients; infertility was found to be associated with anomalous DNA methylation pattern in placentas from pregnancies conceived by IVF/ICSI compared to naturally conception placentas ⁽⁴⁴⁾. At the same time, epigenetic changes (changes in DNA methylation, histone modifications and non-coding RNAs, in miR-210 and genes as SERPINA3 or HI9, among others) have been observed in preeclampsia placentas compared to normal placentas ⁽⁴⁵⁾. Thus, infertility could be the cause of a molecular impairment that predisposes to PE. However, ART per se could also explain this association; the culture conditions used in the embryology laboratory ⁽⁴⁶⁾, the hormones that the patient needs to stimulate the ovary ⁽⁴⁷⁾, or the embryo transfer and manipulations ⁽⁴⁸⁾, all are possible causes for an altered trophoblast invasion.






	Predisposing factors of patients needing ART (advanced age, nulliparity...)
	Multiple gestations
	Cause of the infertility (epigenetic changes, ovarian failure...)
	ART per se (cultures in the lab, hormone therapy...)
	Gamete donation

Figure 5: possible causes of PE in ART patients

Donated gametes and PE

A part from this general association between ART and PE, the use of donated gametes has also been specifically related to PE^(39,40,49–54).

A longer exposure to the partner's sperm and the lack of use of barrier contraceptives, all have been reported to decrease the prevalence of PE^(37,55–57), although the clinical relevance of this protective effect has been questioned^(58,59). Previous pregnancies with the same partner, however, have a demonstrated protective effect against PE: primigravity, or conception with a new partner, on the other hand, are associated with

higher rates of PE, while multiparity or previous abortions with the same partner decrease the PE risk ^(36,60). The reason for this effect could be found in the regulatory T cells (T-reg) of the mother. T-reg suppress the immune response of the mother, allowing an adaptation to the allogenicity of the embryo during pregnancy ⁽⁶¹⁾. Several substances in the seminal fluid, such as TGF β and PGE-related prostaglandins, can induce the expansion of T-reg cells specific to paternal antigens. For this to happen, repeated contact between the seminal fluid and the maternal mucosa is needed ^(62,63).

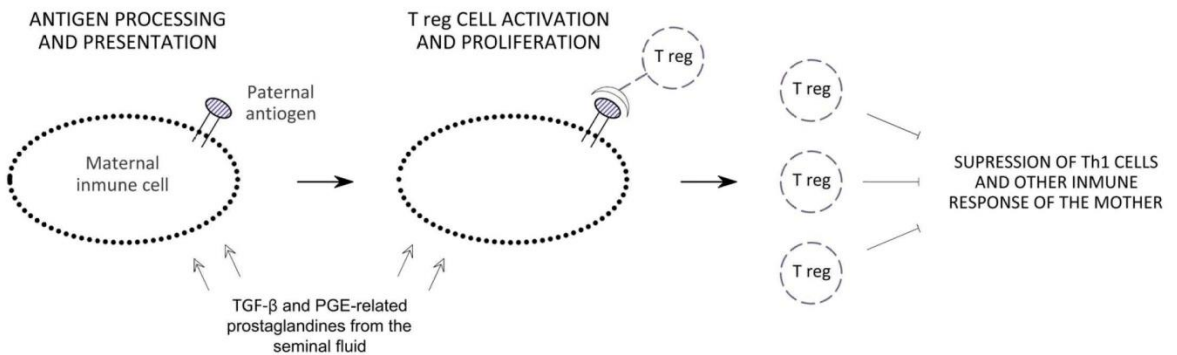


Figure 6: function of Treg cells allowing the implantation of the allogenic trophoblastic cell. Adapted from Robertson, *Am J Reprod Immun* 2013.

Regardless of the role of paternal antigens, the conceptus trophoblastic HLA-C is less recognizable for the immunological system of the mother when donated oocytes are used ⁽⁵¹⁾, because both alleles are non-self to the mother. This could lead to an altered function of the uNK and consequently to an altered maternal blood supply to the

placenta, facilitating disorders like PE and fetal growth restriction^(13,14,51). Hibi shown that the association between a maternal KIR AA with fetal C2, especially if the C2 gene is inherited from the father, increases the risk for PE⁽¹²⁾. In oocyte donation, the oocyte HLA-C behaves as the paternal HLA-C, increasing the chances of finding the combination of a KIR AA recipient with non-maternal HLA-C2 fetus. Additionally, in ART, double embryo transfer (DET) increases the expression of trophoblastic HLA-C in the decidua basalis, multiplying, in cases of oocyte donation, the number of external HLA-C presented to the mother. A study analyzing reproductive outcomes in oocyte donation cycles found that a decreased live birth rate per cycle after DET was observed in mothers KIR AA compared to mothers with KIR AB and KIR BB, but not in SET⁽⁶⁴⁾. They speculate that in DET cases, where 4 possible non-maternal HLA-C could be present (one paternal and one from the oocyte donor per each one of the two embryos), it was more likely to find an external HLA-C2 to the mother than in SET or in own oocytes cycles.

Also, T cells have been involved in the immune down-regulation that allows the fetus to develop in the maternal allogeneic environment⁽⁶⁵⁾. In a study of 26 placentas of oocyte donation pregnancies, an infiltrate of macrophages was found in placentas of pregnancies uncomplicated by PE, and not in the placentas of pregnancies with PE. This lesion in the chorionic plate was associated with intervillitis, chronic deciduitis, higher expression of CD14+ macrophages and fetal HLA-C2. All these data suggested

an immunological protection of the mother to the fetus, which was absent in PE gestations ⁽⁶⁶⁾.

Furthermore, some authors hypothesize an association between the need for oocyte donation per se and PE, as circulating antibodies against granulosa cells and the oocyte's zona pellucida have been detected in patients presenting ovarian failure, a classical indication for oocyte donation (OD) independently from sperm donation ⁽⁶⁷⁾. These authors postulate that the antibodies could cause injury to the trophoblastic cells invading the decidua vessels as well ^(40,53). Moreover, it is not clear whether the altered ovarian function of patients needing OD could be related to vascular or immunological changes that could independently predispose to PE^(53,68).

Frozen embryo transfer and PE

There is strong evidence suggesting that the supraphysiological hormonal levels reached during controlled ovarian stimulation have deleterious effects on the endometrium, causing placental dysfunction and alterations, likely affecting the pregnancy course^(69–71). Different studies relate abnormal levels of progesterone with PE, although with opposite conclusions on whether a high or low level of this hormone is found in preeclamptic pregnancies^(72,73). The same controversy occurs with estradiol levels and pregnancy pathology ^(74,75). Sex steroids play an important role in

the proper blood flow of the placenta, and a variation in their normal concentration could affect the vascularization of the fetus ⁽⁷⁶⁾.

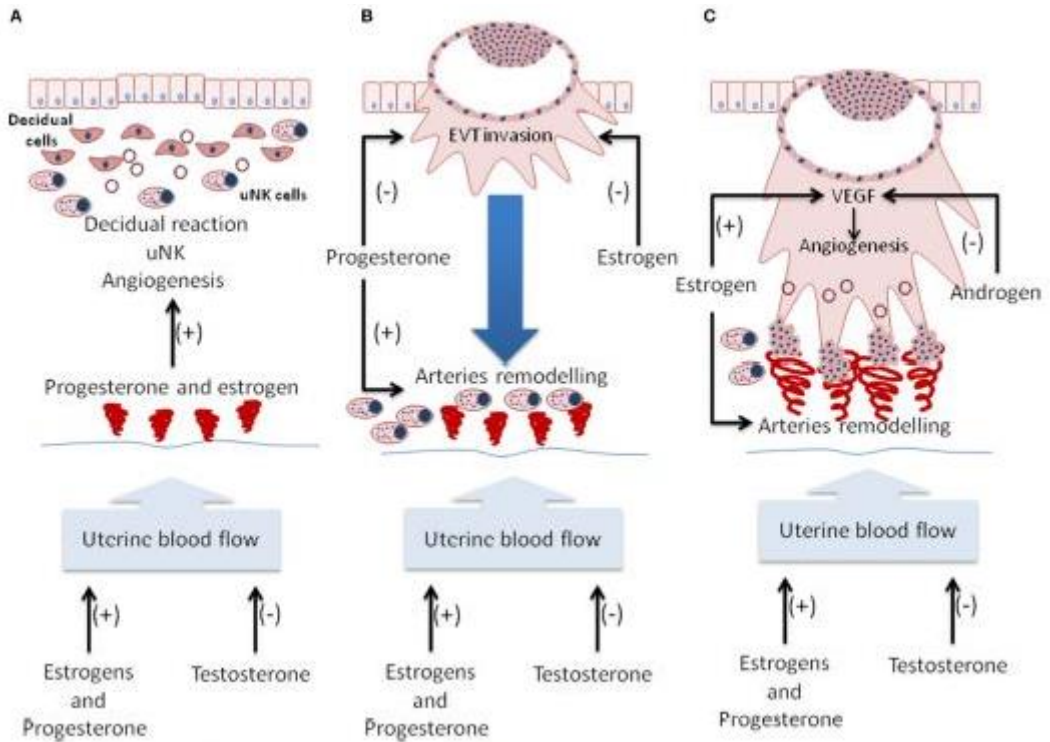


Figure 7: Sex steroids regulate the uterine-placental vasculature. From Maliqueo, *Frontiers in Physiology*, 2016.

This could be one of the reasons for the different perinatal outcomes found after frozen embryo transfers (frozen ET), by definition without a previous ovarian stimulation, and fresh embryo transfers (fresh ET). When compared to fresh ET, frozen ET have been associated with lower rate of low birth weight singletons ^(69,77-80), less preterm birth ^(69,79), less ectopic pregnancy ^(81,82) and higher rates of large for

gestational age singletons^(69,77–79,83). Data are still inconsistent about the perinatal mortality of frozen ET babies: while in some papers the risk is higher compared to fresh ET^(69,77), others do not find differences⁽⁷⁹⁾, or even report less risk⁽⁸⁴⁾.

The effect of frozen ET on the occurrence of placental alteration has been less studied so far: the rate of placenta previa seems either lower⁽⁷⁷⁾, or not different⁽⁷⁸⁾, while the occurrence of placenta accreta seems higher after frozen ET⁽⁷⁸⁾.

Regarding PE, it is the only maternal effect consistently increased in frozen ET compared to fresh ET^(51,77,78,85,86). Some authors also suggest that the process of freezing and thawing per se could bring about some metabolic or epigenetically changes in the embryos, and thus be an adjuvant in the origin of PE in the frozen ET group^(87,88). Some cryoprotectants have been reported to interact with the main enzyme involved in epigenetic reprogramming, methyltransferase⁽⁸⁹⁾. This interaction during the initial embryonic developmental phases could be the cause of variations in the epigenetic burden of the embryo and might affect developmental programming of fetal and placental tissues⁽⁹⁰⁾.

HYPOTHESIS AND OBJECTIVES

GENERAL HYPOTHESIS:

- Assisted reproductive techniques are variably associated to preeclampsia, to the extent of the allogenicity and exposure to paternal agents of each of the techniques.

SECONDARY HYPOTHESIS:

- **Project 1:** In oocyte donation pregnancies, the risk for developing preeclampsia is higher than in pregnancies achieved by IVF with patients own oocytes.
- **Project 2:** In oocyte donation pregnancies, the risk for developing preeclampsia is higher after frozen embryo transfer compared with fresh embryo transfer.
- **Project 3:** Double-donation of oocytes and sperm is increasing worldwide, and those patients have several risk factors for preeclampsia.
- **Project 4:** In double-donation of oocytes and sperm pregnancies, the risk for developing preeclampsia is higher than in patients pregnant by oocyte donation alone.

GENERAL OBJECTIVE:

- To evaluate the incidence of preeclampsia in pregnancies achieved by assisted reproductive technology, especially in donation of gametes, and to define novel risk factors.

SECONDARY OBJECTIVES:

- **Project 1:** to compare the risk for developing preeclampsia between patients pregnant by oocyte donation and by IVF with own gametes.
- **Project 2:** to compare, in pregnancies achieved by oocyte donation, the risk for developing preeclampsia between fresh embryo transfers and frozen embryo transfers.
- **Project 3:** to evaluate patients undergoing double-donation of oocytes and sperm, their characteristics and the trend of this treatment during the period time of the study.
- **Project 4:** to compare the risk for developing preeclampsia between patients pregnant by double-donation of oocytes and sperm and by oocyte donation.

COPY OF THE PUBLISHED MANUSCRIPTS

PAPER 1: Is oocyte donation a risk factor for preeclampsia? A systematic review and meta-analysis

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ASSISTED REPRODUCTION TECHNOLOGIES

Is oocyte donation a risk factor for preeclampsia? A systematic review and meta-analysis

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Abstract

Purpose The objective of this meta-analysis is to determine whether there is a higher incidence of preeclampsia (PE) in pregnancies achieved by oocyte donation (OD) compared with pregnancies achieved by in vitro fertilization with autologous oocytes (IVF).

Methods A systematic review was performed to identify relevant studies published from January 1994 until April 2015 with at least an abstract in English using PubMed, ISI Web of Knowledge, and clinicaltrials.gov. The 11 studies included in this systematic review were retrospective and prospective cohort studies of women reporting results on the association between oocyte donation vs. in vitro fertilization (exposure) and preeclampsia (outcome).

Results Oocyte donation is a risk factor for the development of PE compared to IVF cycles, with a weighted OR of 3.12 under a fixed effects method (FEM: no heterogeneity between the studies). The weighted OR under a random effects model

was 2.9 (REM: heterogeneity between the studies). The meta-regression analysis showed that neither multiple pregnancies (estimate=0.08; $p=0.19$) nor patient age (estimate=-2.29; $p=0.13$) significantly explained the variability of the effect of oocyte donation on PE. Q statistic was 12.78 ($p=0.237$), suggesting absence of heterogeneity between the studies.

Conclusions Pregnancies achieved by oocyte donation confer a threefold increase in the likelihood of developing PE than those achieved by in vitro fertilization with own oocytes. Physicians should be aware of this risk in order to both counsel patients and monitor pregnancies accordingly.

Keywords Induced hypertension · In vitro fertilization · Oocyte donation · Preeclampsia · Pregnancy

Introduction

Preeclampsia (PE), defined as gestational hypertension with either significant proteinuria or end-organ dysfunction after 20 weeks of gestation in a previously normotensive woman, develops in 2–7 % of all pregnancies [1–4], and it is a major contributor to perinatal morbidity and mortality [5].

The involvement of immune mechanisms in the etiology of PE is often suggested. While in normal pregnancy there is a state of tolerance to foreign antigens, in PE, this immunological tolerance may be hampered due to an allogenic mismatch [6]. Oocyte donation is an assisted reproductive treatment with a high success rate ranging from 40 to 60 % [7–9], in which an unnaturally high number of allogenic mismatches have been described [10]. This may explain the higher incidence of PE in oocyte donation pregnancies (OD-P) reported in some series [2, 11–17]. However, whether the transfer of embryos generated from donated oocytes per se represents a risk factor for developing PE is still controversial. Recipients of donated eggs are usually nulliparous, of

Capsule Pregnancies achieved by oocyte donation confer a threefold increase in the likelihood of developing PE than those achieved by in vitro fertilization with own oocytes.

Electronic supplementary material The online version of this article (doi:10.1007/s10815-016-0701-9) contains supplementary material, which is available to authorized users.

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advanced maternal age, and typically suffer from ovarian failure, all factors independently associated with PE [18–21]. Unfortunately, several studies evaluating PE in OD-P compare its incidence with that of patients who conceived naturally [14, 15, 22, 23], thus weakening the robustness of their findings.

Objective

The aim of this systematic review and meta-analysis is to assess the association between oocyte donation pregnancies and the development of preeclampsia, comparing it with preeclampsia in women pregnant with in vitro fertilization with autologous oocytes (IVF-P).

Methods

Eligibility criteria, information sources, and search strategy

This review was carried out following the guidelines for Meta-analysis Of Observational Studies in Epidemiology (MOOSE) [24]. A systematic search was performed using PubMed, ISI Web of Knowledge, and clinicaltrials.gov to identify relevant studies published from January 1994 until April 2015 in English, French, Spanish, or Italian, using the search queries shown in Supplementary Information 1.

References of relevant publications were manually searched for additional potentially relevant published studies.

The relevance of identified abstract was assessed based on these inclusion criteria by two independent evaluators (A.B. and M.J.L.) blinded to authorship, authors' institution, and study results. If the studies were considered to be potentially relevant, the full-text article was read. Any disagreement between the two evaluators was resolved by discussion.

Study selection

The criteria for inclusion in this systematic review were cohort studies (retrospective and prospective) which reported in their results on the association between OD-P vs. IVF-P (exposure) and PE (outcome).

In all the studies selected, PE was defined as hypertension with proteinuria after 20 weeks of gestation; therefore, this was the definition of PE that we used to determine the outcome.

Data extraction

The following data were extracted from the selected studies: country where the study was carried out, study period, inclusion and exclusion criteria, sample size, reproductive

technique used (oocyte donation vs. IVF with autologous oocytes), patient age, outcome definition, and confounders used in the statistical analysis. Unadjusted and adjusted effect estimates for the association between OD-P (vs. IVF-P) and PE were extracted.

Assessment of risk of bias

Two reviewers (A.B. and M.J.L.) independently assessed the risk of bias using the Newcastle-Ottawa Scale (NOS) [25] for studies included in the systematic review. This instrument assessed three specific domains (selection, comparability, and outcome) for each study included in the review.

Data synthesis

Extracted results were pooled in the meta-analysis. Both fixed and random effects models (weighting by inverse of variance) were used. A continuity correction was used for cells with zero values. The between-study heterogeneity was assessed using the χ^2 (Cochrane Q), I^2 , and P^2 statistics. Results were presented using forest plots. An influence analysis was also performed to ascertain the results of the meta-analysis by excluding each of the individual studies. Publication bias was assessed by a funnel plot for meta-analysis and quantified by the Egger method [26]. A meta-regression procedure of the log-OR was performed to evaluate the contribution of multiple pregnancy rates (the prevalence difference between oocyte-donation and IVF groups) and age (the difference in means between oocyte-donation and IVF groups) on the effect.

Statistical analysis were conducted using Stata software v13.0 (Stata Corp., College Station, Texas) (module "meta" [27]) and R v3.1.2 (The R Foundation for Statistical Computing) (package "meta v4.2" [28]).

Results

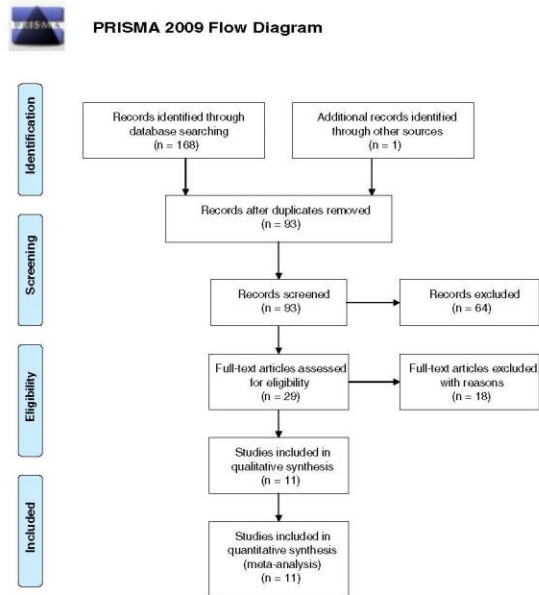
Study selection

The studies identified through database searching were 168, with an extra one included manually. The references analyzed after removing the duplicates were 93. Sixty-four abstracts were excluded because they did not fulfill the inclusion criteria or were systematic reviews. Of the 29 full-text studies selected for eligibility, 18 were excluded because the final outcome was not PE, or the study population included patient with the Turner syndrome, which could modify the result [29].

Study characteristics

Eleven studies were finally included in the meta-analysis (Fig. 1), the characteristics of which are described in Table 1.

Fig. 1 PRISMA flow chart



Risk of bias of included studies

Four studies were considered to have a low risk of bias. Four have a medium risk because the two groups of patients included were comparable only for maternal age or multiple pregnancies, and the last three had a high risk, all of which were due to a lack of comparability in relation both to maternal age and multiple pregnancies (Table 2).

Synthesis of results

Overall, the prevalence of PE in oocyte donation pregnancies (OD-P) was 17.2 % (range 9–29 %) while it was 5.7 % in the in vitro with autologous oocytes pregnancies (IVF-P) (range 0–13 %) (χ^2 246.5; $p < 0.001$). Ten of the 11 articles included in the meta-analysis reported a higher risk of PE in pregnancies achieved after oocyte donation compared with pregnancies achieved with a patient's autologous oocytes (Table 3). There was only one study where OD-P did not have an increased risk for PE, and this might be due to the small number of participants [30].

Both fixed and random effects models were used for this meta-analysis. Under the fixed method, the weighted OR of

OD-P compared to IVF-P was 3.12 (2.56–3.85) (Table 4). Worthy of note was the fact that the Q statistic was 12.78 ($p = 0.23$), suggesting absence of heterogeneity, as it also suggests the result of 21.68 (95 % CI 0, 60.7) of the I^2 , a value that does not depend on the number of the studies included in the analysis. On the other hand, the weighted OR under the random effects model was 2.9 (2.19–3.85); Fig. 2 depicts the forest plot of the ORs extracted from the random effects model.

One of the strengths of the meta-analysis is that the exclusion of any of the published studies did not relevantly change the weighted ORs, as evidenced by the influence analysis, also performed under the random effects model (Supplementary Table 1). In addition, the funnel plot, which helps in assessing visually the publication bias (Fig. 3), does not suggest the existence of unpublished studies which were not included in this meta-analysis. Likewise, the Egger k -coefficient calculated to quantify the publication bias was not significant (-0.33 , 95 % CI -1.81 to 1.15 ; $p = 0.62$).

The meta-regression procedure showed that neither multiple pregnancies (estimate = 0.08; $p = 0.19$) nor patient age (estimate = -2.29 ; $p = 0.13$) significantly contributed to the effect of OD-P on PE. Supplementary Figures 1 and 2 show the

Table 1 Characteristics of the included studies

Author	Year of publication	Country	Design	Study period	Inclusion criteria	Exclusion Criteria	Number	Age (average)	
								FIV	OD
Kilsky	2010	USA	Retrospective cohort	1996–2005	Women who delivered during study period: -IVF matched one-to-one for age, singleton/twin Women who delivered during study period: -All OD -Women >43 years who delivered during study period: -IVF -OD	- Monozygotic twins - Outcomes that not provided or reported by obstetrical provider - IVF ≤ 38 years - Women aged <43 years	158	39.8	40.2
Krug	2008	USA	Retrospective cohort	Jan 1, 2001–Dec 31, 2005	Women who delivered during study period: -All OD -Women >43 years who delivered during study period: -IVF -OD	- IVF ≤ 38 years - Women aged <43 years	179	41.3	42.7
Le Ray	2012	France	Retrospective cohort	Jan 1, 2008–Dec 31, 2010	Women who delivered during study period: -IVF -OD	- Women aged <43 years	144	44.0	46.2
Levan	2014	Israel	Retrospective cohort	2005–2011	Women who delivered during study period: -IVF -OD	- IVF - Pregnancies with congenital anomalies - Pregnancies with chromosomal abnormalities Women who delivered in Denmark but with OD performed in another country	265	41	45
Malchau	2013	Denmark	Retrospective cohort	1995–2010	Women who delivered during study period: -OD -IVF (IVF + ICSI) Women who delivered TWINS pregnancies in the study period: -OD -Age and time of delivery-matched IVF (one-to-one)	- IVF - Women >50 years - Miscarriage or molar pregnancy - Women diagnosed of hypertension before pregnancy or before 20 w of gestation	24,299	33.4	37.1
Schäfer	2014	USA	Retrospective cohort	Jan, 2005–June, 2013	Women who delivered during study period: -OD -Time of ART-matched IVF (two-to-one) Women who underwent ART during the study period and gave birth: -OD -Time of ART-matched IVF (two-to-one)	- Women >50 years - Miscarriage or molar pregnancy - Women diagnosed of hypertension before pregnancy or before 20 w of gestation	112	41.9	43.0
Söderström-Anttila	1998	Finland	Retrospective cohort	Oct, 1991–Dec 1996	Women who delivered during study period: -IVF (one-to-one) Women who underwent ART during the study period and gave birth: -OD -Time of ART-matched IVF (two-to-one)	- Women >50 years - Miscarriage or molar pregnancy - Women diagnosed of hypertension before pregnancy or before 20 w of gestation	148	33.4	33.5
Van Dong	2014	The Netherlands	Retrospective cohort	1992–2009	Women who delivered during study period: -OD -IVF pregnancies matched by date of delivery (≤3 months), maternal age and ZIP code (one-to-one) Pregnancies occurred during the study period: -OD -IVF-matched in terms of age, ethnicity, parity, and plurality (one-to-one)	- Pregnancies after: -Preimplantation genetic diagnosis - Feticular sperm extraction - Donor sperm	411	36.7	36.4
Stoop	2012	Belgium	Retrospective cohort	Jan, 1999–Dec 2008	Women who delivered during study period: -OD -Time of ART-matched IVF (two-to-one)	- Pregnancies after: -Preimplantation genetic diagnosis - Feticular sperm extraction - Donor sperm	408	36.0	36.0
Tranquilli	2013	Italy	Retrospective cohort	1999–May, 2004	Women who delivered during study period: -OD -Time of ART-matched IVF (one-to-one)	- IVF consecutive births (two-to-one) Women who underwent successful ART during study period: -OD -Time-matched IVF (one-to-one)	78	37.5	45.7
Wiggins	2005	USA	Retrospective cohort	1999–May, 2004	Women who delivered during study period: -OD -Time of ART-matched IVF (one-to-one)	- IVF consecutive births (two-to-one) Women who underwent successful ART during study period: -OD -Time-matched IVF (one-to-one)	100	37.7	41.9

Table 2 Risk of bias assessment

Study	Selection		Comparability			Outcome		Stars
	Publication year	Representativeness of the exposed cohort	Selection of the non-exposed cohort	Ascertainment of exposure	Demonstration that outcome of interest was not present at start of study	Study controls for age	Study controls for multiple pregnancies	
Klasky	2010	*	*	*	*	*	*	9
Krieg	2008	*	*	*	*	*	*	7
Le Page	2012	*	*	*	*	*	*	7
Lerou	2014	*	*	*	*	*	*	8
Melchior	2013	*	*	*	*	*	*	8
Sekhon	2014	*	*	*	*	*	*	9
Sorensen	1998	*	*	*	*	*	*	8
Adilla	2014	*	*	*	*	*	*	9
Van Dorp	2012	*	*	*	*	*	*	9
Stoop	2012	*	*	*	*	*	*	7
Trancilli	2013	*	*	*	*	*	*	7
Wiggers	2005	*	*	*	*	*	*	8

* indicates a star, cells with a star indicate that the corresponding item is addressed satisfactorily in the publication in question

bubble graph with the fitted meta-regression line of the multiple pregnancy rates (prevalence difference between oocyte donation and IVF groups) and age (difference between OD-P and IVF-P means). There was a non-significant trend towards a greater association between PE and OD-P as the difference in age between IVF-P and OD increased. On the other hand, there was also a non-significant trend towards a smaller association as the proportion of multiple pregnancies in the OD-P group increases with respect to the IVF-P group.

Discussion

Main findings

With 26,302 cases analyzed, this meta-analysis shows an association between OD-P and PE. This is of a special interest, as fertility treatments with donated oocytes are growing steadily worldwide. In the USA alone, cycles where embryos derived from donated oocytes are transferred account for 15.4 % of the cycles initiated in the country [9], mainly due to the increase in maternal age [31–33].

Strengths and limitations

To our knowledge, this is the first meta-analysis to have focused specifically on PE and including solely cohort studies comparing cycles of in vitro fertilization with OD-P vs. IVF-P. Since we avoided the comparison with natural conception pregnancies, we exclude by design the bias of the assisted reproductive technique per se.

Another relevant strength of our review is the absence of heterogeneity (Supplementary Table 1) and publication bias between the included articles (Fig. 3).

A limitation of this review is the lack of information in the included studies about the cause of infertility that has led to assisted reproductive technology (ART) with either autologous or donor oocytes, because the underlying type of infertility leading to one or the other treatment might itself contribute to the pathophysiology of PE. The factors causing the need of ART certainly vary from patients that will require donated oocytes from the ones that will perform an IVF with autologous oocytes (for instance, tubal infertility is typically a cause of IVF with autologous oocytes, while a premature ovarian failure will most likely lead to OD), thus making it difficult to determine if it is the reception of the oocytes or the cause of its necessity that is associated with the increase in PE incidence.

Another limitation is the lack of detailed information in the included studies on severity or gestational age at onset of PE: the association with OD would be more clinically relevant in early or severe PE, since it is more amenable to prevention by aspirin than mild and late-onset disease [34].

Table 3 Individual odds ratios (and 95 % CI) and relative weights (by inverse of the variance) under fixed and random effects models

Study	Year	OR	95 % CI	Fixed effects	Random effects
Söderström-Anttila	1998	6.09	2.58–14.39	5.9	8.7
Wiggins	2005	3.27	0.63–17.07	1.6	2.7
Krieg	2007	1.5	0.47–3.43	4.4	6.8
Klatsky	2010	3.9	1.22–12.58	3.2	5.2
Le Ray	2012	2.14	0.68–6.72	3.4	5.4
Stoop	2012	1.96	0.97–3.96	8.8	11.8
Malchau	2013	3.76	2.82–5.03	52.1	30.8
Tranquilli	2013	26.86	1.4–507.19	0.5	0.9
Levron	2014	2.06	0.76–5.6	4.4	6.8
Sekhon	2014	2.8	1.05–7.47	4.6	7
Van Dorp	2014	2.04	1.09–3.82	11.1	13.9

Comparison with existing literature

There are numerous studies demonstrating a relation between ART and hypertensive complications [11, 14, 16, 17, 35, 36]. Several differences between natural conception gestations and ART gestations can explain this association: the effects on the endometrium due to the controlled ovarian stimulation therapy, the different implantation due to the transfer of the embryo, and the fact that the process of the formation of the trophoblast begins in vitro instead of in vivo. To establish the risk of PE in OD-P, and thus avoid the bias of the ART, it should be an IVF-P control group. Other factors that can confound the results of the analysis are the advanced maternal age and multiple pregnancies, as both are associated with PE and with the need to undergo oocyte reception. Our review includes a meta-regression procedure that accounts for such potential confounding. Although our analysis did not reach significance

on the association between age and the effect of OD-P on PE, it seems that there is a trend towards it. The difference in the ages between groups in some of the studies and the small number of included studies could explain this limitation of our analysis.

The etiological relationship between OD-P and PE remains unclear. Despite the fact that the cause of developing PE seems to be multifactorial, OD-P is associated with an increased incidence of this disorder. An immunological theory, based on the allogenicity of the fetus to the mother, has been postulated: early on during implantation, the decidua is invaded by trophoblastic cells expressing HLA-C, a ligand for the immunoglobulin receptor (KIR) on the uterine natural killer cells (uNK). The uNK provide regulation of the neovascularization of the decidua, via proangiogenic and endothelial factors, which in turn modulate the spiral arteries. When executed correctly, this process guarantees proper blood flow to the

Table 4 Meta-analysis of the included studies

	Estimation	<i>p</i>	95 % CI	
			Lower	Upper
Fixed effects model				
IoV* weighted OR	3.05	<0.001	2.48	3.74
SE(lnOR)	0.1068			
Relative excess H	1.13		0.8	1.6
% variation <i>I</i> ² due to heterogeneity	21.68		0	60.7
Homogeneity (Q) chi-square	12.78	0.237		
Random effects model				
IoV* weighted OR	2.8	<0.001	2.11	3.72
SE(lnOR)	0.1439			
Relative excess H	0.9746		0.61	1.54
% variation <i>I</i> ² due to heterogeneity	0			
Homogeneity (Q) chi-square	9.5	0.486		

IoV* inverse of variance

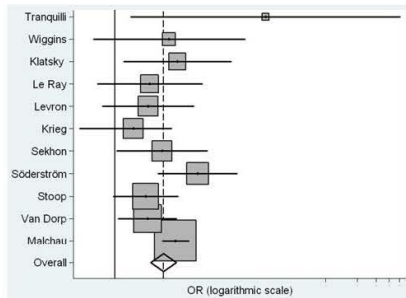


Fig. 2 Forest plot of the risk of preeclampsia in oocyte donation vs. IVF (weighted by the inverse of the variance under a fixed and random effects)

developing fetus. The fetal HLA-C differs from the maternal HLA-C because it also expresses paternal HLA-C alleles. When donated oocytes are used, the trophoblastic HLA-C is less recognizable for the immunological system of the mother because it is completely allogeneic. Speculatively, this can lead to an altered functionality of the uNK and consequently to an altered maternal blood supply to the placenta, facilitating disorders like PE and fetal growth restriction [2, 37, 38]. Also, T cells have been involved in the immune down-regulation that allows the fetus to develop in the maternal allogeneic environment [39]. In a study of 26 placentas of oocyte donation pregnancies, an infiltrate of macrophages was found in placentas of pregnancies uncomplicated by PE and not in the placentas of pregnancies with PE. This lesion in the chorionic plate was associated with intervillitis, chronic deciduitis, higher expression of CD14+ macrophages, and fetal HLA-C2. All these data suggested an immunological protection of the mother to the fetus, which was absent in PE gestations [40].

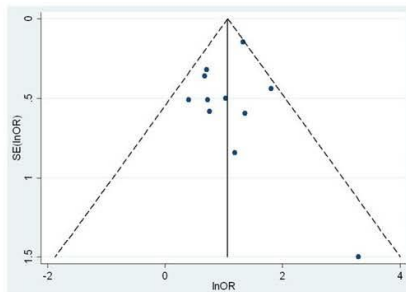


Fig. 3 Funnel plot with pseudo 95 % confidence limits

Some authors hypothesize that a patient needing oocyte donation might have, in addition, an immunologically based condition that predisposes to PE [12, 41]. Circulating antibodies against granulosa cells and zona pellucida have been detected in patients with ovarian failure [42], and as mentioned earlier, it seems that an autoregulation of the immune response by the mother is needed to provide a good placentation and to avoid PE. It is suggested that some autoantibodies or immune dysregulation can cause an injury to the trophoblastic cells invading the decidua.

Conclusions and implications

The rising number of pregnancies achieved by oocyte donation and PE morbidity confers importance to the results of this meta-analysis and necessitates an increase in our understanding of the biochemical and immunological causes of PE in order to develop possible preventive strategies, such as the selection of the oocyte donor immunologically matched to the HLA of the recipient [10, 43]. Nonetheless, the absence of prospective studies from the scientific literature limits the strength of the results of this meta-analysis, which should be interpreted with caution.

Meanwhile, patients undergoing OD cycles should be aware of the possible increased risk of developing PE, and physicians should provide strict obstetrical surveillance for these women, in order to perform early diagnosis and management if the case.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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References

1. Smith GN, Walker M, Tessier JL, Milar KG. Increased incidence of preeclampsia in women conceiving by intrauterine insemination with donor versus partner sperm for treatment of primary infertility. *Am J Obstet Gynecol.* 1997;177(2):455–8.
2. Klatsky PC, Delaney SS, Caughey AB, Tran ND, Schattman GL, Rosenwaks Z. The role of embryonic origin in preeclampsia: a comparison of autologous in vitro fertilization and ovum donor pregnancies. *Obstet Gynecol.* 2010;116(6):1387–92.
3. Kyrou D, Kolibianakis EM, Devroey P, Fatemi HM. Is the use of donor sperm associated with a higher incidence of preeclampsia in women who achieve pregnancy after intrauterine insemination?

- Fertil Steril. 2010;93(4):1124–7. doi:10.1016/j.fertnstert.2008.12.021.
4. American College of O, Gynecologists, Task Force on Hypertension in P. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. *Obstet Gynecol.* 2013;122(5):1122–31. doi:10.1097/01.AOG.0000437382.03963.88.
 5. Flenady V, Koopmans L, Middleton P, Froyen JF, Smith GC, Gibbons K, et al. Major risk factors for stillbirth in high-income countries: a systematic review and meta-analysis. *Lancet.* 2011;377(9774):1331–40. doi:10.1016/S0140-6736(10)62233-7.
 6. Redman CW, Sargent IL. Immunology of pre-eclampsia. *Am J Reprod Immunol.* 2010;63(6):534–43. doi:10.1111/j.1600-0897.2010.00831.x.
 7. Garrido N, Bellver J, Remohi J, Alama P, Pellicer A. Cumulative newborn rates increase with the total number of transferred embryos according to an analysis of 15,792 ovum donation cycles. *Fertil Steril.* 2012;98(2):341–6 e1-2. doi:10.1016/j.fertnstert.2012.04.039.
 8. Group ECW. Failures (with some successes) of assisted reproduction and gamete donation programs. *Hum Reprod Update.* 2013;19(4):354–65. doi:10.1093/humupd/dmt007.
 9. Kupka MS, Ferraretti AP, de Mouzon J, Erb K, D'Hooghe T, Castilla JA, et al. Assisted reproductive technology in Europe, 2010: results generated from European registers by ESHRE. *Hum Reprod.* 2014;29(10):2099–113. doi:10.1093/humrep/det175.
 10. van der Hoorn ML, Lashley EE, Bianchi DW, Claas FH, Schonkeren CM, Scherjon SA. Clinical and immunologic aspects of egg donation pregnancies: a systematic review. *Hum Reprod Update.* 2010;16(6):704–12.
 11. Gundogan F, Bianchi DW, Scherjon SA, Roberts DJ. Placental pathology in egg donor pregnancies. *Fertil Steril.* 2010;93(2):397–404.
 12. Keegan DA, Krey LC, Chang HC, Noyes N. Increased risk of pregnancy-induced hypertension in young recipients of donated oocytes. *Fertil Steril.* 2007;87(4):776–81.
 13. Le Ray C, Scherier S, Anselme O, Marszalek A, Tsatsaris V, Cabrol D, et al. Association between oocyte donation and maternal and perinatal outcomes in women aged 43 years or older. *Hum Reprod.* 2012;27(3):896–901.
 14. Salha O, Sharma V, Dada T, Nugent D, Rutherford AJ, Tomlinson AJ, et al. The influence of donated gametes on the incidence of hypertensive disorders of pregnancy. *Hum Reprod.* 1999;14(9):2268–73.
 15. Wolff KM, McMahon MJ, Kuller JA, Walmer DK, Meyer WR. Advanced maternal age and perinatal outcome: oocyte reciprocity versus natural conception. *Obstet Gynecol.* 1997;89(4):519–23.
 16. Soderstrom-Anttila V, Tiitinen A, Foudila T, Hovatta O. Obstetric and perinatal outcome after oocyte donation: comparison with in-vitro fertilization pregnancies. *Hum Reprod.* 1998;13(2):483–90.
 17. Levron Y, Dviri M, Segol I, Yerushalmi GM, Hourvitz A, Orvieto R, et al. The 'immunologic theory' of preeclampsia revisited: a lesson from donor oocyte gestations. *Am J Obstet Gynecol.* 2014;211(4):19.
 18. Myatt L, Redman CW, Staff AC, Hansson S, Wilson ML, Laivuori H, et al. Strategy for standardization of preeclampsia research study design. *Hypertension.* 2014;63(6):1293–301. doi:10.1161/HYPERTENSIONAHA.113.02664.
 19. Woldringh GH, Frunt MHA, Kremer JAM, Spaanderman MEA. Decreased ovarian reserve relates to pre-eclampsia in IVF/ICSI pregnancies. *Hum Reprod.* 2006;21(11):2948–54. doi:10.1093/humrep/del155.
 20. Paulson RJ, Boostanfar R, Saadat P, Mor E, Tourgeman DE, Slater CC, et al. Pregnancy in the sixth decade of life: obstetric outcomes in women of advanced reproductive age. *JAMA.* 2002;288(18):2320–3.
 21. Yogev Y, Melamed N, Bardin R, Tenenbaum-Gavish K, Ben-Shitrit G, Ben-Haroush A. Pregnancy outcome at extremely advanced maternal age. *Am J Obstet Gynecol.* 2010;203(6). doi:10.1016/j.ajog.2010.07.039.
 22. Porreco RP, Harden L, Gambotto M, Shapiro H. Expectation of pregnancy outcome among mature women. *Am J Obstet Gynecol.* 2005;192(1):38–41.
 23. Shrim A, Levin I, Mallozzi A, Brown R, Salama K, Gamzu R, et al. Does very advanced maternal age, with or without egg donation, really increase obstetric risk in a large tertiary center? *J Perinat Med.* 2010;38(6):645–50.
 24. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA.* 2000;283(15):2008–12.
 25. Wells G, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. [http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp]. Accessed 19/2/2015.
 26. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ.* 1997;315(7109):629–34.
 27. Sterne J. *Meta-analysis in Stata: an updated collection from the Stata Journal.* College Station (Texas): Stata Press; 2009.
 28. Schwarzer G. *Meta-analysis.* The R Foundation for Statistical Computing. 2007.
 29. Hagman A, Loft A, Wennerholm U-B, Pinborg A, Bergh C, Aittomaki K, et al. Obstetric and neonatal outcome after oocyte donation in 106 women with Turner syndrome: a Nordic cohort study. *Hum Reprod.* 2013;28(6):1598–609. doi:10.1093/humrep/det082.
 30. Krieg SA, Henne MB, Westphal LM. Obstetric outcomes in donor oocyte pregnancies compared with advanced maternal age in in vitro fertilization pregnancies. *Fertil Steril.* 2008;90(1):65–70.
 31. de Graaff AA, Land JA, Kessels AG, Evers JL. Demographic age shift toward later conception results in an increased age in the subfertile population and an increased demand for medical care. *Fertil Steril.* 2011;95(1):61–3. doi:10.1016/j.fertnstert.2010.05.013.
 32. Blickstein I. Motherhood at or beyond the edge of reproductive age. *Int J Fertil Women's Med.* 2003;48(1):17–24.
 33. Pal L, Santoro N. Age-related decline in fertility. *Endocrinol Metab Clin N Am.* 2003;32(3):669–88.
 34. Roberge S, Villa P, Nicolaidis K, Giguere Y, Vainio M, Bakthi A, et al. Early administration of low-dose aspirin for the prevention of preterm and term preeclampsia: a systematic review and meta-analysis. *Fetal Diagn Ther.* 2012;31(3):141–6. doi:10.1159/000336662.
 35. Thomopoulos C, Tsioufis C, Michalopoulou H, Makris T, Papademetriou V, Stefanadis C. Assisted reproductive technology and pregnancy-related hypertensive complications: a systematic review. *J Hum Hypertens.* 2013;27(3):148–57. doi:10.1038/jhh.2012.13.
 36. Hernandez-Diaz S, Werler MM, Mitchell AA. Gestational hypertension in pregnancies supported by infertility treatments: role of infertility, treatments, and multiple gestations. *Fertil Steril.* 2007;88(2):438–45. doi:10.1016/j.fertnstert.2006.11.131.
 37. Madeja Z, Yadi H, Apps R, Boulenouar S, Roper SJ, Gardner L, et al. Paternal MHC expression on mouse trophoblast affects uterine vascularization and fetal growth. *Proc Natl Acad Sci U S A.* 2011;108(10):4012–7. doi:10.1073/pnas.1005342108.
 38. Hiby SE, Walker JJ, O'Shaughnessy KM, Redman CW, Carrington M, Trowsdale J, et al. Combinations of maternal KIR and fetal HLA-C genes influence the risk of preeclampsia and reproductive success. *J Exp Med.* 2004;200(8):957–65. doi:10.1084/jem.20041214.
 39. Tilburgs T, Roelen DL, van der Mast BJ, de Groot-Swings GM, Kleijburg C, Scherjon SA, et al. Evidence for a selective migration

- of fetus-specific CD4⁺ CD25^{bright} regulatory T cells from the peripheral blood to the decidua in human pregnancy. *J Immunol.* 2008;180(8):5737–45.
40. Schonkeren D, Swings G, Roberts D, Claas F, de Heer E, Scherjon S. Pregnancy close to the edge: an immunosuppressive infiltrate in the chorionic plate of placentas from uncomplicated egg cell donation. *PLoS One.* 2012;7(3):27.
41. Pecks U, Maass N, Neulen J. Oocyte donation: a risk factor for pregnancy-induced hypertension: a meta-analysis and case series. *Dtsch Arztebl Int.* 2011;108(3):23–31.
42. Kelkar RL, Meherji PK, Kadam SS, Gupta SK, Nandedkar TD. Circulating auto-antibodies against the zona pellucida and thyroid microsomal antigen in women with premature ovarian failure. *J Reprod Immunol.* 2005;66(1):53–67. doi:10.1016/j.jri.2005.02.003.
43. Alecsandru D, Garrido N, Vicario JL, Barrio A, Aparicio P, Requena A, et al. Maternal KIR haplotype influences live birth rate after double embryo transfer in IVF cycles in patients with recurrent miscarriages and implantation failure. *Hum Reprod.* 2014;29(12):2637–43.

PAPER 2: Risk of pre-eclampsia after fresh or frozen embryo transfer in patients undergoing oocyte donation

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Risk of pre-eclampsia after fresh or frozen embryo transfer in patients undergoing oocyte donation



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ABSTRACT

Objectives: Different perinatal and neonatal adverse outcomes have been reported to be increased in frozen embryo transfer pregnancies compared with fresh embryo transfer with patient's own oocytes. Concerning preeclampsia, it has also been reported to be increased after frozen embryo transfer.

The objective of this study is to assess if there is an increased risk of preeclampsia and gestational hypertension in pregnancies achieved with oocyte donation after frozen embryo transfer compared to fresh embryo transfer.

Study design: Retrospective cohort study of 433 patients who underwent a cycle with donated oocytes either after fresh (n = 353) or frozen embryo transfer (n = 80) between March 2013 and April 2016 at a large fertility clinic. Participants are pregnant patients who reached the 20th week of gestation. The risk of preterm preeclampsia (presenting before 37 weeks of gestation), term preeclampsia (presenting at or after 37 weeks of gestation) and gestational hypertension are presented as unadjusted and adjusted odds ratio (OR).

Results: Frozen embryo transfer have similar risk for developing preterm preeclampsia compared to fresh embryo transfer, with an OR of 1.95 (CI 95% 0.72, 5.26, p = 0.18), as well as term preeclampsia (OR 0.3, 95% CI 0.04, 2.35, p = 0.25), and gestational hypertension (OR 1.45, 95% CI 0.75, 2.81, P = 0.27).

Conclusions: Despite a high prevalence of preeclampsia in pregnancies achieved by oocyte donation, the freezing-thawing process does not confer more risk than the fresh embryo transfers in preterm preeclampsia, term preeclampsia or gestational hypertension.

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Introduction

The use of frozen-thawed embryo transfer (frozen ET) is increasing worldwide, in part due to the growing trend towards single embryo transfer, and the implantation of freeze all policies when there is a risk of lower endometrial receptivity or OHSS. The ICMART registry reported a 27.6% increase of non-donor frozen ET between 2008–2010, with a pregnancy rate of 29.1% and a delivery rate of 20.7% in 2010 [1].

Several authors suggest that the supraphysiological hormonal levels reached during controlled ovarian stimulation have deleterious effects on the endometrium, causing placental dysfunction and alterations, thus likely affecting the pregnancy course [2–4]. When compared to fresh embryo transfers (fresh ET), frozen ET have been associated with lower rate of low birth

weight singletons [1–9], less preterm birth; [6,8], and ectopic pregnancy [10,11]. Moreover, while we find higher rates of large for gestational age singletons in frozen ET compared to fresh ET [5–8,12], data are still inconsistent about the perinatal mortality of frozen ET babies: while in some papers the risk is higher compared to fresh ET [5, 6], others do not find differences [8], or even report less risk [13].

The effect of frozen ET on the occurrence of placental alteration has been less studied so far: the rate of placenta previa seems either lower [5], or not different [7], while the occurrence of placenta accreta seems higher after frozen ET [7].

Of note, it seems that an increase in preeclampsia (PE) is the only reported maternal effect increased in frozen ET vs fresh ET [5,7,14–16], even though some authors found that the elective cryopreservation of embryos in IVF cycles at risk of ovarian hyperstimulation syndrome reduces the odds of PE [9].

A common bias in all the studies so far is the comparison of treatments when the patient's own oocytes are used in all transfers. While this is very relevant for the counselling of patients, it does not allow to understand more in detail the source of the

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hypothetical effects of frozen ET, since in fresh ET the endometrium has been affected by the controlled ovarian stimulation, and in frozen ET by hormonal preparation of the endometrium for embryo reception. In this sense, oocyte donation offers a model to isolate the effect of the frozen ET, since all endometrium are prepared, and no ovarian stimulation is performed.

On the other hand, PE incidence has been reported to be higher in OD pregnancies compared to pregnancy achieved with a woman own oocytes (Blazquez, JARG, 2016). The embryo in OD pregnancies is allogeneic to the mother, as both alleles of the HLA expressed by the trophoblastic cells non self. The HLA is the ligand for the KIR, the receptor in the uterine NK (uNK) for the HLA. The function of uNK cells is to modulate proangiogenic and endothelial factors that leads to the proper perfusion of the placenta and the fetus. The immunological theory is based on the dysfunctional response of these uNK that takes place when the HLA is less recognizable for the mother, as it is after an oocyte donation, leading to a hypoperfusion of the placenta and finally to PE.

The aim of our study is therefore to assess whether there is an increased risk of PE in patients recipients of embryos from oocyte donation (OD) undergoing frozen ET compared to recipients undergoing fresh ET, in order to analyze the effect of the freezing-thawing of the embryo and without the bias of the ovarian stimulation.

Material and methods

Study design

This is a retrospective cohort study of 433 consecutive patients that have achieved a pregnancy by IVF/ICSI with donated oocytes and partner's sperm, either after a fresh or a frozen ET, between March 2013 and April 2016 in a large fertility center. Data were obtained through a questionnaire emailed to the patients and filled-in with the help of their physician. This questionnaire was sent automatically as a part of the clinic follow-up protocol to all patients achieving the 20th week of gestation. If patients did not reply, the questionnaire was sent up to three times.

Ethical approval

Permission to conduct the study was obtained from the local Ethical Committee for Clinical Research of the center.

Recipient population

The two study groups were: recipients of oocyte donations with pregnancies achieved through either fresh ET (fresh embryos) or frozen ET (vitrified-warmed embryos). Pregnancies must be ongoing at 20th week to be included in the study.

The primary outcome of this study was the number of PE, defined as gestational hypertension (systolic blood pressure ≥ 140 or diastolic blood pressure ≥ 90 mmHg) associated with proteinuria (≥ 300 mg/24 h) at or after 20 weeks of gestation. PE is classified in two subclasses, preterm and term PE [17], categories of clinical relevance as the consequences of the syndrome are more severe in preterm PE [18,19]. Preterm PE was defined as requiring delivery before 37 weeks of pregnancy, while term PE delivery occurred at or after 37 weeks. The secondary outcome was the number of gestational hypertension (GH) reported by the patients in the questionnaires. GH is defined by hypertension at or after 20 weeks of gestation.

Endometrial preparation for embryo reception

Endometrial preparation of the patients started with oral contraceptives for 21 days if they had irregular menstrual cycles,

with an injection of GnRH agonist (GnRHa; Decapeptyl, Ipsen Pharma, Spain) depot on the 17th day of the oral contraceptive. Recipients with regular menstrual cycles were administered a GnRHa depot in the mesoluteal phase of the cycle. At the first day of menstruation of the cycle immediately following, the estrogen preparation was initiated, with either patches (150 mg/3 days; Estradot, Novartis Pharma, Spain or Vivelledot, Novartis Pharma, Spain) or pills (6 mg/day; Progynova, Bayer Health Care, Spain or Provames, Sanofi-Aventis, France). Vaginal progesterone (400 mg/12 h; Crinone, Merck S. L., Spain) was initiated on the same night as the donor ovum retrieval. In cases in which endometrial thickness did not achieve 6 mm, patients added a higher dose of vaginal estrogens until the endometrium reached at least 6 mm.

Statistical analysis

The risk of preterm PE, term PE and GH in frozen ET compared to fresh ET is presented as odds ratio (OR), with the associated confidence interval (95% CI) and p-value tested by Mantel-Haenszel Chi². In addition, a multivariable analysis has been performed for each study outcome, adjusting for the potential confounding factors: age, primigravity and multiple pregnancy.

All statistical analyses were performed using SPSS version 22.0. A p-value ≤ 0.05 was set as statistically significant.

Results

Demographic characteristics

A total of 1538 patients were sent the questionnaire, and 433 returned it completed, resulting in a response rate of 28.15%. Demographic characteristics and risk factors for PE between responders and non-responders show no significant differences (Supplementary Table 1). Of the 433 patients included, 353 become pregnant after fresh ET and 80 after frozen ET. The mean maternal age was 41.8 and primigravity represented 55.2% of cases. None of the demographic characteristics analyzed differed significantly between groups (Table 1).

Cycle characteristics and pregnancy outcomes

The majority of donor oocytes were inseminated with frozen sperm (85.7%), and most of the embryo transfers were performed on day 2–3 of development (90.8%). The rate of multiple gestations was 22.6%, and 64.6% of deliveries were by C-section.

No significant differences were observed between study groups (Table 2), except for the number of embryos transferred; while in 322 (91.2%) of the fresh ET group two embryos were transferred, in the frozen ET group, 54 cases were double embryo transfer (67.5%), 12 (15%) were single embryo transfers and 14 (17%) triple embryo transfer.

Table 1
Demographic characteristics overall and by study group.

	Overall (n = 433)	Frozen (n = 80)	Fresh (n = 353)	p- value
Age, Mean (SD)	41.86 (4.56)	42.5 (4.2)	41.7 (4.6)	0.15 ^a
BMI, Mean (SD)	23.2 (3.9)	23.2 (3.9)	23.2 (3.9)	1.00 ^a
Primigravity, n (%)	239 (55.2)	45 (56.3)	194 (55.0)	0.83 ^b
Offspring				
- no, n (%)	359 (82.9)	67 (83.8)	292 (82.7)	0.71 ^b
- yes, n (%)	46 (10.6)	9 (11.3)	37 (10.5)	

^a Student's t-test.

^b Pearson's Chi².

Table 2
Cycle characteristics and pregnancy outcome overall and by study group.

	Overall (n = 433)	Frozen (n = 80)	Fresh (n = 353)	p-value ^a
Type of sperm, n (%)				
Fresh	62 (14.3)	13 (16.3)	49 (13.9)	0.58
Frozen	371 (85.7)	67 (83.8)	304 (86.1)	
Day of embryo transfer, n (%)				
2	161 (37.2)	30 (37.5)	131 (37.1)	0.12
3	232 (53.6)	39 (48.8)	193 (54.7)	
5	38 (8.8)	9 (11.3)	29 (8.2)	
Number of transferred embryos, n (%)				
1	39 (9.0)	12 (15.0)	27 (7.6)	<0.001
2	376 (86.8)	54 (67.5)	322 (91.2)	
3	18 (4.2)	14 (17.5)	4 (1.1)	
Multiple pregnancy, n (%)	98 (22.6)	16 (20.0)	82 (23.2)	0.53
Miscarriage, n (%)	3 (0.7)	0 (0.0)	3 (0.8)	0.41
Type of labour, n (%)				
- Vaginal	153 (35.4)	25 (31.3)	128 (36.4)	0.39
- C-section	279 (64.6)	55 (68.8)	224 (63.6)	

^a Pearson's Chi² test.

Table 3
Preeclampsia (PE) and Gestational hypertension (GH) overall and by study group.

	Overall (n = 433)	Frozen (n = 80)	Fresh (n = 353)	p-value
GH, n (%)	62 (14.3)	14 (17.5)	48 (13.6)	0.37 ^a
PE, n (%)	36 (8.3)	7 (8.8)	29 (8.2)	0.88 ^b
- Preterm PE, n (% within PE)	21 (4.8)	6 (7.5)	15 (4.2)	0.22 ^b
- Term PE, n (% within PE)	15 (3.5)	1 (1.3)	14 (4.0)	0.23 ^b

^a Pearson's Chi² test.

^b Fisher's exact test.

Hypertensive disorders

There were 15 cases of preterm PE in the fresh ET group (4.2%) and 6 (7.5%) in the frozen ET group. Fourteen cases of term PE (4.0%) occurred in the fresh ET and 1 (1.3%) in the frozen ET. We found no statistical differences in either preterm or term PE between groups. The rate of GH between groups were also no different, with 48 cases (13.6%) in fresh ET and 14 (17.5%) in frozen ET (Table 3). The multivariate analysis adjusted for confounding factors (age, BMI, primigravity and multiple pregnancy) still showed no difference between fresh and frozen ET for preterm PE (OR 1.95, CI 95% 0.72, 5.26, p = 0.18), term PE (OR 0.3, 95%CI 0.04, 2.35, p = 0.25) or GH (OR 1.45, 95% CI 0.75, 2.81, P = 0.27) (Table 4) (Fig. 1).

Comments

To the best of our knowledge, this is the first study comparing the prevalence of PE in fresh versus frozen ET after oocyte donation. We found no difference in preterm PE, term PE, or gestational hypertension between fresh and frozen ET in recipients of oocyte donation.

In selecting this population, we avoided the possible confounding effect of the hyperestrogenism of the ovarian stimulation in the genesis of PE and GH. This is of special relevance, as several studies have found differences in PE prevalence between fresh and frozen ET in cycles with patient's own oocytes, and this might be due to the freezing and thawing process and/or the different endometrial hormonal environment [2,5,9].

Table 4
Multivariable analysis of frozen embryo transfer in oocyte donation on the likelihood of preeclampsia, adjusted for potential confounders.

		95% CI			p
		OR	Lower	Upper	
Preterm PE	Frozen vs. Fresh	1.95	0.72	5.27	0.19
	Age	0.97	0.88	1.07	0.56
	BMI	1.08	0.98	1.19	0.14
	Primigravity	1.10	0.45	2.71	0.83
	Multiple pregnancy	1.88	0.73	4.86	0.19
Term PE	Frozen vs. Fresh	0.30	0.04	2.35	0.25
	Age	1.02	0.91	1.14	0.74
	BMI	1.00	0.88	1.14	0.98
	Primigravity	2.33	0.73	7.49	0.16
	Multiple pregnancy	0.86	0.23	3.14	0.82
GH	Frozen vs. Fresh	1.45	0.75	2.81	0.27
	Age	1.00	0.94	1.06	0.91
	BMI	1.05	0.99	1.13	0.13
	Primigravity	1.14	0.65	1.99	0.64
	Multiple pregnancy	1.74	0.95	3.19	0.07

PE: Preeclampsia.

GH: Gestational Hypertension.

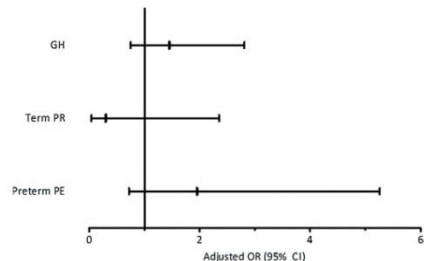


Fig. 1. Risk of preeclampsia in frozen ET vs. fresh ET in pregnancies originated from oocyte donation.

Some authors suggest that the process of freezing and thawing per se could bring about some metabolic or epigenetically changes in the embryos, and thus be an adjuvant in the origin of PE in the frozen ET group [20,21]. Some cryoprotectants have been reported to interact with the methylation state of embryonic genes, and these variations in the epigenetic burden of the embryo might affect developmental programming of fetal and placental tissues [22]. Our results seem to indicate that the clinical relevance of those changes, at least up to birth, is relatively minor, given that, when the endometrial preparation and hormonal state is the same in the recipients, the prevalence of preterm PE, term PE or GH does not change between pregnancies achieved with fresh or frozen ETs. Our findings are in agreement with the current PE etiological theory which assigns more weight to the defective vascularization of the placenta, and not necessarily to the embryo characteristics and manipulations [23,24]. Female sex hormones play an important role in placental vascularization, as they promote placental angiogenesis and decrease the resistance of spiral uterine arteries [25,26]. Several studies underscore the relationship between variations in levels of progesterone and estrogens and PE, despite inconsistent results: while some authors report that low levels of estrogens are correlated with PE development [1–28], others found no differences or even increased estrogen levels in PE [29,30], as well as with progesterone concentrations [31,32]. It seems then that certain ranges of estrogens and progesterone are

needed for normal placentation process. In the studies published so far, frozen ET pregnancies carry an increased risk of PE than the fresh ET ones [5,7,14–16], possibly indicating that the protocols currently used for endometrial reception of embryos have a deleterious effect on placentation, maybe due to the long exposure of hormone replacement.

We do recognize some limitations to this study: data have been obtained through a questionnaire that was completed by patients and their physicians. Giving the importance of the syndrome and the specificity of the information asked, a mistake in the data provided by the patients is unlikely, especially in the preterm group. Despite the fact that we had a low response rate in our questionnaire of pregnancy outcome, this is unlikely to result in a selection bias since the baseline characteristics did not differ between responders and non-responders. In any case, any potential selection bias would not likely operate differently between women with fresh and frozen ET. The number of cases of PE in the frozen ET group is relatively low, but the total number of patients included and the fact that it has never been before reported in the literature, gives strength to this report. The embryo score in the frozen ET group was likely lower than the fresh ET group, as embryos are vitrified after a fresh ET, and transferred if the fresh ET has not been successful. Despite these frozen embryos might present poorer morphology than the ones selected for the fresh ET, the incidence of PE should not change. Oron showed that poor embryo quality is not associated with adverse obstetric outcomes such as PE [33], as did other studies that after comparing pregnancies from cleavage or blastocyst transfer, did not find differences in PE incidence [13,34,35]. The fact that are second embryo transfers of a whole embryo cohort could represent a bias if the pregnancy rate was the outcome, since the frozen ET group are by definition patients with worse prognosis when pregnancy rate is the outcome, but once the pregnancy is ongoing, it should not change the PE outcome.

In conclusion, despite the high prevalence of PE in pregnancies from oocyte donation, the freezing-thawing process in the embryo does not seem to be adding more risk compared with fresh ET. Even though the sequential freezing and thawing of embryos is in general not recommended, as it decreases embryo viability, the freezing of all embryos on a donation cycle does not seem to affect the development of hypertensive disorders during the pregnancy. The endometrial hormonal environment can have a role in the pathogenesis of PE, and further studies are required to determine if levels of estrogens and progesterone could be used as biomarkers for the early diagnosis of PE, or if different hormonal replacement therapies could decrease the incidence of this disorder.

Authorship

A.B.: involved in study design, data analysis, and manuscript preparation. D.G.: involved in data analysis and manuscript preparation. R.V.: involved in study implementation and supervision, expert knowledge, manuscript preparation. FF: involved in data analysis, expert knowledge and manuscript revision. A.R.: expert knowledge, manuscript revision.

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Conflict of interest

A.B. has nothing to declare. D.G. has nothing to declare. R.V. has nothing to declare. FF. has nothing to declare. A.R. has nothing to declare.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.ejogrb.2018.05.030>.

References

- [1] Dyer S, Chambers GM, de Mouzon J, Nygren KG, Zegers-Hochschild F, Mansour R, et al. International Committee for monitoring assisted reproductive technologies world report: assisted reproductive technology 2008, 2009 and 2010. *Hum Reprod* 2013;31(7):1588–609. Epub 2015/05/22.
- [2] Wennerholm UB, Soderstrom-Anttila V, Bergh C, Aitomaki K, Hazekamp J, Nygren KG, et al. Children born after cryopreservation of embryos or oocytes: a systematic review of outcome data. *Hum Reprod* 2009;24(9):2158–72. Epub 2009/05/22.
- [3] Soderstrom-Anttila V, Tiitinen A, Foudila T, Hovatta O. Obstetric and perinatal outcome after oocyte donation: comparison with in-vitro fertilization pregnancies. *Hum Reprod* 1998;13(2):483–90. Epub 1998/04/29.
- [4] Roque M, Lattes K, Serra S, Sola I, Geber S, Carreras R, et al. Fresh embryo transfer versus frozen embryo transfer in vitro fertilization cycles: a systematic review and meta-analysis. *Fertil Steril* 2013;99(1):156–62. Epub 2012/10/09.
- [5] Sazonova A, Kallen K, Thurin-Kjellberg A, Wennerholm UB, Bergh C. Obstetric outcome in singletons after in vitro fertilization with cryopreserved/thawed embryos. *Hum Reprod* 2012;27(5):1343–50. Epub 2012/03/01.
- [6] Wennerholm UB, Henningsen AK, Romundstad LB, Bergh C, Pinborg A, Sjaevanen R, et al. Perinatal outcomes of children born after frozen-thawed embryo transfer: a Nordic cohort study from the CoNARTAS group. *Hum Reprod* 2013;28(9):2545–53. Epub 2013/07/09.
- [7] Ishihara O, Araki R, Kuwahara A, Itakura A, Saito H, Adamson GD. Impact of frozen-thawed single-blastocyst transfer on maternal and neonatal outcome: an analysis of 277,042 single-embryo transfer cycles from 2008 to 2010 in Japan. *Fertil Steril* 2014;101(1):128–33. Epub 2013/11/25.
- [8] Pelkonen S, Koivunen R, Gissler M, Nuojua-Huttunen S, Suikkari AM, Hyden-Granskog C, et al. Perinatal outcome of children born after frozen and fresh embryo transfer: the Finnish cohort study 1995–2006. *Hum Reprod* 2010;25(4):914–23. Epub 2010/02/04.
- [9] Imudia AN, Awonuga AO, Kaimal AJ, Wright DL, Styer AK, Toth TL. Elective cryopreservation of all embryos with subsequent cryothaw embryo transfer in patients at risk for ovarian hyperstimulation syndrome reduces the risk of adverse obstetric outcomes: a preliminary study. *Fertil Steril* 2013;99(1):168–73. Epub 2012/10/03.
- [10] Ishihara O, Kuwahara A, Saitoh H. Frozen-thawed blastocyst transfer reduces ectopic pregnancy risk: an analysis of single embryo transfer cycles in Japan. *Fertil Steril* 2011;95(6):1966–9. Epub 2011/03/08.
- [11] Roque M. Freeze-all policy: is it time for that? *J Assist Reprod Gen* 2015;32(2):171–6. Epub 2014/11/28.
- [12] Pinborg A, Henningsen AA, Loft A, Malchau SS, Forman J, Andersen AN. Large baby syndrome in singletons born after frozen embryo transfer (FET): is it due to maternal factors or the cryotechnique? *Hum Reprod* 2014;29(3):618–27. Epub 2014/01/15.
- [13] Maheshwari A, Pandey S, Shetty A, Hamilton M, Bhattacharya S. Obstetric and perinatal outcomes in singleton pregnancies resulting from the transfer of frozen thawed versus fresh embryos generated through in vitro fertilization treatment: a systematic review and meta-analysis. *Fertil Steril* 2012;98(2):368–77. Epub 2012/06/16.
- [14] Klatsky PC, Delaney SS, Caughy AB, Tran ND, Schattman GL, Rosenwaks Z. The role of embryonic origin in preeclampsia: a comparison of autologous in vitro fertilization and ovum donor pregnancies. *Obstet Gynecol* 2010;116(5):1387–92. Epub 2010/11/26.
- [15] Barsky M, St Marie P, Rahil T, Markenson GR, Sites CK. Are perinatal outcomes affected by blastocyst vitrification and warming? *Am J Obstet Gynecol* 2016;215(3):603 e1–e5. Epub 2016/10/30.
- [16] Chen S, Gong E, Kazi DS, Gates AB, Karaye KM, Girerd N, et al. Development of a mobile phone-based intervention to improve adherence to secondary prevention of coronary heart disease in China. *J Med Eng Technol* 2016;40(7–8):372–82. Epub 2016/10/01.
- [17] Myatt L, Redman CW, StaffAC, Hansson S, Wilson ML, Laiuori H, et al. Strategy for standardization of preeclampsia research study design. *Hypertension* 2014;63(6):1293–301. Epub 2014/04/02.
- [18] Xiong X, Demianczuk NN, Saunders LD, Wang FL, Fraser WD. Impact of preeclampsia and gestational hypertension on birth weight by gestational age. *Am J Epidemiol* 2002;155(3):203–9. Epub 2002/02/01.
- [19] Mongraw-Chaffin ML, Cirillo PM, Cohn BA. Preeclampsia and cardiovascular disease death: prospective evidence from the child health and development studies cohort. *Hypertension* 2010;56(1):166–71. Epub 2010/06/03.

- [20] Shih W, Rushford DD, Bourne H, Garrett C, McBain JC, Healy DL, et al. Factors affecting low birthweight after assisted reproduction technology: difference between transfer of fresh and cryopreserved embryos suggests an adverse effect of oocyte collection. *Hum Reprod* 2008;23(7):1644–53 Epub 2008/04/30.
- [21] Young LE, Sinclair KD, Wilmut I. Large offspring syndrome in cattle and sheep. *Rev Reprod* 1998;3(3):155–63 Epub 1998/11/26.
- [22] De Geyter C, De Geyter M, Steimann S, Zhang H, Holzgreve W. Comparative birth weights of singletons born after assisted reproduction and natural conception in previously infertile women. *Hum Reprod* 2006;21(3):705–12 Epub 2005/11/15.
- [23] Staff AC, Benton SJ, von Dadelszen P, Roberts JM, Taylor RN, Powers RW, et al. Redefining preeclampsia using placenta-derived biomarkers. *Hypertension* 2013;61(5):932–42 Epub 2013/03/06.
- [24] Thilagathanan B. Placental syndromes: getting to the heart of the matter. *Ultrasound Obstet Gynecol* 2017;49(1):7–9 Epub 2017/01/10.
- [25] Maliqueo M, Echiburú B, Crisosto N. Sex steroids modulate uterine-placental vasculature: implications for obstetrics and neonatal outcomes. *Front Physiol* 2016;7:152 Epub 2016/05/21.
- [26] Berkane N, Liere P, Oudinet JP, Hertig A, Lefevre G, Pluchino N, et al. From pregnancy to preeclampsia: a key role for estrogens. *Endocr Rev* 2017;38(2):123–44 Epub 2017/03/23.
- [27] Hertig A, Fort J, Lefevre G, Chabbert-Buffet N, Uzan M, Rondeau E, et al. Soluble endoglin in preclampsic patients with or without HELLP syndrome. *Am J Obstet Gynecol* 2010;202(6):594 e1–e4. Epub 2010/05/01.
- [28] Jobe SO, Ramadoss J, Wargin AJ, Magness RR. Estradiol-17beta and its cytochrome P450- and catechol-O-methyltransferase-derived metabolites selectively stimulate production of prostacyclin in uterine artery endothelial cells: role of estrogen receptor-alpha versus estrogen receptor-beta. *Hyperfine Interact* 2013;61(2):509–18 Epub 2013/01/16.
- [29] Bussen S, Bussen D. Influence of the vascular endothelial growth factor on the development of severe pre-eclampsia or HELLP syndrome. *Arch Gynecol Obstet* 2011;284(3):551–7 Epub 2010/10/14.
- [30] Su EJ, Ernst L, Abdallah N, Chatterton R, Xin H, Monsivais D, et al. Estrogen receptor-beta and fetoplacental endothelial prostanoid biosynthesis: a link to clinically demonstrated fetal growth restriction. *J Clin Endocrinol Metab* 2011;96(10):E1558–67 Epub 2011/08/13.
- [31] Walsh SW, Coulter S. Increased placental progesterone may cause decreased placental prostacyclin production in preeclampsia. *Am J Obstet Gynecol* 1989;161(6 Pt 1):1586–92 Epub 1989/12/01.
- [32] Uddin MN, Horvat D, Jones RO, Beeram MR, Zawieja DC, Perger L, et al. Suppression of aldosterone and progesterone in preeclampsia. *J Maternal-Fetal Neonatal Med* 2014;1–6 Epub 2014/08/29.
- [33] Oron G, Son WY, Buckett W, Tulandi T, Holzer H. The association between embryo quality and perinatal outcome of singletons born after single embryo transfers: a pilot study. *Hum Reprod* 2014;29(7):1444–51 Epub 2014/05/09.
- [34] Oron G, Nayot D, Son WY, Holzer H, Buckett W, Tulandi T. Obstetric and perinatal outcome from single cleavage transfer and single blastocyst transfer: a matched case-control study. *Gynecol Endocrinol* 2015;31(6):469–72 Epub 2015/04/22.
- [35] Oron G, Sokal-Arnon T, Son WY, Demirtas E, Buckett W, Zeadna A, et al. Extended embryo culture is not associated with increased adverse obstetric or perinatal outcome. *Am J Obstet Gynecol* 2014;211(2):165 e1–e7. Epub 2014/03/19.

PAPER 3: Characteristics and clinical outcomes of patients undergoing fertility treatment by double gamete donation

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ORIGINAL ARTICLE

Characteristics and clinical outcomes of patients undergoing fertility treatment by double gamete donation

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ABSTRACT

The number of women receiving *in vitro* fertilization cycles with both oocyte and sperm donation (double donation; DD) has grown globally in the last decade. The aim of this retrospective study, which included 1139 DD cycles, was to describe the characteristics of patients receiving DD and the outcomes of this assisted reproductive treatment. A cluster analysis identified couple 'status' as the main variable in dividing patients into categories. Three such status groups were identified for further analysis: (i) single women (SW), that is women without a partner either male or female; (ii) women with a male partner (MP); (iii) women with a female partner (FP). SW were significantly older (43.9) than patients with a MP (40.4) and a FP (41.3). Women with a male or FP comprised fewer patients with no previous assisted reproductive technology cycles (18.4% and 25.7%, respectively) compared to SW (43.5%). The proportion of patients without children before treatment was significantly different between SW (94.7%) and women with a MP (87.2%). There were no differences in clinical outcomes among the three groups studied. Biochemical pregnancy rate was 58.2% in SW, 58.4% in women with a MP and 64.9% in women with a FP. For the same groups, clinical pregnancy rates were 50.2%, 49.4% and 55.4%, while 'take-home baby' rates were 36.6%, 38.9% and 40.3%. Multiple birth and caesarean section rates were not different among the groups, with twinning rates 21.1%, 30.4% and 36%, and caesarean section rates 25.6%, 24% and 26.4% for SW, women with MP and women with FP, respectively.

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Introduction

With the development of assisted reproductive technology (ART) and gamete donation programs, a larger portion of the population can access advanced therapeutic options to become pregnant. One such example is double gamete donation (DD), that is the transfer to a recipient's uterus of an embryo resulting from the fertilization of a donor oocyte with donor sperm. Although it is still comparatively rare in the assisted reproduction landscape, in the last few years DD has become the path to pregnancy for thousands of women worldwide.

Although a report has dealt with the psychological and socioeconomic characteristics of women undergoing double gamete donation (Landau, Weissenberg, & Madgar, 2008), no studies have as yet addressed the detailed characteristics of the patient population undergoing this fertility treatment to any reasonable extent, with the exception of one small case series of seven couples (Sills et al., 2010). To date, a description

of the patients undergoing DD is lacking, efficiency studies and analysis of pitfalls have not been reported and no clear indication for this treatment option has been established. This study of 1139 DD cycles aims (i) to bridge this knowledge gap, as well as identify the characteristics of the cohort of patients receiving DD for fertility treatment and (ii) to report the efficiency of the technique on reproductive outcomes including pregnancy and delivery rate, in order to provide information for physicians and patients when evaluating this therapeutic option.

Materials and methods

Study design and ethical approval

This is a retrospective analysis of anonymized cohort data from 1139 DD cycles between January 2001 and August 2010 at a large private fertility centre. Permission to conduct the study was obtained from the Institutional Review Board.

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Supplemental data for this article can be accessed [here](#).

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DD cycles inclusion criteria

The inclusion criteria of the study were consecutive cycles of DD resulting in a fresh embryo transfer (ET) during the study period. Cycles where the woman was positive to HIV or HCV were excluded. All embryo recipients were between 18 and 50 years old. The unit of analysis for this study is the ET, with an average of 1.37 (SD 0.7) cycles per patient who reached ET. In 821 patients, the cycle reported was the first one to involve DD, while in 319 the patients had undergone at least one previous DD attempt.

Statistical analysis

Recorded variables

The following variables were recorded as binary: relationship status [single women (reference category), SW; with a male partner, MP; with a female partner, FP]; age (years); history of infertility [continuous (years) and categorical (more than 5 years)]; previous ART cycles (yes/no and categorical [0, 1, 2, 3, 4+]); previous pregnancies (yes/no); previous live births (yes/no); endometrial preparation (oral/patch); biochemical pregnancy (yes/no); clinical pregnancy (yes/no); ongoing pregnancy at 12, 20 and 37 weeks (yes/no); multiple pregnancy at delivery (yes/no); caesarean section (yes/no); baby at home (yes/no).

Cluster analysis

A cluster analysis was undertaken designed to reveal natural groupings based on demographic [maternal age, relationship status (SW, women with MP or with FP)] and reproductive history (number of previous children, number of previous ART cycles and years of infertility) within the cohort of patients.

A two-step procedure was used. In the first step, cases were assigned to an automatically determined number of pre-clusters, which were treated as single cases in the second step. In the second step, a hierarchical algorithm based on the log-likelihood of the distances between variables was used to cluster the pre-clusters. The clustering was done by a procedure based on Bayesian Information Criteria. The analyses were performed using the statistical package SPSS 20.0 (Chicago, IL).

Regression analysis

Categorical variables were analysed by the Pearson chi-squared test and continuous variables by one-way ANOVA. Logistic regression models were performed for the pregnancy outcomes (biochemical pregnancy,

clinical pregnancy, ongoing pregnancy and child at home) to evaluate the effect of the relationship status, adjusted by the potential confounders (age, years of infertility, previous ART cycles, endometrial preparation and previous children). The statistical software SPSS 20.0 was used for the analyses. A p value <0.05 was considered statistically significant.

Results

Double gamete donation patients throughout the years

DD cycles were analysed from 2001 to 2010 based on the relationship status of the patients. There were few cases for the years 2001 and 2002, and there was no visible trend in the patient population during the study period.

The overall number of DD cycles increased steadily throughout the study. On the one hand, there was a progressive increase in the proportion of FP couples accessing this technique (from 0% in 2001 to 9.1% in 2010). The number of SW seeking DD also increased, comprising in 2010 about 50% of those accessing the technique. The proportion of patients reaching DD as part of a MP couple therefore decreased from 2003 to 2010 to about 40% of the patients (Figure 1).

Double gamete donation patients' characteristics

Patient population characteristics are reported in Table 1. On average, patients accessing DD were 42.2 (SD 4.2) years old, and reported a history of infertility

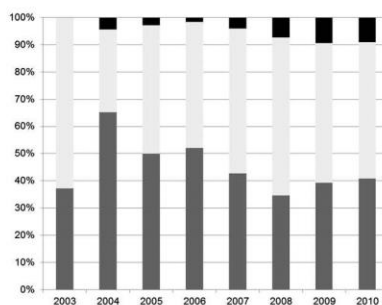


Figure 1. Trends in proportions of double gamete donation according to relationship status. Data from 2001–2002 comprises less than 10 cycles/year. Grey bar: women with a male partner. White bar: single women. Black bar: women with a female partner. X-axis: year; Y-axis: percentage of cycles.

of 3.41 years (SD 4.0; range: 0–21). Twenty-two percent (22.5%) of them had a history of more than 5 years of infertility. Most patients (91.7%) did not have a child at the time of treatment, with the notable exception of one mother of seven children. The decision to perform a DD cycle came, on average, after 3.21 (SD 3.8) previous unsuccessful ART cycles, although 31.8% of the patients underwent a DD cycle directly without having had a previous ART cycle; these patients were on average 43.3 (SD 4.1) years old, with means of 44.2 (SD 3.3) for SW, 41.0 (SD 5.1) for MP and 41.8 (SD 4.1) for FP.

Most DD patients were European (92.1%), while 3.5% were African and 0.5% from North and South America. A cumulative 3.9% of patients came from other regions of the world. Consistent with these data, the ethnicity of the patient was 91.6% non-Hispanic white, 3.9% black, 2.0% Hispanic white, 1.6% mulattos, 0.6% Asian and 0.3% of other ethnicities (Table 2).

Clusters

The clustering procedure resulted in the definition of three groups of patients, representing 34.6% (cluster I), 43% (cluster II) and 22.4% (cluster III) of the population; the clustering overall showed a 'fair' quality (Silhouette measure of cohesion and separation of 0.4).

Cluster I ($n = 393$) comprised 18.8% of women with FP and 81.2% of SW. They had no children (97.7%), despite having had previous ART cycles (95.2%). Cluster II ($n = 488$) was made up mainly of women with MP (97.5%); 85% of them were childless, and about half (49.6%) had had 3 or more previous ART cycles. They tended to be younger and with a longer history of infertility. Cluster III ($n = 254$) comprised SW (100%) with no previous ART treatment. Most were childless (94.9%) and tended to be older and with a shorter history of infertility (Table 3).

Classification by relationship status

We identified 'relationship status' as the most relevant variable defining sub-populations among DD patients in the cluster analysis (Supplementary Figure 1) and therefore chose to do a quantitative analysis of the three types of couple relationship in our sample: namely, SW (used as the reference category); women with MP and women with FP (Table 1).

Although MP women were younger than SW when they started a DD cycle: 40.4 (SD 4.7) versus 43.9 (SD 3.18) years, ($p < 0.001$), they had undergone more ART cycles: 4.3 (SD 4.1) versus 2.3 (SD 3.4) ($p < 0.001$), and reported a longer history of sterility: 5.2 (SD 4.2) versus 2.2 (SD 3.5) years ($p < 0.001$). The proportion of patients without children was lower in MP women than SW (87.2% vs. 94.7%, $p < 0.001$).

FP women were younger than SW when accessing DD: 41.3 (SD 3.2) versus 43.9 (SD 3.18), ($p < 0.001$). The proportion of FP patients with more than 5 years of infertility was lower than SW (2.7% vs. 12%, $p = 0.016$), as was the case for the proportion of patients without any previous ART cycle (25.7% vs. 43.5%, $p = 0.030$). Data on region of origin and ethnicity by relationship status are provided in Table 2.

Table 2. Area of origin and ethnicity of women accessing DD.

	Overall	Single women	Male partner	Female partner
Origin, n (%)				
Europe	1050 (92.1)	536 (91.4)	443 (92.7)	70 (94.6)
Africa	40 (3.5)	22 (3.7)	16 (3.3)	2 (2.7)
America	5 (0.5)	3 (0.5)	2 (0.4)	0 (0)
Other	46 (3.9)	26 (4.4)	17 (3.6)	2 (2.7)
Ethnicity, n (%)				
Non-Hispanic whites	1045 (91.6)	531 (90.4)	443 (92.6)	70 (94.5)
Black	44 (3.9)	25 (4.3)	17 (3.6)	2 (2.7)
Hispanic Whites	23 (2.0)	17 (2.9)	6 (1.3)	0 (0)
Mulattos	18 (1.6)	8 (1.4)	9 (1.9)	1 (1.4)
Asian	7 (0.6)	4 (0.7)	2 (0.4)	1 (1.4)
Other	3 (0.3)	2 (0.3)	1 (0.2)	0 (0)

Table 1. Demographic characteristics of patients accessing double gamete donation by relationship status.

	Total	SW	MP	FP
N	1139	587	478	74
Age, years, mean (SD)	42.2 (4.2)	43.9 (3.18)	40.4 (4.7) ^a	41.3 (3.2) ^b
History of infertility, years, mean (SD)	3.41 (4)	2.2 (3.5)	5.2 (4.2) ^a	1.8 (1.9)
More than 5 years of infertility, n (%)	255 (22.5)	70 (12)	183 (38.4) ^a	2 (2.7) ^b
Previous ART cycle (SD)	3.2 (3.8)	2.3 (3.4)	4.3 (4.1) ^a	3.0 (3.1)
No previous ART cycle, n (%)	363 (31.8)	256 (43.5)	88 (18.4) ^a	19 (25.7) ^b
No previous pregnancies, n (%)	471 (41.3)	246 (41.8)	199 (41.6) ^a	26 (35.1)
No previous children, n (%)	1043 (91.7)	557 (94.7)	415 (87.2) ^a	71 (95.9)

SW: single women; MP: male partner; FP: female partner. Different superscripts indicate a statistically significant difference between groups in rows ($p < 0.05$).

^aMP versus SW.

^bFP versus SW.

Table 3. Demographic characteristics and reproductive history of each cluster.

	Cluster I	Cluster II	Cluster III	<i>p</i>
<i>N</i> (%)	393 (34.6%)	488 (43%)	254 (22.4%)	
SW	319 (81.2)	12 (2.5)	254 (100)	<0.001
MP	0	476 (97.5)	0	
FP	74 (18.8)	0	0	
Age, years, mean (SD)	43.1 (3.1)	40.5 (4.7)	44.3 (4.2)	<0.001
Years of infertility, mean (SD)	2.7 (3.4)	5.2 (4.3)	1.04 (2.6)	<0.001
No previous ART cycle, <i>n</i> (%)	19 (4.8)	86 (17.6)	254 (100)	<0.001
Childless, <i>n</i> (%)	384 (97.7)	415 (85)	241 (94.9)	<0.001

SW: single women; MP: male partner; FP: female partner.

Table 4. Endometrial preparation and pregnancy outcomes.

	Total 1139	SW 587	MP 478	FP 74
Oral endometrial preparation, <i>n</i> (%)	840 (73.9)	399 (67.9)	286 (80.8) ^a	55 (74.3)
Patch endometrial preparation, <i>n</i> (%)	297 (26.1)	186 (31.8)	92 (19.2) ^a	19 (25.7)
Biochemical pregnancy, <i>n</i> (%)	669 (58.7)	342 (58.2)	279 (58.4)	48 (64.9)
7 weeks pregnancy, <i>n</i> (%)	572 (50.2)	295 (50.2)	236 (49.4)	41 (55.4)
14 weeks pregnancy, <i>n</i> (%)	563 (49.4)	283 (48.1)	242 (50.6)	38 (51.4)
20 weeks pregnancy, <i>n</i> (%) (total <i>n</i> = 1031)	402 (39)	205 (38.8)	172 (39)	25 (40.3)
37 weeks pregnancy, <i>n</i> (%) (total <i>n</i> = 1030)	383 (37.2)	198 (37.5)	161 (36.6)	24 (38.7)
Caesarean section, <i>n</i> (%) (total <i>n</i> = 945)	236 (25)	125 (25.6)	97 (24.0)	14 (26.4)
Baby at home, <i>n</i> (%) (total <i>n</i> = 1030)	400 (38.8)	204 (38.6)	171 (38.9)	25 (40.3)

SW: single women; MP: male partner; FP: female partner. Different superscripts indicate a statistically significant difference between groups in rows (*p* < 0.05).

^aMP versus SW.

^bFP versus SW.

Pregnancy outcomes

Details about ET and pregnancy outcomes are reported in Tables 4 and 5. On average, 73.9% of patients used oral oestrogen in their protocol for endometrial preparation, while 26.1% used transdermal oestrogen patches. The overall rates of biochemical, clinical and ongoing pregnancy were 58.7%, 50.2% and 49.4%, respectively, with multiple delivery rates of 25.5% for twins and 0.5% for triplets. Overall, 25% of the patients gave birth by caesarean section.

There were no differences in the biochemical, clinical and ongoing pregnancy rates between the three groups (SW, MP and FP), neither in the multiple pregnancy rates nor the proportion of caesarean sections. These differences remained non-significant after adjustment for potential confounders (Supplementary Table 1).

Discussion

In the last few decades, the number of people accessing fertility treatments has been rising (Chandra & Stephen, 1998; Dyer et al., 2016; Stephen & Chandra, 1998) and DD treatments follow this trend. We have observed that the clinical profile of ART patients has shifted in the last 10 years, with more FP and SW being treated currently, as well as older patients overall.

Assisted reproductive care in general has become more widespread and socially accepted, while technical

Table 5. Multiple births by relationship status.

	Total (400)	SW (204)	MP (171)	FP (25)
Singleton birth, <i>n</i> (%)	296 (74)	161 (78.9)	119 (69.6)	16 (64)
Twin birth, <i>n</i> (%)	102 (25.5)	9 (21.1)	50 (29.4)	9 (36)
Triplet birth, <i>n</i> (%)	2 (0.5)	0	2 (1)	0

SW: single women; MP: male partner; FP: female partner.

advances allow for the treatment of increasing numbers of people. The main factor for the increase in the demand for ART in developed countries is the increasing age at which women have their first child (de Graaff, Land, Kessels, & Evers, 2011; INE, 2014). In addition, more women are waiting to have children until they have completed higher education degrees, and participation of women in the skilled workforce is increasing, however, delaying motherhood can reduce the possibility of using one's own oocytes to achieve a pregnancy (Blickstein, 2003; Pal & Santoro, 2003).

Although the number of MP couples requiring DD has risen in absolute terms over the years, the relative proportion has not. One reason for this shift is that while DD is for the moment the only successful ART treatment available for SW or FP couples experiencing ovarian failure, research has advanced such that we are now able to offset, to a great extent the influence of a mild to moderate male factor (Nangia et al., 2011; Palermo, Cohen, & Rosenwaks, 1996), thus lowering their relative need to access DD. The proportion of FP women and SW electing double gamete donation has increased over the years, which may be due to the

progressive acceptance in society of new social relationships such as lesbian and single parent families.

With the clustering procedure the aim was to divide the patients into groups, the profiles of the patients being as close as possible when they were within a group and as different as possible when between groups.

This analysis confirmed that relationship status is the best variable to analyse groups accessing DD. Situations requiring DD are: (i) SW and FP suffering from premature ovarian failure (primary ovarian insufficiency, mainly due to ovarian aging), (ii) recurrent unexplained infertility in MP couples and (iii) MP couples with a medical indication not to use their own gametes; this category can be divided further into two main groups: couples that came to DD gradually; via oocyte donation with multiple implantation failure although the sperm of partner was of acceptable quality for IVF/ICSI), and in couples who opted for DD without prior experience of single gamete donation (a health issue other than infertility that discourages the use of one's own gametes such as cancer treatment or genetic disease, or in older women whose partner has a very severe male factor).

Women with a MP tend to be younger, with the longest history of infertility, and more previous ART cycles. Heterosexual couples therefore reach the decision to have a DD at a younger age; it is likely that they start at an earlier age than other DD patients to try and have children since their relationship is the social norm, all of which leads to an earlier request for ART if a pregnancy is not achieved spontaneously, but without an initial requirement to resort to gamete donation apart from cases of a severe male factor. Such couples experience more ART treatments and a longer duration of infertility than SW and FP women, as they start having ART treatments earlier. It is also noticeable that women in this group have had more children than SW and FP women. This could be due to their having started to have children earlier, when their fertility is still unaffected by ovarian ageing, or resuming their wish for motherhood later in their reproductive years, sometimes with a different MP.

More heterogeneity can be found among SW, although they are in general the oldest amongst the relationship groups. SW may have waited to have children within a traditional family structure before accessing ART as single parents, and are then unable to conceive with their own gametes. In the cluster analyses, they tend to distribute into two groups; in the first, some SW profiles overlap with FP couples: younger women who have had previous ART treatments, either alone or with a previous partner. Younger SW might

be more open to the prospect of single parenthood as a result of shifting social norms. However, there are also some SW women who are older, have a short history of infertility and no previous infertility treatments; this subgroup of patients decide to have their first child as their reproductive age increases, and so reach DD faster.

FP women access DD earlier, possibly because lesbian patients in a stable relationship do not wait as long as SW to address the issue of maternity. A woman who is openly in a lesbian relationship and cohabiting with her partner might also live in a more accepting social environment, which in turn would offer emotional and practical support for parenthood. However, FP patients do wait slightly longer than those with a MP; again possibly due to the social acceptance of the relationship. Society might accept the homosexual relationship with more difficulty, which may slightly delay motherhood for lesbian couples.

The reproductive outcomes of DD treatments were no different among the three groups. The proportion of deliveries with twins and triplets are comparable with those reported from IVF and ICSI cycles worldwide (Sullivan et al., 2013), and the rate of caesarean section is similar to that reported for spontaneous pregnancies in the European population (OECD, 2013). Spontaneous miscarriage/abortion rate (loss of a clinical pregnancy that occurs before the 21st week of gestation) was 19.7% overall. This rate is also comparable with the general population (Wang et al., 2003; Wilcox et al., 1988) and for pregnancies achieved by ART taken as a whole in a region (FIVCAT, 2011, p. 14).

To the best of our knowledge, this is the first large study reporting patients' characteristics and reproductive outcomes of DD cycles. We recognize some limitations, the most important being that it was carried out in a single private centre, which could have skewed the patient population. The inclusion of 100% of the DD patients in the study period notwithstanding the absence of publicly available literature on the subject makes it impossible to know how representative our cohort might be of worldwide DD trends. To address this issue partially we have obtained data for all DD cycles performed in the USA in the 2000–2010 decade from the US Centers for Disease Control and Prevention (R.V. personal inquiry) from which growth of DD cycles over time, average age at access and reproductive results, are similar to those observed here (Supplementary Table 2). In conclusion, DD is a reasonable treatment option for selected patients. The results are encouraging; however, perinatal and neonatal outcomes should be reported and monitored more widely,

as has been done for outcomes of ART cycles with couples' own or donor oocytes.

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

- Blockstein, I. (2003). Motherhood at or beyond the edge of reproductive age. *International Journal of Fertility and Women's Medicine*, 48, 17–24. doi: 10.1515/ijfm-2012-0019.
- Chandra, A., & Stephen, E.H. (1998). Impaired fecundity in the United States: 1982–1995. *Family Planning Perspectives*, 30, 34–42. doi: 10.2307/2991524.
- de Graaff, A.A., Land, J.A., Kessels, A.G., & Evers, J.L. (2011). Demographic age shift toward later conception results in an increased age in the subfertile population and an increased demand for medical care. *Fertility and Sterility*, 95, 61–63. doi: org/10.1016/j.fertnstert.2010.05.013.
- Dyer, S., Chambers, G.M., de Mouzon, J., Nygren, K.G., Zegers-Hochschild, F., Mansour, R., ... Adamson, G.D. (2016). International Committee for Monitoring Assisted Reproductive Technologies world report: Assisted Reproductive Technology 2008, 2009 and 2010. *Human Reproduction*, 31, 1588–1609. doi: 10.1093/humrep/dew082.
- FIVCAT. (2011). *FIVCAT.NET: statistical assisted human reproduction in Catalonia 2011*. Barcelona: Department of Health. Retrieved from http://www20.gencat.cat/docs/salut/Home/EI%20Departament/Estadistiques%20sanitaries/Dades%20de%20salut%20i%20serveis%20sanitaris/Reproduccio%20humana%20assistida/documents/fivcat_2011_resum_resultats.pdf.
- INE. (2014). *Instituto Nacional de Estadística (España)*. Retrieved from <http://www.ine.es/jaxiT3/Tabla.htm?t=1579>
- Landau, R., Weissenberg, R., & Madgar, I. (2008). A child of "hers": Older single mothers and their children conceived through IVF with both egg and sperm donation. *Fertility and Sterility*, 90, 576–583. doi: 10.1016/j.fertnstert.2007.07.1296.
- Nangia, A.K., Luke, B., Smith, J.F., Mak, W., & Stern, J.E., the SART Writing Group. (2011). National study of factors influencing assisted reproductive technology outcomes with male factor infertility. *Fertility and Sterility*, 96, 609–614. doi: 10.1016/j.fertnstert.2011.06.026.
- OECD. (2013). *Health at a Glance 2013: OECD indicators*. OECD Publishing. Retrieved from <https://www.oecd.org/els/health-systems/Health-at-a-Glance-2013.pdf>.
- Pal, L., & Santoro, N. (2003). Age-related decline in fertility. *Endocrinology and Metabolism Clinics of North America*, 32, 669–688. doi: org/10.1016/S0889-8529(03)00046-X.
- Palermo, G.D., Cohen, J., & Rosenwaks, Z. (1996). Intracytoplasmic sperm injection: A powerful tool to overcome fertilization failure. *Fertility and Sterility*, 65, 899–908. doi: org/10.1016/S0015-0282(16)58257-0.
- Sills, E.S., Mykhaylyshyn, L.O., Dorofeyeva, U.S., Walsh, D.J., Salma, U., Omar, A.B., ... Walsh, A.P. (2010). The long path to pregnancy: Early experience with dual anonymous gamete donation in a European *in vitro* fertilisation referral centre. *Reproductive Health*, 7, 20. doi: 10.1186/1742-4755-7-20.
- Stephen, E.H., & Chandra, A. (1998). Updated projections of infertility in the United States: 1995–2025. *Fertility and Sterility*, 70, 30–34. doi: 10.1016/S0015-0282(98)00103-4.
- Sullivan, E.A., Zegers-Hochschild, F., Mansour, R., Ishihara, O., de Mouzon, J., Nygren, K.G., & Adamson, G.D. (2013). International Committee for Monitoring Assisted Reproductive Technologies (ICMART) world report: Assisted reproductive technology 2004. *Human Reproduction*, 28, 1375–1390. doi: 10.1093/humrep/det036.
- Wang, X., Chen, C., Wang, L., Chen, D., Guang, W., & French, J., The Reproductive Health Study Group. (2003). Conception, early pregnancy loss, and time to clinical pregnancy: A population-based prospective study. *Fertility and Sterility*, 79, 577–584. doi: org/10.1016/S0015-0282(02)04694-0.
- Wilcox, A.J., Weinberg, C.R., O'Connor, J.F., Baird, D.D., Schlatterer, J.P., Canfield, R.E., ... Nisula, B.C. (1988). Incidence of early loss of pregnancy. *The New England Journal of Medicine*, 319, 189–194. doi: 10.1056/NEJM198807283190401.

PAPER 4: Risk of preeclampsia in pregnancies resulting from double gamete donation and from oocyte donation alone

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Risk of preeclampsia in pregnancies resulting from double gamete donation and from oocyte donation alone

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ABSTRACT

Introduction: Pregnancies after gamete donation are at higher risk of developing pre-eclampsia (PE) than those achieved by IVF with patient's own gametes. We aim to assess whether pregnancies achieved with both oocyte and sperm donation (double donation, DD) are at an increased risk of developing PE and gestational hypertension (GH) compared to those achieved by oocyte donation alone (OD).

Materials and methods: Retrospective cohort study of 433 patients who reached the 20th week of gestation with either DD (n = 81) or OD (n = 352) between March 2013 and April 2016 at a fertility clinic. The risk of preterm PE, term PE, and gestational hypertension (GH) are presented as unadjusted and adjusted odds ratio (OR).

Results: DD have a higher risk of preterm PE than OD, with an OR of 3.02 (95%CI 1.11–8.24; p = 0.031). We found no difference in the risk of term PE (OR 0.26, 95%CI 0.03–1.98; p = 0.19) or of GH (OR 1.23, 95% CI 0.63–2.43; p = 0.55).

Discussion: Pregnancies with DD are at higher risk of developing preterm PE than OD alone. Patients, and physicians treating them, should be made aware of the elevated risk of PE in these gestations, in order to start prophylactic measures during the first weeks of pregnancy.

1. Introduction

Despite pre-eclampsia (PE) complicates between 2 and 7% of all gestations [1–3], its still unknown etiology can be explained by two different theories: the vascular theory suggests that oxidative stress and other related factors cause endothelial damage and impaired trophoblast invasion of the myometrial arteries, which in turn leads to disrupted placentation [4]. On the other hand, the immunological theory postulates that the vascular dysfunction is the result of a maladaptation of the mother to fetal agents, and rests on the observation that the trophoblastic cells invading the decidua during early pregnancy express HLA-C antigens (both maternal and paternal), which are polymorphic. The HLA-C is a strong ligand for the killer immunoglobulin receptor (KIR) which is present on the surface of the uterine natural killer cells (uNK) and modulates proangiogenic and endothelial factors that promote changes in the spiral arteries to supply proper blood flow to the fetus. Some maternal KIR genotypes (especially the AA genotype) combined with certain trophoblastic HLA-C allotypes (particularly HLA-C2) can favor a dysfunction of uNK, which is associated with an altered maternal blood supply to the placenta, inducing disorders like

PE and fetal growth restriction [5,6]. Several histological findings of chronic villitis of immune origin have been associated with PE [7].

Some epidemiological evidence supports this theory: a longer exposure to the partner's sperm and the lack of barrier contraceptives use have both been reported to decrease the prevalence of PE [8–11], although the clinical relevance of this protective effect has been questioned [12,13]. Similarly, previous pregnancies or miscarriages with the same partner have been reported to have a protective effect against PE [14,15]. Furthermore, primigravity or conception with a new partner are associated with higher rates of PE [15].

Numerous studies have reported higher rates of PE in women who achieve pregnancy after either oocyte donation (OD) [1–3,16–20] or sperm donation [1,3,17]. The conceptus trophoblastic HLA-C is less recognizable to the immunological system of the mother when donated oocytes are used [2]. Furthermore, some authors hypothesize an association between the need for oocyte donation *per se* and PE, as circulating antibodies against granulosa cells and the zona pellucida have been detected in patients presenting ovarian failure, a classical indication for OD independently from sperm donation [21]. Moreover, it is not clear whether the altered ovarian function of patients needing OD

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or double donation (DD) could be related to vascular or immunological changes that could independently predispose to PE [19,22].

Assisted reproduction treatments by which embryos obtained from both donated sperm and oocytes are transferred to a woman uterus have increased in the last decades due to demographic and societal changes such as increased maternal age, single motherhood, and same sex family formation [23]. If poor immunological recognition causes PE, it would be reasonable to assume an increase of this pathology in pregnancies achieved by DD, since in these cases the mother has not been in contact with the fetal antigens, and there is no protective effect provided by the continuous exposure to the partner semen.

The aim of this study is to assess whether there is an increased risk of PE and in pregnancies achieved with both OD and sperm donation compared to pregnancies achieved by OD and partner sperm. An additional analysis of gestational hypertension (GH) risk has been also carried out.

2. Materials and methods

2.1. Study design

This is a retrospective cohort study of 433 patients having achieved a pregnancy through assisted reproductive technology (OD or DD) between March 2013 and April 2016 at a large referral fertility center. Data has been obtained through a questionnaire emailed to the patients at their 20th week of gestation in preparation for the delivery and sent for up to 3 times to patients that failed to reply after having delivered. The questionnaire was filled in by the patient with the help of her obstetrician/gynecologist

2.2. Ethical approval

Permission to conduct the study was granted by the Ethics Committee for Clinical Research of Clinica Eugin.

2.3. Study population and participants

Patients of OD and DD with a fresh embryo transfer during the timeframe of the study, with a pregnancy reaching the 20th week of gestation were included in the study.

PE was defined as hypertension (arterial tension > 140/90 mmHg in at least 2 determinations taken at least 6 h apart) associated with proteinuria (> 300 mg protein in a 24 h urine), diagnosed at or after 20 weeks of pregnancy (Bulletins–Obstetrics 2002). Preterm PE was defined as that requiring delivery before 37 weeks of pregnancy, while in term PE delivery occurred at or after 37 weeks.

GH was defined by hypertension diagnosed by the same criteria than for PE but without proteinuria.

All patients underwent endometrial preparation with either oral (6mg/24 h) or transdermal (150 mcg/72 h) estrogens. In the menstrual cycle previous to the embryo transfer, they were administered a GnRHα depot (3.75 mg of triptorelin depot) in the mesoluteal phase, if they had regular cycles, or in the 17th day of oral contraceptives administration in case their periods were irregular. Progesterone (400 mg/12 h) was started the day of the donor’s oocytes pick-up.

2.4. Statistical analysis

The risk of preterm PE, term PE and GH in DD compared to OD is presented as odds ratio (OR), with the associated confidence interval (95% CI) and p-value (Mantel-Haenszel Chi²). In addition, a multivariable analysis has been performed for each study outcome, adjusting for the potential confounding factors age, primigravida and multiple pregnancy.

All statistical analyses were performed using SPSS version 22.0. A p-value ≤ 0.05 was set as statistically significant.

Table 1 Demographic characteristics overall and by study group.

	Overall (n = 433)	DD (n = 81)	OD (n = 352)	p-value ^a
Age, Mean (SD)	41.9 (4.5)	42.6 (3.7)	41.7 (4.6)	NS
BMI, Mean (SD)	23.2 (3.9)	23.4 (3.9)	23.2 (3.9)	NS
Primigravida, n (%)	251 (58.0)	58 (71.6)	193 (54.8)	0.006
Miscarriage history, n (%)	157 (36.3)	21 (25.9)	136 (38.6)	NS
Previous ART treatment offspring				
- 0, n (%)	366 (85.3)	78 (96.3)	291 (82.7)	
- 1, n (%)	39 (9.1)	2 (2.5)	37 (10.5)	NS
- 2+, n (%)	24 (5.6)	1 (1.2)	24 (6.8)	

DD: double donation (oocyte and sperm).

OD: oocyte donation.

^a Student’s t-test or Pearson’s Chi².

3. Results

3.1. Demographic characteristics

A total of 1793 patients met the inclusion criteria and were sent the questionnaire, and 434 completed and returned it. The response rate was 24.2%. Supplementary Table 1 compares responders and non-responders characteristics and known PE risk factors. To note, no higher incidence of PE risk were found in non-responders. Information about non responders was obtained as part of the standard protocol of our center, where general information about patient’s characteristics and pregnancy outcomes are registered in the clinic’s database. Demographic characteristics of patients included in the study are presented in Table 1. On average, women were 41.9 years old, and 84.3% of them were childless.

3.2. Cycle and pregnancy characteristics

All oocytes in DD cycles were inseminated with frozen donor sperm, while 86.8% of frozen partner sperm was used in OD cycles. The proportion of frozen semen used is a consequence of patients living out of state, thus storing a semen sample on their first visit for future use. In most cases (90.3%), 2 embryos were transferred, and the majority of embryo transfers were on day 2–3 of development (90.3%). All embryos were transferred fresh (Table 2).

Overall, 22.6% of pregnancies were multiple, 93.5% ended in a live birth and C-section delivery was performed in 64.3% of cases. No significant differences were observed between study groups (Table 2).

3.3. Preeclampsia and gestational hypertension

Thirty-six cases of PE (8 in DD and 28 in OD) were registered. Of those, 16 were preterm PE, a condition statistically higher in the DD group (7/8 in DD and 12/28 in OD, p = 0.044). Regarding GH, 60 cases were reported (13 in DD and 47 in OD) (Table 3). DD pregnancies resulted in an OR of 2.68 [95%CI 1.02, 7.04, p = 0.038] for preterm PE, and OR of 0.26 [95%CI 0.03, 2.01, p = 0.17] for term PE; GH in DD pregnancies resulted in an OR of 1.24 [95%CI 0.64, 2.42, p = 0.53].

The risk of preterm PE associated to DD remained significant after adjustment for age, primigravida and multiple pregnancy, with an OR of 3.02 (95%CI 1.11–8.24; p = 0.031), and non significant for term PE and GH (Table 4).

4. Discussion

In the present study we found a high overall risk of PE and GH in the population studied, as expected after our previous report [16]. Interestingly, we also found an increased incidence of preterm PE in DD gestations compared to OD gestations, a finding not previously reported

Table 2
Cycle and pregnancy characteristics overall and by study group.

	Overall (n = 433)	DD (n = 81)	OD (n = 352)	p-value
Type of sperm, n (%)				
Fresh	46 (10.7%)	0	49 (13.9%)	< 0.001
Frozen	385 (89.3%)	81 (100%)	303 (86.1%)	
Day of embryo transfer, n (%)				
2–3	392 (90.3%)	67 (82.7%)	324 (92.0%)	< 0.011
5	42 (9.7%)	14 (17.3%)	28 (8.0%)	
Number of transferred embryos, n (%)				
1	38 (8.8%)	10 (12.3%)	27 (7.7%)	
2	392 (90.3%)	71 (87.7%)	321 (91.2%)	NS
3	4 (0.9%)	0	4 (1.1%)	
Embryo's morphological score of transferred embryos [†] , Mean (SD)	8.11 (1.10)	8.18 (1.09)	8.10 (1.10)	NS
Multiple pregnancy, n (%)	98 (22.6%)	16 (19.8%)	82 (23.3%)	NS
Miscarriage, n (%)	28 (6.5%)	3 (3.7%)	25 (7.1%)	NS
Type of labour, n (%)				
- Vaginal	154 (35.7%)	26 (32.1%)	128 (36.6%)	NS
- C-section	277 (64.3%)	55 (67.9%)	222 (63.4%)	
Gestational age at birth, Mean (SD)	37.8 (3.66)	37.8 (2.90)	37.8 (3.81)	NS

DD: double donation (oocyte and sperm).

OD: oocyte donation.

[†] Student's t-test or Pearson's Chi².

** Blastocysts excluded.

Table 3
Preeclampsia (PE) and Gestational hypertension (GH) overall and by study group.

	Overall (n = 433)	DD (n = 81)	OVODON (n = 352)	p
GH, n (%)	60 (13.9%)	13 (16.0%)	47 (13.4%)	0.53 [†]
PE, n (%)	36 (8.3%)	8 (9.9%)	28 (8.0%)	0.57 [†]
- Preterm PE, n (%)	19 (52.8%)	7 (87.5%)	12 (42.9%)	0.044 [‡]
within PE				
- Term PE, n (%)	17 (47.2%)	1 (12.5%)	16 (57.1%)	
within PE				

DD: double donation (oocyte and sperm).

OD: oocyte donation.

[†] Pearson's Chi² test.

[‡] Fisher's exact test.

Table 4
Multivariable analysis of the effect of sperm donation in addition to oocyte donation on the likelihood of preeclampsia, adjusted for potential confounders.

		95% CI			p
		OR	Lower	Upper	
Preterm PE	DD vs. OD	3.02	1.11	8.24	0.031
	Age	0.91	0.83	1.01	0.06
	Primigravity	1.34	0.48	3.76	0.58
	Multiple pregnancy	2.40	0.90	6.43	0.08
Term PE	DD vs. OD	0.25	0.03	1.98	0.19
	Age	1.01	0.90	1.12	0.91
	Primigravity	1.15	0.42	3.12	0.79
	Multiple pregnancy	1.03	0.33	3.26	0.96
GH	DD vs. OD	1.23	0.63	2.43	0.55
	Age	1.01	0.94	1.07	0.88
	Primigravity	1.12	0.63	1.98	0.70
	Multiple pregnancy	1.45	0.78	2.69	0.24

PE: Preeclampsia.

GH: Gestational hypertension.

DD: double donation (oocyte and sperm).

OD: oocyte donation.

in the medical literature.

Several studies have reported an elevated risk of PE and hypertensive disorders in OD pregnancies, discussing the hypothesis of an

immunological pathogenesis of this disorder based on the semi-allogenicity of the fetus with the mother [2,16,18,19,24,25]. Also, histological studies have demonstrated association between gestational hypertension and increased immune activity in placentas of OD pregnancies [26]. Additionally, intrauterine insemination with donated sperm has been associated with an increase in PE when compared with insemination with a partner's sperm; specifically, the fewer the cycles of intrauterine insemination, the higher the incidence of PE [3]; moreover, an exposure of more than 1 year to the partner sperm (by unprotected intercourse) was associated to lower PE [1]. Since both the partner and the donor semen are non-self with respect to the woman, these data support the hypothesis that an extended exposure to the partner seminal fluid induces some tolerance mechanisms in the future mother [27], while a pregnancy achieved without this previous exposure might be at a higher risk of PE. It seems logical to hypothesize that, in DD, the complete allogenicity of the fetal genome, compiled with the "novelty" of the paternal antigens will increase the risk of PE, but so far only one study has analyzed the incidence of PE in embryo donation, and found an increased incidence of PE in pregnancies by embryo donation [20]; unfortunately, those pregnancies were compared to women who became pregnant naturally, thus introducing several unaccounted for variables in the experimental design such as, for instance, the effect of hormonal manipulation, the laboratory handling of gametes, and the need to resort to third party assisted reproduction itself.

We found that the addition of the donor's sperm to the oocyte donation increases the risk of developing preterm PE, but not term PE or GH. A possible explanation for these results may reside in the different etiology of preterm PE and term PE. While preterm PE seems to be related to the deficient placentation due to immunological maladaptation and impaired decidualisation, maternal predisposing pathology such cardiovascular and inflammatory disorders are believed to trigger the onset of term PE [28,29]. Thus, in cases of DD pregnancies, it would be logical to expect a higher risk of only preterm PE, due to allogenicity of the gestation and the immunological process that it implies. This is especially outstanding, as preterm PE is associated to a more serious disease, with higher incidence of fetal growth restriction, gestational diabetes and risk of future cardiovascular disease [29–33]. Early identification of women at risk of preeclampsia is key in prenatal care, especially for cases with preterm onset. There is now good evidence [34] that low-dose aspirin started before 16 weeks of pregnancy halves the incidence of preeclampsia in high risk women, especially for early-onset and severe preeclampsia, which are the major contributors to

maternal and neonatal complications. Our study indicates that the risk of preterm PE of DD triples that of OD. Thus, prophylaxis with aspirin might be warranted in these women.

Although our findings confirm an elevated rate of PE in recipients of donated gametes, as reported [24,35,36], the proportion of pregnancies with PE that we report is lower than some studies [37–41]. A reason for this discrepancy could be the inclusion in several studies of frozen-thawed embryo transfers, which seem to confer and additional higher risk of PE compared to the fresh embryo transfers [2,42–45]. Alternatively, the reliance of our data on the patient and physician reports of the pregnancy, rather than direct diagnosis, might underestimate the incidence of PE and especially GH in patients with access to lower quality care in their environment. This bias, however, should be independent from the type of treatment that generated the pregnancy and should therefore be mitigated in our study.

We recognize some limitations to the current study; a significant one, as mentioned, is the collection of data by means of a questionnaire rather than directly from the observation of the patient throughout the pregnancy. Although patients were instructed to fill in the questionnaire with the help of their physician, the investigators had no direct control over the information provided. A recall bias is therefore possible, although unlikely, given the importance of PE/GH. Another limitation is caused by the retrospective design of the study which does not allow for the control of unaccounted for variables. Although the overall number of study events is relatively low, the elevated number of DD cases included, never before reported in the scientific literature, together with the selection of only fresh embryo transfers, are two important strengths of this report.

5. Conclusion

Women undergoing treatments with donated gametes should be made aware of the higher risk of developing gestational hypertensive disorders, as should be their attending physicians, and particularly women pregnant by double gamete donation, as they have an increased risk of developing preterm PE. These pregnancies should be identified early in order to start prophylactic measures appropriately, and decrease the risk of PE.

Authors roles

A.B.: involved in study design, data analysis, and manuscript preparation. D.G.: involved in data analysis and manuscript preparation. R.V.: involved in study implementation and supervision, expert knowledge, manuscript preparation. F.F.: involved in data analysis, expert knowledge and manuscript revision. A.R.: expert knowledge, manuscript revision.

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Declaration of interest

A.B. has nothing to declare. D.G. has nothing to declare. R.V. has nothing to declare. F.F. has nothing to declare. A.R. has nothing to declare.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.preghy.2018.06.010>.

References

- [1] G.N. Smith, M. Walker, J.L. Tessier, K.G. Millar, Increased incidence of preeclampsia in women conceiving by intrauterine insemination with donor versus partner sperm for treatment of primary infertility, *Am. J. Obstetrics Gynecol.* 177 (2) (1997) 455–458.
- [2] P.C. Klatsky, S.S. Delaney, A.B. Caughey, N.D. Tran, G.L. Schattman, Z. Rosenwaks, The role of embryonic origin in preeclampsia: a comparison of autologous in vitro fertilization and ovum donor pregnancies, *Obstetrics Gynecol.* 116 (6) (2010) 1387–1392.
- [3] D. Kyrou, E.M. Kolliakakis, F. Devroey, H.M. Fatemi, Is the use of donor sperm associated with a higher incidence of preeclampsia in women who achieve pregnancy after intrauterine insemination? *Fertil. Steril.* 93 (4) (2010) 1124–1127.
- [4] A.F. Tanbe, R.A. Khalil, Circulating and vascular bioactive factors during hypertension in pregnancy, *Curr. Bioactive Compounds* 6 (1) (2010) 60–75.
- [5] Z. Madeja, H. Yadi, R. Apps, S. Bouleznour, S.J. Roper, I. Gardner, A. Moffett, F. Colucci, M. Hemberger, Paternal MHC expression on mouse trophoblast affects uterine vascularization and fetal growth, *PNAS* 108 (10) (2011) 4012–4017.
- [6] S.E. Hilys, J.L. Walker, M. O'Shaughnessy, C.W. Redman, M. Carrington, J. Truswell, A. Moffett, Combinations of maternal HLA genes influence the risk of preeclampsia and reproductive success, *J. Exp. Med.* 200 (8) (2004) 957–965.
- [7] C.J. Kim, R. Romero, P. Chamsathong, J.S. Kim, Chronic inflammation of the placenta: definition, classification, pathogenesis, and clinical significance, *Am. J. Obstetrics Gynecol.* 213 (4 Suppl.) (2015) S53–S69.
- [8] A.F. Saifas, L. Rubenetein, K. Prater, K.K. Hariand, E. Field, E.W. Triche, Gametative exposure to paternal seminal fluid prior to conception and subsequent risk of preeclampsia, *J. Reprod. Immunol.* 101–102 (2014) 104–110.
- [9] E.M. Kho, L.M. McGowan, R.A. North, C.T. Roberts, E. Chan, M.A. Black, R.S. Taylor, G.A. Dekker, S. Consortium, Duration of sexual relationship and its effect on preeclampsia and small for gestational age perinatal outcome, *J. Reprod. Immunol.* 82 (1) (2009) 66–73.
- [10] J.I. Einarsson, H. Sangi-Hagheykar, M.O. Gardner, Sperm exposure and development of preeclampsia, *Am. J. Obstetrics Gynecol.* 188 (5) (2003) 1241–1243.
- [11] Z. Saidati, M. Abediadeh Kalahroudi, F. Saberi, The effect of short duration sperm exposure on development of preeclampsia in primigravida women, *Iran. Red Crescent Med. J.* 14 (1) (2012) 20–24.
- [12] G. Hall, W. Noble, S. Lindow, E. Masson, Long-term sexual co-habitation offers no protection from hypertensive disease of pregnancy, *Human Reprod.* 16 (2) (2001) 349–352.
- [13] R.B. Ness, N. Markovic, G. Harger, R. Day, Barrier methods, length of preconception intercourse and preeclampsia, *Hypertens. Pregnancy* 23 (3) (2004) 227–235.
- [14] P.Y. Robillard, T.C. Huisey, J. Perianita, E. Janjey, E.H. Miri, E. Papiernik, Association of pregnancy-induced hypertension with duration of sexual cohabitation before conception, *Lancet* 344 (8928) (1994) 973–975.
- [15] A.F. Saifas, R.J. Levine, M.A. Kiebanoff, K.L. Martz, M.G. Ewell, C.D. Morris, B.M. Sibai, Abortion, changed paternity, and risk of preeclampsia in nulliparous women, *Am. J. Epidemiol.* 157 (12) (2003) 1108–1114.
- [16] A. Blazquez, D. Garcia, A. Rodriguez, R. Vassena, F. Figueras, V. Vernaev, Is oocyte donation a risk factor for preeclampsia? A systematic review and meta-analysis, *J. Assisted Reprod. Genetics* 33 (7) (2016) 855–863.
- [17] M. Gonzalez-Comadran, J. Urresta Avila, A. Saavedra Tascón, R. Jimenez, I. Sola, M. Brasseur, R. Carreiras, M.A. Checa, The impact of donor insemination on the risk of preeclampsia: a systematic review and meta-analysis, *Eur. J. Obstetrics Gynecol. Reprod. Biol.* 182 (2014) 160–166.
- [18] U. Pecks, N. Maass, J. Neulen, Oocyte donation: a risk factor for pregnancy-induced hypertension: a meta-analysis and case series, *Deutsches Arzteblatt Int.* 108 (3) (2011) 29–31.
- [19] D.A. Keegan, L.C. Krey, H.C. Chang, N. Noyes, Increased risk of pregnancy-induced hypertension in young recipients of donated oocytes, *Fertil. Steril.* 87 (4) (2007) 776–781.
- [20] O. Salha, V. Sharma, T. Dada, D. Nugent, A.J. Rutherford, A.J. Tomlinson, S. Phillips, V. Allgar, J.J. Walker, The influence of donated gametes on the incidence of hypertensive disorders of pregnancy, *Human Reprod.* 14 (9) (1999) 2268–2273.
- [21] R.L. Keizer, P.K. Meherji, S.S. Kadam, S.K. Gupta, T.D. Nandekar, Circulating auto-antibodies against the zona pellucida and thyroid microsomal antigen in women with premature ovarian failure, *J. Reprod. Immunol.* 66 (1) (2005) 53–67.
- [22] G.H. Woldringh, M.H. Frunt, J.A. Kremer, M.E. Spaanderman, Decreased ovarian reserve relates to pre-eclampsia in IVF/ICSI pregnancies, *Human Reprod.* 21 (11) (2006) 2948–2954.
- [23] A. Blazquez, R. Vassena, A. Rodriguez, F. Figueras, V. Vernaev, Characteristics and clinical outcomes of patients undergoing fertility treatment by double gamete donation, *Human Fertil.* 19 (3) (2016) 180–185.
- [24] Y. Levron, M. Dvir, I. Segol, G.M. Yerushalmi, A. Hourvitz, R. Orvieto, S. Mazaki-Tovi, Y. Yinon, The 'immunologic theory' of preeclampsia revisited: a lesson from donor oocyte gestations, *Am. J. Obstetrics Gynecol.* 211 (4) (2014) 383 e1–5.
- [25] N.S. Fox, A.S. Roman, D.H. Saltzman, T. Hourizadeh, J. Hastings, A. Rebarber, Risk factors for preeclampsia in twin pregnancies, *Am. J. Perinatol.* 31 (2) (2014) 163–166.

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- [26] F. Gundogan, D.W. Bianchi, S.A. Scherjon, D.J. Roberts, Placental pathology in egg donor pregnancies, *Fertil. Steril.* 93 (2) (2010) 397–404.
- [27] S.A. Robertson, J.R. Prins, B.J. Sharkey, L.M. Moldenhauer, Seminal fluid and the generation of regulatory T cells for embryo implantation, *Am. J. Reprod. Immunol.* 69 (4) (2013) 315–330.
- [28] E.A. Steegers, P. von Dadelzen, J.J. Duvekot, R. Pijnenborg, Pre-eclampsia, *Lancet* 376 (9741) (2010) 631–644.
- [29] J.K. Phillips, M. Janowiak, G.J. Badger, I.M. Bernstein, Evidence for distinct preterm and term phenotypes of preeclampsia, *J. Maternal-Fetal Neonatal Med.* 23 (7) (2010) 622–626.
- [30] L. Myatt, C.W. Redman, A.C. Staff, S. Hansson, M.L. Wilson, H. Lalvaori, L. Poston, J.M. Roberts, C. Global Pregnancy, Strategy for standardization of preeclampsia research study design, *Hypertension* 63 (6) (2014) 1293–1301.
- [31] X. Xiong, N.N. Demianczuk, L.D. Saunders, F.L. Wang, W.D. Fraser, Impact of preeclampsia and gestational hypertension on birth weight by gestational age, *Am. J. Epidemiol.* 155 (3) (2002) 203–209.
- [32] M.L. Moongrwa-Chaffin, F.M. Cirillo, B.A. Cohn, Preeclampsia and cardiovascular disease death: prospective evidence from the child health and development studies cohort, *Hypertension* 56 (1) (2010) 166–171.
- [33] G.T. Manten, M.J. Slikema, H.A. Voorbij, G.H. Visser, H.W. Bruinse, A. Franx, Risk factors for cardiovascular disease in women with a history of pregnancy complicated by preeclampsia or intrauterine growth restriction, *Hypertens. Pregnancy* 26 (1) (2007) 39–50.
- [34] S. Roberg, K. Nicolaidis, S. Demers, J. Hyett, N. Chaillet, E. Bujold, The role of aspirin dose on the prevention of preeclampsia and fetal growth restriction: systematic review and meta-analysis, *Am. J. Obstetrics Gynecol.* 216 (2) (2017) 110–120 e6.
- [35] D. Stoop, M. Baumgarten, P. Haentjens, N.P. Polyzos, M. De Vos, G. Verheyen, M. Camus, P. Devroey, Obstetric outcome in donor oocyte pregnancies: a matched-pair analysis, *Reprod. Biol. Endocrinol.: RB&E* 10 (2012) 42.
- [36] S.S. Malchaire, A. Loft, E.C. Larsen, A.K. Aaris Henningsen, S. Rasmussen, A.N. Andersen, A. Pinborg, Perinatal outcomes in 375 children born after oocyte donation: a Danish national cohort study, *Fertil. Steril.* 99 (6) (2013) 1637–1643.
- [37] L.H. Seckhon, R.S. Gerber, A. Rehder, D.H. Salzman, C.K. Klaster, S. Gupta, N.S. Fox, Effect of oocyte donation on pregnancy outcomes in in vitro fertilization twin gestations, *Fertil. Steril.* 101 (5) (2014) 1326–1330.
- [38] V. Soderstrom-Anutila, A. Tiitinen, T. Foudila, O. Hovatta, Obstetric and perinatal outcome after oocyte donation: comparison with in-vitro fertilization pregnancies, *Human Reprod.* 13 (2) (1998) 483–490.
- [39] A.L. Tranquilli, V. Biondini, S. Talebi Chalvar, A. Coradetti, D. Tranquilli, S. Giannubilo, Perinatal outcomes in oocyte donor pregnancies, *J. Maternal-Fetal Neonatal Med.* 26 (13) (2013) 1263–1267.
- [40] C. Le Ray, S. Scherier, O. Anzelem, A. Marszalek, V. Tsatsaris, D. Cabrol, F. Goffinet, Association between oocyte donation and maternal and perinatal outcomes in women aged 43 years or older, *Human Reprod.* 27 (3) (2012) 896–901.
- [41] D.A. Wiggins, E. Main, Outcomes of pregnancies achieved by donor egg in vitro fertilization – a comparison with standard in vitro fertilization pregnancies, *Am. J. Obstetrics Gynecol.* 192 (6) (2005) 2002–2006 discussion 2006–8.
- [42] A. Szaszova, K. Kallen, A. Thurn-Kjellberg, I.B. Wennerholm, C. Bergh, Obstetric outcome in singletons after in vitro fertilization with cryopreserved/thawed embryos, *Human Reprod.* 27 (5) (2012) 1343–1350.
- [43] O. Ishihara, R. Araki, A. Kuwahara, A. Itakura, H. Saito, G.D. Adamson, Impact of frozen-thawed single-blastocyst transfer on maternal and neonatal outcome: an analysis of 277,042 single-embryo transfer cycles from 2008 to 2010 in Japan, *Fertil. Steril.* 101 (1) (2014) 128–133.
- [44] M. Barsky, P. S. Marie, T. Rabih, G.R. Markenson, C.K. Sites, Are perinatal outcomes affected by blastocyst vitrification and warming? *Am. J. Obstetrics Gynecol.* 215 (5) (2016) 603 e1–603 e5.
- [45] Z.J. Chen, Y. Shi, Y. Sun, B. Zhang, X. Liang, Y. Cao, J. Yang, J. Liu, D. Wei, N. Weng, L. Tian, C. Hao, D. Yang, F. Zhou, J. Shi, Y. Xu, J. Li, J. Yan, Y. Qin, H. Zhao, H. Zhang, R.S. Legro, Fresh versus Frozen Embryos for Infertility in the Polycystic Ovary Syndrome, *N. Engl. J. Med.* 375 (6) (2016) 523–533.

DISCUSSION

This thesis aims to elucidate the relationship between preeclampsia and assisted reproductive technology, especially with donated gametes.

From 2008 to 2010, around 4.5 million ART cycles were initiated around the world, with an annual increase every year. The number of cycles with donated oocytes reported increased a 35.8% in this triennium, with about 50,000 oocyte donation embryo transfers performed in 2010 ⁽⁴²⁾. Assisted reproductive care in general has become more widespread and socially accepted, while technical advances allow for the treatment of more people than ever before. The main factor for the increase in the demand of ART in developed countries is the increasing age at which women have their first child ^(91,92). More women are waiting to have children until they have completed higher education degrees, and participation of women in the skilled workforce is increasing; however, delaying motherhood can reduce the possibility of using one's own oocytes to achieve a pregnancy ^(93,94).

As a first insight into the relationship between gamete donation and PE, the design of the meta-analysis focused specifically on PE and included solely cohort studies comparing cycles of in vitro fertilization with OD vs. IVF. Since we avoided the comparison with natural conception pregnancies, we excluded the bias of the assisted reproductive technique per se, avoiding then the hormone therapy or the cultures in

the laboratory as an explanation of the difference in PE incidence. With 26.302 cases analyzed, this meta-analysis shows a clear association between OD and PE.

A limitation of this review is the lack of information in the included studies about the cause of infertility that has led to assisted reproductive technology (ART) with either autologous or donor oocytes, because the underlying type of infertility leading to one or the other treatment might itself contribute to the pathophysiology of PE. The factors causing the need of ART certainly vary from patients that will require donated oocytes from the ones that will perform an IVF with autologous oocytes (for instance, tubal infertility is typically a cause of IVF with autologous oocytes, while a premature ovarian failure will most likely lead to OD), thus making it difficult to determine if it is the reception of the oocytes or the cause of its necessity that is associated with the increase in PE incidence. Another limitation is the lack of detailed information in the included studies on severity or gestational age at onset of PE: the association with OD would be more clinically relevant in early onset PE, since it is more amenable to prevention by aspirin than late-onset disease.

The etiological relationship between OD and PE remains unclear. Despite the fact that the cause of developing PE seems to be multifactorial, OD is associated with an increased incidence of this disorder. As with the IVF control group we avoid the bias of the ART as a cause of the higher risk for PE, and with the meta regression procedure of our review we account for the confounding factors of maternal age and multiple

pregnancy, the immunological theory reinforces. However, there is a need of an increase in our understanding of the biochemical and immunological causes of PE, in order to develop possible preventive strategies, such as the selection of the oocyte donor immunologically matched to the HLA of the recipient ⁽⁹⁵⁾.

Examining the studies reported so far regarding PE and frozen embryo transfers, we realized that a common bias in all these papers is that they have only included embryo transfers of treatments with patient's own oocytes. Including only IVF with own oocytes, it does not allow to understand more in detail the source of the hypothetical effects of frozen ET, since in fresh ET the endometrium has been affected by the controlled ovarian stimulation, and in frozen ET by hormonal preparation of the endometrium for embryo reception. In this sense, oocyte donation offers a model to isolate the effect of the frozen ET, since all endometrium are prepared, and no ovarian stimulation is performed. So, when selecting this population, we avoided the possible confounding effect of the hyperestrogenism of the ovarian stimulation in the genesis of PE and GH^(69,77,80).

To the best of our knowledge, this is the first study comparing the prevalence of PE in fresh versus frozen ET after oocyte donation. We found no difference in preterm PE,

term PE, or gestational hypertension between fresh and frozen ET in recipients of oocyte donation.

Our results seem to indicate that the clinical relevance of the epigenetic changes supposed to be a possible etiology for PE in frozen ET, at least up to birth, is relatively minor, given that, when the endometrial preparation and hormonal state is the same in the recipients, the prevalence of preterm PE, term PE or GH does not change between pregnancies achieved with fresh or frozen ETs. Our findings are in agreement with the current PE etiological theory which assigns more weight to the defective vascularization of the placenta, and not necessarily to the embryo characteristics and manipulations ^(7,96). Female sex hormones play an important role in placental vascularization, as they promote placental angiogenesis and decrease the resistance of spiral uterine arteries ^(76,97). Studies about the relationship between female sex hormones and PE report opposite results: while some authors report that low levels of estrogens are correlated with PE development^(97–99), others found no differences or even increased estrogen levels in PE^(100,101), as well as with progesterone concentrations^(72,102). It seems then that certain ranges of estrogens and progesterone are needed for normal placentation process. In the studies published so far, frozen ET pregnancies carry an increased risk of PE compared to fresh ET^(51,77,78,85,86), possibly indicating that the protocols currently used for endometrial

reception of embryos have a deleterious effect on placentation, maybe due to the long exposure of hormone replacement.

We recognize some limitations to the current study; a significant one is the collection of data by means of a questionnaire rather than from a direct diagnosis, despite patients were instructed to fill in the questionnaire with the help of their physician. A recall bias is therefore possible, although unlikely, given the importance of PE/GH. In any case, any potential selection bias would likely operate similarly between women with fresh and frozen ET. The number of cases of PE in the frozen ET group is relatively low, but the total number of patients included and the fact that it has never been reported before in the literature, gives strength to this report. The embryo score in the frozen ET group was likely lower than the fresh ET group, as embryos are vitrified after a fresh ET, and transferred if the fresh ET has not been successful. Despite these frozen embryos might present poorer morphology than the ones selected for the fresh ET, the incidence of PE should not change. Oron showed that poor embryo quality is not associated with adverse obstetric outcomes such as PE⁽¹⁰³⁾, as did other studies that after comparing pregnancies from cleavage or blastocyst transfer, did not find differences in PE incidence^(84,104,105). The fact that are second embryo transfers of a whole embryo cohort could represent a bias if the pregnancy rate was the outcome, since the frozen ET group are by definition patients with worse prognosis when

pregnancy rate is the outcome, but once the pregnancy is ongoing, it should not change the PE outcome.

One observation from our study of patients undergoing double-donation of gametes treatment is that the shift in ART patients in general is not only due to an increase in older women planning to have a child but also in single women and women with female partner. The indications to perform an assisted reproductive technique with donor oocytes have extended: to the classical ovarian failure (due to iatrogenic or spontaneous menopause) we have to add the repeated IVF failures with own oocytes, the genetic causes that discourage to use own's oocytes, and maybe the most common, ovarian aging. Although the number of MP couples needing DD has risen in absolute numbers over the years, the relative proportion of MP couples in the total of DD cycles has not. One reason for this shift might be ascribed to the fact that, while DD is for the moment the only successful ART treatment available for SW or FP couples experiencing ovarian failure, research in male factor infertility has advanced in such a way that we are now able to offset the influence of a mild to moderate male factor to a great extent ^(106,107), thus lowering their relative need to access DD. The proportion of FP women and SW electing double gamete donation has increased over the years,

which may be due to the progressive acceptance in society of new structures such as lesbian and single parent families.

Our clustering analysis found that relationship status was the best variable to analyze the groups accessing DD. On average, patients accessing DD were 42 years old, and most of them (91.7%) did not have a child at the time of treatment. The reproductive outcomes of DD treatments was no different among the three groups, with a proportion of multiple pregnancies comparable with those reported from IVF and ICSI cycles worldwide (25.5% for twins and 0.5% for triplets) ⁽¹⁰⁸⁾, a rate of caesarean section similar to that reported for spontaneous pregnancies in the European population ⁽¹⁰⁹⁾, and a spontaneous miscarriage/abortion rate (loss of a clinical pregnancy that occurs before the 21st week of gestation) also comparable both with the general population ^(110,111) or pregnancies achieved by ART taken as a whole in the region⁽¹¹²⁾.

Upon revision of the list of risk factors for PE, we found that DD patients have several of them, like advanced maternal age, primigravity, the use of ART to achieve a pregnancy, the use of donated gametes, and a high rate of multiple pregnancy. Despite the fact that DD cycles are increasing worldwide (Rita Vassena personal inquiry, data from USA in the 2000-2010 decade from the US Centers for Disease Control and Prevention), there is an absence of publicly available literature on the matter. This is,

in fact, to the best of our knowledge, the first large study reporting patients' characteristics and reproductive outcomes of DD cycles. So, in conclusion, DD cycles are increasing, and it is a reasonable treatment option for selected patients, with encouraging results. However, perinatal and neonatal outcomes should be reported and monitored more widely, as has been done with the outcomes of ART cycles with own or donor oocytes.

If poor immunological recognition causes PE, it would be reasonable to assume an increase of this pathology in pregnancies achieved by DD, since in these cases the mother has not been in contact with the fetal antigens, and there is no protective effect provided by the continuous exposure to the partner semen. We tested this hypothesis in the fourth project, and found an increased incidence of preterm PE in DD gestations compared to OD gestations, a finding not previously reported in the medical literature. So far there are two studies analyzing the incidence of PE in double gamete donation: the first found an increased risk of PE in embryo donation pregnancies ⁽³⁹⁾, but unfortunately, those pregnancies were compared to women who became pregnant naturally, thus introducing several unaccounted for variables in the experimental design, such as the effect of hormonal manipulation, the laboratory handling of gametes, or the need to resort to third party assisted reproduction itself.

The second study⁽¹¹³⁾ did not find differences in the incidence of GH or PE between DD and OD pregnancies, reporting only an increase in gestational diabetes mellitus that could not be explained by the authors.

We found that the addition of the donor's sperm to the oocyte donation increases the risk of developing preterm PE, but not term PE or GH. A possible explanation for these results may reside in the different etiology of preterm PE and term PE. While preterm PE seems to be related to the deficient placentation due to immunological maladaptation and impaired decidualization, maternal predisposing pathology such as cardiovascular and inflammatory disorders are believed to trigger the onset of term PE^(6,114). Thus, in cases of DD pregnancies, it would be logical to expect a higher risk of only preterm PE, due to allogenicity of the gestation and the immunological process that it implies. This is especially outstanding, as preterm PE is associated to a more serious disease, with higher incidence of fetal growth restriction, gestational diabetes and risk of future cardiovascular disease^(8,9,114–116). Nowadays studies about PE should always report preterm PE and term PE separately, and probably this could be the cause of the difference in the results found by Preaubert. Early identification of women at risk of preeclampsia is key in prenatal care, and there is now good evidence⁽¹¹⁷⁾ that low-dose aspirin started before 16 weeks of pregnancy halves the incidence of preeclampsia in high risk women, especially for early-onset and severe preeclampsia,

which are the major contributors to maternal and neonatal complications. Our study indicates that the risk of preterm PE in DD triples the on in OD.

This study shares one limitation with the second project: data have been obtained through a questionnaire, but again, giving the importance of the syndrome and the specificity of the information asked, a mistake in the data provided by the patients is unlikely, especially in the preterm group, and should be independent from the type of treatment that generated the pregnancy. Despite the fact that we had a low response rate in our questionnaire of pregnancy outcome, this is unlikely to result in a selection bias since the baseline characteristics did not differ between responders and non-responders. The elevated number of DD cases included, never before reported in the scientific literature, together with the selection of only fresh embryo transfers, are two important strengths of this report. Other studies reporting PE in ART included fresh and frozen embryo transfers, and the lasts seem to confer and additional higher risk of PE compared to the fresh embryo transfers^(51,77,78,85,86).

In conclusion, women undergoing treatments with donated gametes should be made aware of the higher risk of developing gestational hypertensive disorders, as should be their attending physicians. Despite this high prevalence of PE, the freezing-thawing process in the embryo does not seem to be adding more risk compared with fresh ET.

Particularly women pregnant by double gamete donation should receive strict obstetrical surveillance, giving the shift in this reproductive treatment, as they have an increased risk for developing preterm PE. These pregnancies should be identified early in order to start prophylactic measures appropriately and decrease the risk of PE. Also, as the endometrial hormonal environment can have a role in the pathogenesis of PE, further studies are required to determine if levels of estrogens and progesterone could be used as biomarkers for the early diagnosis of PE.

Reducing the prevalence of PE will not only improve maternal outcomes, but also neonatal outcomes. There is evidence that the adverse perinatal outcomes reported in OD pregnancies, specially preterm birth and low birth weight ^(118,119), improve after adjusting for PE^(120–127). So, an important part of this prematurity is due to induced labors that obstetricians perform in order to finish the pregnancy, the only curative treatment for PE.

NICE guidelines recommend the prophylaxis with aspirin in those pregnant women with 1 high risk factor or 2 moderate risk factors for PE ⁽¹²⁸⁾. The U.S. Preventive Services Task Force recommends the use of low-dose aspirin (81mg per day) as preventive medication after 12 weeks of gestation in women who are at high risk of preeclampsia⁽¹²⁹⁾. Age of 40 years or older, multiple pregnancy and primigravity are some of these risk factors, but nowadays gamete donation is not included as an

independent risk factor. Probably, giving the increase in the risk for PE that oocyte donation confers to the pregnancy, this ART treatment in itself should be included as a risk factor and, thus, prophylaxis with aspirin might be warranted in these women. Additionally, giving the role that hormonal environment also has in the pathogenesis of PE, different hormonal replacement therapies could decrease the incidence of this disorder.

CONCLUSIONS

- OD is associated with a higher incidence of PE than IVF, even adjusting for maternal age and multiple pregnancy.
- These data support that in OD, the origin of PE is related with a maladaptation of the mother to embryonic antigens.
- Frozen ET does not confer more risk for developing PE than fresh ET in OD cycles.
- The hormonal milieu in the endometrium has probably a role in the pathogenesis of PE.
- Double-donation cycles are increasing, with positive results for selected patients.
- Patients pregnant with double-donation of gametes have several risk factors for developing PE.
- Double-donation is associated with a higher incidence of preterm PE compared to OD, but not of term PE or GH, even adjusting for maternal age, primigravity and multiple pregnancy.
- In reproduction, the more the gametes are donated, the more the risk for developing PE.

BIBLIOGRAPHY

1. Phipps E, Prasanna D, Brima W, Jim B. Preeclampsia: Updates in pathogenesis, definitions, and guidelines. *Clinical Journal of the American Society of Nephrology*. 2016;11(6):1102-13.
2. Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. *Lancet*. 2005;365(9461):785–99.
3. Ventura SJ, Mosher WD, Curtin SC, Abma JC, Henshaw S. Trends in pregnancy rates for the United States, 1976-97: an update. *Natl Vital Stat Rep*. 2001;49(4):1–9.
4. ACOG Committee on Practice Bulletins--Obstetrics. ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002. *Obstet Gynecol*. 2002; 99(1):159-67.
5. American College of Obstetricians and Gynecologists, Task Force on Hypertension in Pregnancy. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. *Obstet Gynecol*. 2013;122(5):1122-31.
6. Steegers EAP, Von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. *Lancet*. 2010; 376(9741):631-44.

7. Thilaganathan B. Placental syndromes: getting to the heart of the matter. *Ultrasound Obstet Gynecol.* 2017;49: 7–9.
8. Xiong X, Demianczuk NN, Saunders LD, Wang F-L, Fraser WD. Impact of preeclampsia and gestational hypertension on birth weight by gestational age. *Am J Epidemiol.* 2002;155(3):203-9.
9. Mongraw-Chaffin ML, Cirillo PM, Cohn BA. Preeclampsia and cardiovascular disease death: Prospective evidence from the child health and development studies cohort. *Hypertension.* 2010; 56(1):166-71.
10. Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction hypertension, and proteinuria in preeclampsia. *J Clin Invest.* 2003;111(5):649-58.
11. Venkatesha S, Toporsian M, Lam C, Hanai JJ, Mammoto T, Kim YM, et al. Soluble endoglin contributes to the pathogenesis of preeclampsia. *Nat Med.* 2006; 12(6):642-9.
12. Hiby SE, Apps R, Sharkey AM, Farrell LE, Gardner L, Mulder A, et al. Maternal activating KIRs protect against human reproductive failure mediated by fetal

- HLA-C2. *J Clin Invest.* 2010; 120(11):4102-10.
13. Madeja Z, Yadi H, Apps R, Boulenouar S, Roper SJ, Gardner L, et al. Paternal MHC expression on mouse trophoblast affects uterine vascularization and fetal growth. *Proc Natl Acad Sci.* 2011; 108(10):4012-7.
 14. Hiby SE, Walker JJ, O'Shaughnessy KM, Redman CWG, Carrington M, Trowsdale J, et al. Combinations of Maternal KIR and Fetal HLA-C Genes Influence the Risk of Preeclampsia and Reproductive Success. *J Exp Med.* 2004; 200(8):957-65.
 15. Giovanna Ogge, Tinnakorn Chaiworapongsa, Roberto Romero, Youssef Hussein, Juan Pedro Kusanovic, Lami Yeo, Chong Jai Kim, MD and SSH. Placental lesions associated with maternal underperfusion are more frequent in early-Onset than in late-onset preeclampsia. *J Perinat Med.* 2011;39(6):641–52.
 16. Yu CKH, Khouri O, Onwudiwe N, Spiliopoulos Y, Nicolaides KH. Prediction of pre-eclampsia by uterine artery Doppler imaging: Relationship to gestational age at delivery and small-for-gestational age. *Ultrasound Obstet Gynecol.* 2008; 31(3):310-3.
 17. Jelin AC, Cheng YW, Shaffer BL, Kaimal AJ, Little SE, Caughey AB. Early-onset preeclampsia and neonatal outcomes. *J Matern Neonatal Med Off J Eur Assoc*

- Perinat Med Fed Asia Ocean Perinat Soc Int Soc Perinat Obstet. 2010;23(5):389-92.
18. Espinoza J, Lee W, Martin SR, Belfort MA. Customized growth curves for identification of large-for-gestational age neonates in pre-eclamptic women. *Ultrasound Obstet Gynecol.* 2014; 43(2):165-9.
 19. Verlohren S, Melchiorre K, Khalil A, Thilaganathan B. Uterine artery Doppler, birth weight and timing of onset of pre-eclampsia: providing insights into the dual etiology of late-onset pre-eclampsia. *Ultrasound Obstet Gynecol.* 2014; 44(3):293-8.
 20. Ray JG, Vermeulen MJ, Schull MJ, Redelmeier DA. Cardiovascular health after maternal placental syndromes (CHAMPS): Population-based retrospective cohort study. *Lancet.* 2005; 366(9499):1797-803.
 21. Bellamy L, Casas J-P, Hingorani AD, Williams DJ. Pre-eclampsia and risk of cardiovascular disease and cancer in later life: systematic review and meta-analysis. *BMJ.* 2007; 335(7627):974.
 22. Brown MC, Best KE, Pearce MS, Waugh J, Robson SC, Bell R. Cardiovascular disease risk in women with pre-eclampsia: Systematic review and meta-

- analysis. *European Journal of Epidemiology*. 2013;28(1):1-19.
23. Magnussen EB, Vatten LJ, Lund-Nilsen TI, Salvesen KA, Davey Smith G, Romundstad PR. Prepregnancy cardiovascular risk factors as predictors of pre-eclampsia: population based cohort study. *BMJ*. 2007; ;335(7627):978.
 24. Romundstad PR, Magnussen EB, Smith GD, Vatten LJ. Hypertension in pregnancy and later cardiovascular risk: Common antecedents? *Circulation*. 2010; 122(6):579-84.
 25. Enkhmaa D, Wall D, Mehta PK, Stuart JJ, Rich-Edwards JW, Merz CNB, et al. Preeclampsia and Vascular Function: A Window to Future Cardiovascular Disease Risk. *J Women's Heal*. 2016; 25(3):284-91.
 26. Sibai BM, El-Nazer A, Gonzalez-Ruiz A. Severe preeclampsia-eclampsia in young primigravid women: Subsequent pregnancy outcome and remote prognosis. *Am J Obstet Gynecol*. 1986; 155(5):1011-6.
 27. Makkonen N, Heinonen S, Kirkinen P. Obstetric prognosis in second pregnancy after preeclampsia in first pregnancy. *Hypertens Pregnancy*. 2000; 19(2):173-81.
 28. Duckitt K, Harrington D. Risk factors for pre-eclampsia at antenatal booking:

- Systematic review of controlled studies. *British Medical Journal*. 2005;330(7491):565.
29. Skomsvoll JF, Ostensen M, Irgens LM, Baste V. Pregnancy complications and delivery practice in women with connective tissue disease and inflammatory rheumatic disease in Norway. *Acta Obstet Gynecol Scand*. 2000; 79(6):490-5.
 30. Yogev Y, Xenakis EMJ, Langer O. The association between preeclampsia and the severity of gestational diabetes: The impact of glycemic control. *Am J Obstet Gynecol*. 2004;191(5):1655-60.
 31. Baeten JM, Bukusi EA, Lambe M. Pregnancy complications and outcomes among overweight and obese nulliparous women. *Am J Public Health*. 2001; ;91(3):436-40.
 32. Coonrod D V, Hickok DE, Zhu K, Easterling TR, Daling JR. Risk factors for preeclampsia in twin pregnancies: a population-based cohort study. *Obstet Gynecol*. 1995; 85(5 Pt 1):645-50.
 33. Qiu C, Williams MA, Leisenring WM, Sorensen TK, Frederick IO, Dempsey JC, et al. Family history of hypertension and type 2 diabetes in relation to preeclampsia risk. *Hypertension*. 2003; 41(3):408-13.

34. Cincotta RB, Brennecke SP. Family history of pre-eclampsia as a predictor for pre-eclampsia in primigravidas. *Int J Gynecol Obstet*. 1998; 60(1):23-7.
35. Bianco A, Stone J, Lynch L, Lapinski R, Berkowitz G, Berkowitz RL. Pregnancy outcome at age 40 and older. *Obstet Gynecol [Internet]*. 1996 Jun;87(6):917–22.
36. Saftlas AF, Levine RJ, Klebanoff MA, Martz KL, Ewell MG, Morris CD, et al. Abortion, changed paternity, and risk of preeclampsia in nulliparous women. *Am J Epidemiol*. 2003; 157(12):1108-14.
37. Einarsson JI, Sangi-Haghpeykar H, Gardner MO. Sperm exposure and development of preeclampsia. *Am J Obstet Gynecol*. 2003; 188(5):1241-3.
38. Bibbins-Domingo K, Grossman DC, Curry SJ, Barry MJ, Davidson KW, Doubeni CA, et al. Screening for Preeclampsia: US Preventive Services Task Force Recommendation Statement. *JAMA [Internet]*. 2017 Apr 25;317(16):1661.
39. Salha O, Sharma V, Dada T, Nugent D, Rutherford AJ, Tomlinson AJ, et al. The influence of donated gametes on the incidence of hypertensive disorders of pregnancy. *Hum Reprod*. 1999; 14(9):2268-73.
40. Pecks U, Maass N, Neulen J. Oocyte donation: a risk factor for pregnancy-

- induced hypertension: a meta-analysis and case series. *Dtsch Arztebl Int.* 2011; 108(3):23-31.
41. Cnattingius S, Lambe M. Trends in smoking and overweight during pregnancy: Prevalence, risks of pregnancy complications, and adverse pregnancy outcomes. *Semin Perinatol.* 2002; 26(4):286-95.
 42. Dyer S, Chambers GM, De Mouzon J, Nygren KG, Zegers-Hochschild F, Mansour R, et al. International committee for monitoring assisted reproductive technologies world report: Assisted reproductive technology 2008, 2009 and 2010†. *Hum Reprod.* 2016;31(7):1588–609.
 43. Tandberg A, Klungsoyr K, Romundstad LB, Skjærven R. Pre-eclampsia and assisted reproductive technologies: Consequences of advanced maternal age, interbirth intervals, new partner and smoking habits. *BJOG An Int J Obstet Gynaecol.* 2015; 122(7):915-22.
 44. Nelissen ECM, Dumoulin JCM, Daunay A, Evers JLH, Tost J, van Montfoort APA. Placentas from pregnancies conceived by IVF/ICSI have a reduced DNA methylation level at the H19 and MEST differentially methylated regions†. *Hum Reprod [Internet].* 2013 Apr;28(4):1117–26.

45. Nelissen ECM, van Montfoort APA, Dumoulin JCM, Evers JLH. Epigenetics and the placenta. *Hum Reprod Update*. 2011; 17(3):397-417.
46. Mann MRW. Selective loss of imprinting in the placenta following preimplantation development in culture. *Development*. 2004; 131(15):3727-35.
47. Fortier AL, Lopes FL, Darricarrère N, Martel J, Trasler JM. Superovulation alters the expression of imprinted genes in the midgestation mouse placenta. *Hum Mol Genet*. 2008; 17(11):1653-65.
48. Rivera RM, Stein P, Weaver JR, Mager J, Schultz RM, Bartolomei MS. Manipulations of mouse embryos prior to implantation result in aberrant expression of imprinted genes on day 9.5 of development. *Hum Mol Genet*. 2008; ;17(1):1-14.
49. Tarlatzi TB, Imbert R, Alvaro Mercadal B, Demeestere I, Venetis CA, Englert Y, et al. Does oocyte donation compared with autologous oocyte IVF pregnancies have a higher risk of preeclampsia? *Reprod Biomed Online*. 2017; 34(1):11-18.
50. Smith GN, Walker M, Tessier JL, Millar KG. Increased incidence of preeclampsia in women conceiving by intrauterine insemination with donor versus partner

- sperm for treatment of primary infertility. *Am J Obstet Gynecol.* 1997; 177(2) 455-8.
51. Klatsky PC, Delaney SS, Caughey AB, Tran ND, Schattman GL, Rosenwaks Z. The role of embryonic origin in preeclampsia: A comparison of autologous in vitro fertilization and ovum donor pregnancies. *Obstet Gynecol.* 2010; 116(6)1387-92.
52. Kyrou D, Kolibianakis EM, Devroey P, Fatemi HM. Is the use of donor sperm associated with a higher incidence of preeclampsia in women who achieve pregnancy after intrauterine insemination? *Fertil Steril.* 2010;93(4) 1124-7.
53. Keegan DA, Krey LC, Chang HC, Noyes N. Increased risk of pregnancy-induced hypertension in young recipients of donated oocytes. *Fertil Steril.* 2007; 87(4)776-81.
54. González-Comadran M, Avila JU, Tascón AS, Jimenéz R, Solà I, Brassesco M, et al. The impact of donor insemination on the risk of preeclampsia: A systematic review and meta-analysis. *European Journal of Obstetrics Gynecology and Reproductive Biology.* 2014; 182:160-6.
55. Saftlas AF, Rubenstein L, Prater K, Harland KK, Field E, Triche EW. Cumulative

- exposure to paternal seminal fluid prior to conception and subsequent risk of preeclampsia. *J Reprod Immunol*. 2014; 101-102:104-10.
56. Kho EM, McCowan LME, North RA, Roberts CT, Chan E, Black MA, et al. Duration of sexual relationship and its effect on preeclampsia and small for gestational age perinatal outcome. *J Reprod Immunol*. 2009; 82(1):66-73.
57. Sadat Z, Abedzadeh Kalahroudi M, Saberi F. The effect of short duration sperm exposure on development of preeclampsia in primigravid women. *Iran Red Crescent Med J*. 2012; 14(1):20-4.
58. Hall GH. Long-term sexual co-habitation offers no protection from hypertensive disease of pregnancy. *Hum Reprod*. 2001; 16(2) 349-52.
59. Ness RB, Markovic N, Harger G, Day R. Barrier methods, length of preconception intercourse, and preeclampsia. *Hypertens Pregnancy*. 2004; 23(3):227-35.
60. Robillard PY, Périanin J, Janky E, Miri EH, Hulse TC, Papiernik E. Association of pregnancy-induced hypertension with duration of sexual cohabitation before conception. *Lancet*. 1994; 344(8928) 973-5.
61. Aluvihare VR, Kallikourdis M, Betz AG. Regulatory T cells mediate maternal

- tolerance to the fetus. *Nat Immunol.* 2004; 5(3):266-71.
62. Robertson SA, Guerin LR, Moldenhauer LM, Hayball JD. Activating T regulatory cells for tolerance in early pregnancy - the contribution of seminal fluid. *J Reprod Immunol.* 2009; 83(1-2):109-16.
63. Johansson M, Bromfield JJ, Jasper MJ, Robertson SA. Semen activates the female immune response during early pregnancy in mice. *Immunology.* 2004;112(2):290-300.
64. Alecsandru D, Garrido N, Vicario JL, Barrio A, Aparicio P, Requena A, et al. Maternal KIR haplotype influences live birth rate after double embryo transfer in IVF cycles in patients with recurrent miscarriages and implantation failure. *Hum Reprod.* 2014; 29(12):2637-43.
65. Tilburgs T, Roelen DL, van der Mast BJ, de Groot-Swings GM, Kleijburg C, Scherjon SA, et al. Evidence for a Selective Migration of Fetus-Specific CD4+CD25bright Regulatory T Cells from the Peripheral Blood to the Decidua in Human Pregnancy. *J Immunol.* 2008; 180(8):5737-45.
66. Schonkeren D, Swings G, Roberts D, Claas F, de Heer E, Scherjon S. Pregnancy close to the edge: An immunosuppressive infiltrate in the chorionic plate of

- placentas from uncomplicated egg cell donation. *PLoS One*. 2012; 7(3):e32347.
67. Kelkar RL, Meherji PK, Kadam SS, Gupta SK, Nandedkar TD. Circulating auto-antibodies against the zona pellucida and thyroid microsomal antigen in women with premature ovarian failure. *J Reprod Immunol*. 2005; 66(1) 53-67.
68. Woldringh GH, Frunt MHA, Kremer JAM, Spaanderman MEA. Decreased ovarian reserve relates to pre-eclampsia in IVF/ICSI pregnancies. *Hum Reprod*. 2006; 21(11)2948-54.
69. Wennerholm UB, Söderström-Anttila V, Bergh C, Aittomäki K, Hazekamp J, Nygren KG, et al. Children born after cryopreservation of embryos or oocytes: A systematic review of outcome data. *Hum Reprod*. 2009; 24(9):2158-72.
70. Söderström-Anttila V, Tiitinen A, Foudila T, Hovatta O. Obstetric and perinatal outcome after oocyte donation: Comparison with in-vitro fertilization pregnancies. *Hum Reprod*. 1998; 13(2):483-90.
71. Roque M, Lattes K, Serra S, Solà I, Geber S, Carreras R, et al. Fresh embryo transfer versus frozen embryo transfer in in vitro fertilization cycles: A systematic review and meta-analysis. *Fertil Steril*. 2013; 99(1):156-62.
72. Uddin MN, Horvat D, Jones RO, Beeram MR, Zawieja DC, Perger L, et al.

- Suppression of aldosterone and progesterone in preeclampsia. *J Matern Neonatal Med.* 2015;
73. He P, Chen Z, Sun Q, Li Y, Gu H, Ni X. Reduced Expression of 11 β -Hydroxysteroid Dehydrogenase Type 2 in Preeclamptic Placentas Is Associated With Decreased PPAR γ but Increased PPAR α Expression. *Endocrinology.* 2014; 28:1-6.
74. Wu J, Hellerstein S, Lipworth L, Wide L, Xu B, Yu GP, et al. Correlates of pregnancy oestrogen, progesterone and Sex Hormone-binding Globulin in the USA and China. *European Journal of Cancer Prevention.* 2002; 11(3):283-93.
75. Matsuura S, Itakura A, Ohno Y, Nakashima Y, Murata Y, Takeuchi M, et al. Effects of estradiol administration on feto-placental growth in rat. *Early Hum Dev.* 2004; 77(1-2):47-56.
76. Maliqueo M, Echiburú B, Crisosto N. Sex steroids modulate uterine-placental vasculature: Implications for obstetrics and neonatal outcomes. *Frontiers in Physiology.* 2016;26;7:152.
77. Sazonova A, Kllen K, Thurin-Kjellberg A, Wennerholm UB, Bergh C. Obstetric outcome in singletons after in vitro fertilization with cryopreserved/thawed embryos. *Hum Reprod.* 2012; 27(5):1343-50.

78. Ishihara O, Araki R, Kuwahara A, Itakura A, Saito H, Adamson GD. Impact of frozen-thawed single-blastocyst transfer on maternal and neonatal outcome: An analysis of 277,042 single-embryo transfer cycles from 2008 to 2010 in Japan. *Fertility and Sterility*. 2014;101(1):128-33.
79. Pelkonen S, Koivunen R, Gissler M, Nuojuua-Huttunen S, Suikkari AM, Hydén-Granskog C, et al. Perinatal outcome of children born after frozen and fresh embryo transfer: The Finnish cohort study 1995-2006. *Hum Reprod*. 2010; 25(4):914-23.
80. Imudia AN, Awonuga AO, Kaimal AJ, Wright DL, Styer AK, Toth TL. Elective cryopreservation of all embryos with subsequent cryothaw embryo transfer in patients at risk for ovarian hyperstimulation syndrome reduces the risk of adverse obstetric outcomes: A preliminary study. *Fertil Steril*. 2013; 99(1):168-73.
81. Ishihara O, Kuwahara A, Saitoh H. Frozen-thawed blastocyst transfer reduces ectopic pregnancy risk: An analysis of single embryo transfer cycles in Japan. *Fertil Steril*. 2011; 95(6):1966-9.
82. Roque M. Freeze-all policy: is it time for that? *J Assist Reprod Genet*. 2015;32(2):171-6.

83. Pinborg A, Henningsen AA, Loft A, Malchau SS, Forman J, Andersen AN. Large baby syndrome in singletons born after frozen embryo transfer (FET): Is it due to maternal factors or the cryotechnique? *Hum Reprod.* 2014; 29(3):618-27.
84. Maheshwari A, Pandey S, Shetty A, Hamilton M, Bhattacharya S. Obstetric and perinatal outcomes in singleton pregnancies resulting from the transfer of frozen thawed versus fresh embryos generated through in vitro fertilization treatment: A systematic review and meta-analysis. *Fertility and Sterility.* 2012;98(2):368-77 e1-9.
85. Barsky M, St. Marie P, Rahil T, Markenson GR, Sites CK. Are perinatal outcomes affected by blastocyst vitrification and warming? In: *American Journal of Obstetrics and Gynecology.* 2016;215(5):603 e1- e5.
86. Chen Z, Shi Y, Sun Y, et al. Fresh versus Frozen Embryos for Infertility in the Polycystic Ovary Syndrome. *New EnglandJ Medicine.* 2016;11;375(6):523-33;
87. Shih W, Rushford DD, Bourne H, Garrett C, McBain JC, Healy DL, et al. Factors affecting low birthweight after assisted reproduction technology: Difference between transfer of fresh and cryopreserved embryos suggests an adverse

- effect of oocyte collection. *Hum Reprod.* 2008; 23(7):1644-53.
88. Young L. Large offspring syndrome in cattle and sheep. *Rev Reprod.* 1998; 3(3):155-63.
89. Yokochi T, Robertson KD. Dimethyl sulfoxide stimulates the catalytic activity of de novo DNA methyltransferase 3a (Dnmt3a) in vitro. *Bioorg Chem.* 2004; 32(4):234-43.
90. De Geyter C, De Geyter M, Steimann S, Zhang H, Holzgreve W. Comparative birth weights of singletons born after assisted reproduction and natural conception in previously infertile women. *Hum Reprod.* 2006; 21(3):705-12.
91. De Graaff AA, Land JA, Kessels AGH, Evers JLH. Demographic age shift toward later conception results in an increased age in the subfertile population and an increased demand for medical care. *Fertil Steril.* 2011; 95(1):61-3.
92. Instituto Nacional de Estadística.
<http://www.ine.es/jaxiT3/Tabla.htm?t=1579>.
93. Blickstein I. Motherhood at or beyond the edge of reproductive age. *Int J Fertil Womens Med.* 2003; 48(1):17-24.

94. Pal L, Santoro N. Age-related decline in fertility. *Endocrinology and Metabolism Clinics of North America*. 2003;32(3):669-88.
95. van der Hoorn MLP, Lashley EELO, Bianchi DW, Claas FHJ, Schonkeren CMC, Scherjon SA. Clinical and immunologic aspects of egg donation pregnancies: A systematic review. *Hum Reprod Update*. 2010; 16(6):704-12.
96. Staff AC, Benton SJ, Von Dadelszen P, Roberts JM, Taylor RN, Powers RW, et al. Redefining preeclampsia using placenta-derived biomarkers. *Hypertension*. 2013; 61(5):932-42..
97. Berkane N, Liere P, Oudinet J-P, Hertig A, Lefèvre G, Pluchino N, et al. From Pregnancy to Preeclampsia: A Key Role for Estrogens. *Endocr Rev*. 2017; 38(2):123-44.
98. Hertig A, Fort J, Lefevre G, Chabbert-Buffet N, Uzan M, Rondeau E, et al. Soluble endoglin in preeclamptic patients with or without HELLP syndrome. *Am J Obstet Gynecol*. 2010; 202(6):594 e1-4.
99. Jobe SO, Ramadoss J, Wargin AJ, Magness RR. Estradiol-17 and its Cytochrome P450- and Catechol-O-Methyltransferase-Derived Metabolites Selectively Stimulate Production of Prostacyclin in Uterine Artery Endothelial Cells: Role of

- Estrogen Receptor- Versus Estrogen Receptor-. Hypertension. 2013; 61(2):509-18.
100. Bussen S, Bussen D. Influence of the vascular endothelial growth factor on the development of severe pre-eclampsia or HELLP syndrome. Arch Gynecol Obstet. 2011;284(3):551-7.
101. Su EJ, Ernst L, Abdallah N, Chatterton R, Xin H, Monsivais D, et al. Estrogen receptor- β and fetoplacental endothelial prostanoid biosynthesis: A link to clinically demonstrated fetal growth restriction. J Clin Endocrinol Metab. 2011;96(10):E1558-67.
102. Walsh SW, Coulter S. Increased placental progesterone may cause decreased placental prostacyclin production in preeclampsia. Am J Obstet Gynecol. 1989;161(6 Pt 1):1586-92.
103. Oron G, Son WY, Buckett W, Tulandi T, Holzer H. The association between embryo quality and perinatal outcome of singletons born after single embryo transfers: A pilot study. Hum Reprod. 2014; 29(7):1444-51.
104. Oron G, Nayot D, Son WY, Holzer H, Buckett W, Tulandi T. Obstetric and

- perinatal outcome from single cleavage transfer and single blastocyst transfer:
A matched case-control study. *Gynecol Endocrinol*. 2015; 31(6):469-72.
105. Oron G, Sokal-Arnon T, Son WY, Demirtas E, Buckett W, Zeadna A, et al. Extended embryo culture is not associated with increased adverse obstetric or perinatal outcome. *Am J Obstet Gynecol*. 2014; 211(2):165 e1-7.
106. Palermo GD, Cohen J, Rosenwaks Z. Intracytoplasmic sperm injection: a powerful tool to overcome fertilization failure. *Fertil Steril*. 1996; 65(5):899-908.
107. Nangia AK, Luke B, Smith JF, Mak W, Stern JE. National study of factors influencing assisted reproductive technology outcomes with male factor infertility. *Fertil Steril*. 2011; 96:609–614.
108. Sullivan EA, Zegers-Hochschild F, Mansour R, Ishihara O, De Mouzon J, Nygren KG, et al. International Committee for Monitoring Assisted Reproductive Technologies (ICMART) world report: Assisted reproductive technology 2004. *Hum Reprod*. 2013; 28(5):1375-90.
109. OECD (2013), *Health at a Glance 2013: OECD Indicators*, OECD Publishing. http://dx.doi.org/10.1787/health_glance-2013-en.

110. Wang X, Chen C, Wang L, Chen D, Guang W, French J, et al. Conception, early pregnancy loss, and time to clinical pregnancy: A population-based prospective study. *Fertil Steril*. 2003; 79(3):577-84.
111. Wilcox AJ, Weinberg CR, O'Connor JF, Baird DD, Schlatterer JP, Canfield RE, et al. Incidence of Early Loss of Pregnancy. *N Engl J Med*. 1988;28;319(4):189-94.
112. FIVCAT 2011.
http://www20.gencat.cat/docs/salut/Home/EI%20Departament/Estadistiques%20sanitaries/Dades%20de%20salut%20i%20serveis%20sanitaris/Reproduccio%20humana%20assistida/documents/fivcat_2011_resum_resultats.pdf.
113. Preaubert L, Vincent-Rohfritsch A, Santulli P, Gayet V, Goffinet F, Le Ray C. Outcomes of pregnancies achieved by double gamete donation: A comparison with pregnancies obtained by oocyte donation alone. *Eur J Obstet Gynecol Reprod Biol*. 2018; 222:1-6.
114. Phillips JK, Janowiak M, Badger GJ, Bernstein IM. Evidence for distinct preterm and term phenotypes of preeclampsia. *J Matern Neonatal Med*. 2010; ;23(7)622-6.
115. Myatt L, Redman CW, Staff AC, Hansson S, Wilson ML, Laivuori H, et al. Strategy

- for standardization of preeclampsia research study design. *Hypertension*. 2014;63(6):1293–301.
116. Manten GTR, Sikkema MJ, Voorbij HAM, Visser GHA, Bruinse HW, Franx A. Risk factors for cardiovascular disease in women with a history of pregnancy complicated by preeclampsia or intrauterine growth restriction. *Hypertens Pregnancy*. 2007;26(1)39-50.
117. Roberge S, Nicolaides K, Demers S, Hyett J, Chaillet N, Bujold E. The role of aspirin dose on the prevention of preeclampsia and fetal growth restriction: systematic review and meta-analysis. In: *American Journal of Obstetrics and Gynecology*. 2017; 216(2) 110-120 e6.
118. Storgaard M, Loft A, Bergh C, Wennerholm UB, Söderström-Anttila V, Romundstad LB, et al. Obstetric and neonatal complications in pregnancies conceived after oocyte donation: a systematic review and meta-analysis. *BJOG An Int J Obstet Gynaecol*. 2017;124(4):561–72.
119. Adams DH, Clark RA, Davies MJ, De Lacey S. A meta-analysis of neonatal health outcomes from oocyte donation. *J Dev Orig Health Dis*. 2016;7(3):257–72.
120. Malchau SS, Loft A, Larsen EC, Aaris Henningsen AK, Rasmussen S, Andersen

- AN, et al. Perinatal outcomes in 375 children born after oocyte donation: A Danish national cohort study. *Fertil Steril*. 2013;99(6):1637–1643.e3.
121. Nejdet S, Bergh C, Källén K, Wennerholm UB, Thurin-Kjellberg A. High risks of maternal and perinatal complications in singletons born after oocyte donation. *Acta Obstet Gynecol Scand*. 2016;95(8):879–86.
122. Dildy, Jackson, Fowers, Oshiro, Varner, Clark. Very advanced maternal age: Pregnancy after age 45. *Am J Obstet Gynecol*. 1996; 175(3 Pt 1):668-74.
123. Shrim A, Levin I, Mallozzi A, Brown R, Salama K, Gamzu R, et al. Does very advanced maternal age, with or without egg donation, really increase obstetric risk in a large tertiary center? *J Perinat Med*. 2010;38(6):645–50.
124. Clua E, Meler E, Rodríguez D, Coroleu B, Rodríguez I, Martínez F, et al. Obstetric and perinatal complications in an oocyte donation programme. Is it time to limit the number of embryos to transfer? *Gynecol Endocrinol*. 2016;32(4):267–71.
125. Guesdon E, Vincent-Rohfritsch A, Bydlowski S, Santulli P, Goffinet F, Le Ray C. Oocyte donation recipients of very advanced age: perinatal complications for singletons and twins. *Fertil Steril*. 2017;107(1):89–96.
126. Le Ray C, Scherier S, Anselem O, Marszalek A, Tsatsaris V, Cabrol D, et al.

Association between oocyte donation and maternal and perinatal outcomes in women aged 43 years or older. *Hum Reprod.* 2012;27(3):896–901.

127. Tranquilli AL, Biondini V, Talebi Chahvar S, Corradetti A, Tranquilli D, Giannubilo S. Perinatal outcomes in oocyte donor pregnancies. *J Matern Neonatal Med.* 2013;26(13):1263–7.
128. NICE Guideline in Hypertension in Pregnancy.
<https://www.nice.org.uk/guidance/cg107/resources/hypertension-in-pregnancy-diagnosis-and-management-pdf-35109334011877>.
129. LeFevre ML. Low-dose aspirin use for the prevention of morbidity and mortality from preeclampsia: U.S. Preventive Services Task Force recommendation statement. *Ann Intern Med.* 2014; 161(11):819-26.

SUMMARY IN SPANISH

INTRODUCCIÓN:

PREECLAMPSIA:

Epidemiología:

La preeclampsia (PE) es un trastorno hipertensivo del embarazo que afecta entre un 2 y un 8% de las gestaciones, siendo además la causa del 16% de muertes en gestantes. En países en desarrollo su incidencia está aumentando ya que sus factores de riesgo también lo están haciendo, como se verá posteriormente.

Definición:

Clásicamente se define por hipertensión de nueva aparición (tensión arterial > 140/90 mmHG en al menos 2 determinaciones separadas 6h entre sí) asociada a proteinuria (>300mg de proteína en orina de 24h), diagnosticado a o a partir de las 20 semanas de gestación.

Clasificación:

Se clasifica en 2 subclases:

1. PE precoz o de debut temprano: se inicia antes de las 34 semanas de gestación.
Se debe a una mala adaptación inmunológica de la madre al feto que tiene lugar en la placenta, por eso también es llamada “PE placentaria”.

Habitualmente es la cara más severa del síndrome, ya que se asocia a retraso de crecimiento intrauterino y el parto suele ser antes de término (< 37 semanas).

2. PE de debut tardío: se inicia a partir de las 34 semanas y se postula que su origen es por una disfunción cardiovascular materna que secundariamente da lugar a una disfunción placentaria. Por ello se la llama “PE materna”. A pesar que no es tan grave como la anterior, su incidencia supera en 5-20 veces la de la PE precoz. El parto suele ser a término (\geq 37 semanas).

Patogénesis:

La etiología de la PE es una etiología de teorías, y probablemente en la mayoría de los casos no existe una única vía causante del trastorno, sino que se solapan varias de ellas.

El punto en común en todas las hipótesis es que existe una insuficiencia placentaria. El trofoblasto invade la decidua de forma anómala y esto conlleva una deficiente remodelación de las arterias espirales, hecho que deriva a una incorrecta vascularización de la placenta y a su disfunción.

Las dos líneas etiológicas divergen en el desencadenante de la alteración en la invasión del trofoblasto en las primeras fases de la gestación.

Teoría inmunológica:

Postula que la hipoperfusión placentaria es secundaria a una mala adaptación de la madre a agentes fetales.

Las células trofoblásticas expresan HLA-C, muy polimórficos, y que presentan alelos tanto paternos como maternos. El HLA-C es el ligando para el receptor Killer-immunoglobulin (KIR) presente en las células Natural Killers uterinas (uNK). Estas células tienen una función distinta a las NK sistémicas, ya que modulan factores proangiogénicos y endoteliales (como PIGF, VEGF y TGF- β), que a su vez estimulan la neovascularización, y que finalmente promueven los cambios en las arterias espirales responsables de la correcta perfusión fetal.

La combinación de algunos genotipos de KIR maternos (especialmente KIR AA) con algunos alotipos de HLA-C de las células trofoblásticas (particularmente HLA-C2) ocasionan la disfunción de estas uNK, lo que deriva a una alteración de la perfusión placentaria y, finalmente, a alteraciones durante el embarazo, como la PE o el retraso de crecimiento intrauterino en el feto.

Esta teoría respalda la PE de debut temprano, con origen placentario, y que se caracteriza por placentas de pequeño tamaño, con signos histológicos de hipoperfusión, y asociado a restricción del crecimiento fetal. Pero en los numerosos

casos de PE de debut tardío las lesiones de hipoperfusión pueden ser ausentes en la placenta, siendo ésta de tamaño normal con también fetos de peso normal.

Teoría cardiovascular:

Muchos estudios hallan factores de riesgo cardiovascular en pacientes que han sufrido PE, creando una relación causal entre ambos hechos. Otros autores defienden que estos factores de riesgo existen previos a la gestación, y que incluso se asocian a la futura patología cardiovascular de la paciente con más solidez que la PE en sí misma.

Sabemos que el embarazo es un estado donde hay un incremento de las necesidades metabólicas y hemodinámicas. Esta teoría sostiene que, en determinadas pacientes con factores de riesgo cardiovascular, que a menudo son inadvertidos, estas demandas pueden ser excesivas y, secundariamente, esto va a conllevar una hipoperfusión placentaria causante de la PE. Esta disfunción cardiovascular es la etiología de la PE de debut tardío.

Factores de riesgo:

La siguiente lista ordena los factores de riesgo según el riesgo relativo que suponen, de mayor a menor:

1. Historia de PE en gestación previa. Incrementa 7 veces el riesgo de PE.
2. Patología médica de base:

- a. Enfermedad autoinmune. Concretamente el síndrome antifosfolípido aumenta 6 veces el riesgo de desarrollar PE.
 - b. Diabetes Mellitus. Incrementa en 4 el riesgo de PE.
 - c. Hipertensión arterial pre-existente. Dobra el riesgo de padecer PE.
 - d. Enfermedad renal
3. Obesidad: un IMC de > 35 triplica el riesgo de desarrollar PE.
 4. Paridad: la nuliparidad incrementa casi 3 veces el riesgo de PE.
 5. Embarazo múltiple: el embarazo gemelar confiere un riesgo 3 veces superior de PE que el embarazo de feto único.
 6. Historia en familiar de primer grado de PE, hipertensión o diabetes mellitus: casi triplica el riesgo de PE.
 7. Edad materna avanzada: sobre todo a partir de los 40 años el riesgo de PE se dobla.
 8. Cambio de pareja: el embarazo con una nueva pareja incrementa el riesgo de desarrollar PE.
 9. Raza: las gestantes afro-americanas tienen más riesgo que las mujeres blancas.
 10. Modo de concepción: las embarazadas mediante técnicas de reproducción asistida (TRA) tienen más riesgo que las embarazadas de forma natural. Adicionalmente, los embarazos tras donación de gametos tienen un riesgo incrementado respecto a los embarazos con gametos propios de la pareja.

TECNICAS DE REPRODUCCIÓN ASISTIDA I PREECLAMPSIA:

Está extensamente demostrada la asociación entre TRA y el riesgo de PE. La controversia está en la justificación de esta asociación, ya que tanto los factores de riesgo de las pacientes que necesitan TRA, la causa de la infertilidad o la hormonoterapia que reciben, todos son posibles causas del aumento de este trastorno hipertensivo. Además, en reproducción asistida encontramos un aumento de la tasa de embarazo gemelar.

Tandberg comparó embarazos únicos tras TRA con embarazos únicos tras gestación espontánea y concluyó que el aumento en el riesgo de PE tras TRA permanecía incluso ajustando por edad materna y paridad. Una de las hipótesis que explica esta asociación es la que se basa en la infertilidad de estas pacientes; la infertilidad se ha asociado a patrones de metilación anómalos en ADN de placentas de embarazos obtenidos tras TRA comparados con embarazos naturales y, por otro lado, cambios epigenéticos se han asociado con placentas con PE también por patrones anómalos de metilación. La infertilidad, por tanto, puede causar cambios moleculares y genéticos que finalmente desencadenen la PE. Pero la TRA de por sí, por los cultivos embrionarios, los tratamientos hormonales y la transferencia embrionaria, puede ser causa de una alteración en la correcta invasión trofoblástica.

Donación de gametos y PE:

Además de la asociación entre TRA y PE, la donación de gametos también ha sido relacionada con un incremento del riesgo de PE.

La exposición al semen de la pareja masculina o tener hijos con la misma pareja protege de la PE; usar métodos anticonceptivos barrera o el cambio de pareja aumenta la tasa de este síndrome. La explicación la encontramos en las células T regulatorias de la madre: estas células suprimen la respuesta inmunológica materna, permitiendo la adaptación a la alogenicidad del embrión. Diferentes factores en el líquido seminal, como el TGF β o prostaglandinas relacionadas con la PGE, son capaces de inducir la expansión de las células T específicas a antígenos paternos, facilitando así la implantación embrionaria. Para que esto ocurra debe haber contacto entre líquido seminal y mucosa vaginal.

A parte de los antígenos paternos, el HLA-C fetal es menos reconocible por la madre cuando se trata de donación de óvulos (OD), ya que ambos alelos son externos a ella, tanto el del padre como el de la donante de óvulos. Esta alogenicidad conllevará una disfunción de las uNK y finalmente una hipoperfusión placentaria responsable de la PE. Además, se han hallado infiltrados de macrófagos, signos de intervellitis y deciduitis crónica en placentas no complicadas por PE tras donación de ovocitos, que no se han

observado en placentas de pacientes con PE. Estos hallazgos histológicos sugieren una respuesta inmunológica protectora en la madre.

Sumado a lo anterior, algunos autores relacionan la necesidad de recibir óvulos de donante con la PE; se han hallado autoanticuerpos contra la zona pellúcida y las células de la granulosa en pacientes con fallo ovárico, que es una indicación clásica para la recepción de ovocitos. La hipótesis es que estos autoanticuerpos pueden afectar también las células trofoblásticas cuando invaden la decidua. Además, la alteración ovárica de estas pacientes puede a su vez estar relacionada con cambios vasculares o inmunológicos que independientemente predispongan a la PE.

Transferencia de embriones vitrificados y PE:

Son numerosos los estudios que muestran diferentes resultados obstétricos y neonatales tras la transferencia de embriones en fresco comparados con la transferencia de embriones vitrificados. La mayoría concluyen que la transferencia de embriones que han sido criopreservados aumenta la tasa de PE. Algunos autores sugieren que los criopreservantes pueden interactuar con enzimas responsables de la metilación del ADN, alterando así la epigenética embrionaria y conduciendo a una variación de la programación del desarrollo fetal y placentario.

Estos estudios se han realizado siempre con pacientes que realizan ciclos de fecundación in vitro con ovocitos propios (FIV), por tanto se les transfiere el embrión

fresco tras la estimulación ovárica o, en caso de criotransfer, tras una preparación endometrial. Secundario a la estimulación ovárica encontramos un hiperestrogenismo a nivel endometrial. Este ambiente hormonal es, pues, distinto al que encontramos en la transferencia de embriones vitrificados, donde no ha habido estimulación ovárica sino preparación endometrial aislada. Los esteroides gonadales tienen un papel en la correcta vascularización placentaria. Sabemos que hay relación entre variaciones en los niveles adecuados de estrógenos y progesterona con la PE, se cree que precisamente por la alteración de la perfusión corial. Esta distinción entre perfil hormonal endometrial es, pues, un sesgo en estos estudios que concluyen que el criotransfer incrementa la PE, ya que también ello puede justificar las diferencias perinatales entre un tipo de transferencia y otra.

HIPOTESIS:

Hipótesis General:

- Las técnicas de reproducción asistida están relacionadas con la PE, en relación al grado de alogenicidad y la exposición a antígenos paternos de cada una de las técnicas.

Hipótesis secundarias:

- **Proyecto 1:** En embarazos con ovocitos de donante, el riesgo de desarrollar PE es mayor que tras ciclos de FIV con óvulos propios.
- **Proyecto 2:** En embarazos con ovocitos de donante, el riesgo de desarrollar PE es mayor tras la transferencia de embriones criopreservados que tras la transferencia de embriones en fresco.
- **Proyecto 3:** La doble donación de gametos está en aumento en todo el mundo, y las pacientes tiene varios factores de riesgo para PE.
- **Proyecto 4:** En embarazos con doble-donación de gametos, el riesgo de desarrollar PE es mayor que tras solo donación de ovocitos.

OBJETIVOS:

Objetivo general:

- Evaluar la incidencia de PE en embarazos conseguidos tras TRA, especialmente tras donación de gametos, y definir nuevos factores de riesgo.

Objetivos secundarios:

- **Proyecto 1:**comparar el riesgo de desarrollar PE entre pacientes embarazadas tras donación de ovocitos y tras FIV con ovocitos propios.
- **Proyecto 2:**comparar, en embarazos obtenidos con óvulos de donante, el riesgo de desarrollar PE entre transferencia de embriones en fresco y embriones vitrificados.
- **Proyecto 3:**evaluar pacientes que realizan doble donación de gametos, sus características y la tendencia de este tipo de tratamiento durante el periodo de tiempo del estudio.
- **Proyecto 4:**comparar el riesgo de desarrollar PE entre pacientes embarazadas tras doble donación de gametos y tras donación de solo ovocitos.

RESULTADOS:

Proyecto 1:

- Tras el proceso de selección, 11 estudios fueron incluidos para el análisis, con el resultado de una prevalencia de PE del 17,2% en OD comparada con un 5,7% en FIV.
- La OR de PE en OD comparada con FIV fue del 3.12 (2.56-3.85).
- La Q statistic y el valor I^2 confirmaron ausencia de heterogeneidad. Un análisis de influencia demostró también que la exclusión de algún estudio no cambiaría los resultados.
- Tras ajustar por edad materna y embarazo múltiple, la diferencia se mantenía significativa.

Proyecto 2:

- En total se registraron 62 casos de hipertensión gestacional, 36 de PE (dividida en 21 PE pretérmino y 15 PE a término) de un total de 433 pacientes incluidas en el estudio.
- Los casos de criotransfers comparados con transfers en fresco resultaron en una OR de 1.95 [95%CI 0.72, 5.27, $p=0.19$] para PE pretérmino, 0.30 [95%CI 0.04, 2.35, $p=0.25$] para PE a término y 1.45 [95%CI 0.75, 2.81, $p=0.27$] para hipertensión gestacional.

- Tras ajustar por edad materna, primigravidad, IMC y embarazo múltiple, el análisis continuó sin demostrar diferencias significativas entre grupos para todos los resultados analizados.

Proyecto 3:

- Los tratamientos de doble donación de gametos (DD) aumentaron considerablemente durante la década del estudio.
- La media de edad de las pacientes era de 42,2 años, con un 91,7% de pacientes sin hijos. La media de ciclos de TRA previos sin éxito era de 3.21.
- Tras el proceso de “cluster”, se definieron 3 grupos según el estado de relación de pareja: mujeres con pareja masculina, mujeres con pareja femenina y mujeres sin pareja.
- Las tasas de embarazo bioquímico, clínico y embarazo en curso fueron del 58,7%, 50,2% y 49,4% respectivamente, con un 26% de embarazo múltiple.

Proyecto 4:

- En total se registraron 60 casos de hipertensión gestacional, 36 de PE (dividida en 19 PE pretérmino y 17 PE a término) de un total de 433 pacientes incluidas en el estudio.
- Los casos de DD comparados con OD resultaron en una OR de 2.68 [95%CI 1.02, 7.04, p=0.038] para PE pretérmino, 0.26 [95%CI 0.03, 2.01, p=0.17] para PE a término y 1.24 [95%CI 0.64, 2.42, p=0.53] para hipertensión gestacional.

- Tras ajustar por edad materna, primigravidad y embarazo múltiple, la OR para preterm PE permaneció significativa con 3.02 (95%CI 1.11-8.24; p=0.031), y no significativa para PE a término o hipertensión gestacional.

DISCUSIÓN:

Esta tesis estudia la relación entre PE y la TRA, especialmente con gametos donados.

Las TRA son cada vez más accesibles en todo el mundo, como indican los 4,5 millones de ciclos realizados entre 2008 y 2010 a nivel mundial. Especialmente la donación de ovocitos ha incrementado su necesidad, puesto que actualmente las mujeres tienen los hijos en unas edades cada vez más avanzadas, impidiendo en muchos casos la utilización de los ovocitos propios.

Para iniciar el estudio de la relación entre PE y donación de gametos, el metaanálisis se diseñó de forma que se comparó embarazos tras OD con embarazos tras FIV. Excluyendo embarazos espontáneos evitamos el sesgo de la TRA como causa de PE.

El metaanálisis nos confirma que la OD triplica el riesgo de la FIV para desarrollar PE. Habiendo descartado la TRA como causa de PE en estas pacientes, y también habiendo descartado la edad materna y el embarazo múltiple tras el análisis multivariado, la teoría inmunológica gana peso en la etiología de la PE en donación de ovocitos.

Las dos limitaciones del metaanálisis son el no saber la causa de la infertilidad, ya que diferentes etiologías podrían contribuir por sí solas a la fisiopatología de la PE, y no

disponer de la información acerca del momento del debut de la PE, ya que la PE precoz tiene más relevancia a nivel clínico por disponer de tratamientos preventivos como la aspirina.

Examinando los estudios publicados hasta el momento sobre PE y transferencia de embriones vitrificados, nos dimos cuenta que un sesgo común en todos es la comparación de ciclos de FIV con ovocitos propios. En estas pacientes, el ambiente hormonal del endometrio es distinto en TE en fresco y con embriones previamente congelados. Seleccionando esta población no podemos analizar con detalle el efecto de la vitrificación en los embriones ya que, a parte de esta diferencia, los ciclos en fresco tienen un endometrio influido por la hiperestimulación ovárica que es diferente a la preparación endometrial que encontramos en los ciclos donde se transfieren embriones vitrificados. En este sentido la donación de ovocitos es un modelo ideal ya que la preparación endometrial es la misma cuando hacemos TE de embriones frescos o congelados.

Hasta el momento, y que nosotros sepamos, es el primer estudio que compara los trastornos hipertensivos del embarazo en OD tras TE en fresco y congelado, sin hallar diferencias entre grupos en ninguno de los resultados analizados.

Nuestro estudio va en acorde con la actual teoría etiológica de la PE que asigna más peso a la vascularización de la placenta que a las características del embrión. Hemos comprobado que, con el mismo ambiente hormonal a nivel endometrial, las probabilidades de desarrollar PE son iguales cuando transferimos embriones previamente congelados o no. Consecuentemente podemos concluir que un adecuado nivel hormonal en el endometrio es imprescindible para una correcta placentación, y que los protocolos que actualmente usamos para preparar el endometrio para la transferencia de embriones tienen un efecto deletéreo en este proceso, de allí los resultados encontrados en los estudios publicados hasta el momento con pacientes que realizan FIV con óvulos propios.

La principal limitación de este estudio es el uso de un cuestionario para la recogida de datos. Aunque se pidió a las pacientes que lo rellenaran con ayuda de su obstetra, no podemos negar un posible sesgo de recuerdo, aunque dada la importancia de la PE y la hipertensión gestacional, sería poco probable, e independiente del tipo de tratamiento.

Una observación de nuestro estudio sobre pacientes realizando doble-donación de gametos fue que a pesar que la incidencia de este tratamiento aumentó durante la década del estudio, la proporción de mujeres con pareja masculina se mantuvo

constante, y en cambio la de las mujeres sin pareja o con pareja femenina aumentó considerablemente. El aumento relativo de estos dos grupos se puede deber a que, estas pacientes, a partir de cierta edad y con el fallo ovárico asociado, van a requerir siempre de una doble-donación para conseguir embarazo. En cambio, la ICSI es una herramienta que solventa un porcentaje muy alto de infertilidad masculina, de forma que cuando existe pareja masculina la donación de semen puede no ser necesaria. Como ya se ha comentado, el aumento de la edad en la maternidad, junto con una mayor aceptación social de la familia monoparental o con madres homosexuales, ha conllevado un incremento absoluto y relativo de estas pacientes llevando a cabo tratamientos con doble-donación de gametos.

Revisando los factores de riesgo para PE, observamos que las pacientes de doble-donación tienen varios de ellos, como puede ser una edad materna avanzada, primigravidad, el uso de TRA para conseguir la gestación, el uso de gametos donados y una tasa alta de embarazo gemelar. A pesar que este tipo de tratamientos está aumentando a nivel mundial, falta literatura acerca de los resultados obstétricos en estas pacientes. Este es, hasta el momento y que nosotros sepamos, el primer estudio amplio que describe las características de las pacientes y los resultados de la doble-donación.

Basándonos en que el deficiente reconocimiento inmunológico es causa de PE, sería entonces razonable asumir un aumento de esta patología en la DD, puesto que en estos casos la madre no ha estado expuesta a ninguno de los antígenos fetales ni ha estado en contacto con el espermatozoides del donante. En el cuarto proyecto hallamos un aumento de PE precoz en DD respecto a la OD, un hallazgo nunca antes descrito en la literatura, pero no de PE a término ni de hipertensión gestacional. La explicación sobre estos resultados la podemos encontrar en la diferente etiología de la PE precoz y tardía: mientras que la PE tardía tiene el origen en un sistema cardiovascular materno alterado, la precoz se debe a una mala implantación corial secundaria a una mala adaptación inmunológica de la madre al embrión. Por tanto, en los embarazos tras doble donación de gametos, es lógico que sea la PE precoz la que incremente. Esto es especialmente relevante ya que las consecuencias de la PE precoz son más graves, tanto para la gestante como para el feto, pero además su diagnóstico precoz puede permitir iniciar medidas preventivas. El inicio de la aspirina a dosis bajas antes de la semana 16 de embarazo se ha demostrado eficaz en la reducción de la incidencia de la PE precoz.

Este estudio comparte la misma limitación que el proyecto 2, que es que los datos han sido recogidos mediante un cuestionario enviado a las pacientes, pero por el mismo motivo que anteriormente, no debería alterar los resultados hallados. Además, nunca antes se habían descrito tantos casos de DD, y como segundo punto fuerte solo se

incluyeron transferencias de embriones en fresco, evitando el posible sesgo de incluir TE en fresco y vitrificados.

En conclusión, las pacientes embarazadas tras donación de gametos, y sobre todo tras doble donación, deben ser informadas y conscientes del alto riesgo que tienen de desarrollar PE, al igual que sus médicos. Tras la transferencia de embriones vitrificados, estas pacientes que han recibido óvulos donados siguen teniendo un riesgo elevado, pero no mayor que las que han recibido embriones frescos. Estas embarazadas deben recibir un control gestacional estricto para poder ser identificadas, iniciar medidas preventivas cuanto antes y, en caso de desarrollo del síndrome hipertensivo, recibir un tratamiento adecuado. Varios estudios demuestran que la prematuridad y el bajo peso al nacer en embarazos tras donación de ovocitos mejoran al ajustar por PE. Esto significa que parte de estas complicaciones neonatales son consecuencia de la inducción prematura del parto que el obstetra lleva a cabo para tratar de la única forma definitiva que hay la PE, que es finalizando la gestación. Identificando a las pacientes de riesgo e iniciando medidas preventivas como la administración de aspirina antes de las 16 semanas, pues, podríamos evitar resultados perinatales adversos para madre y para el neonato. Además, sabiendo que el ambiente hormonal

tiene un papel en la PE, el desarrollo de protocolos con diferentes terapias hormonales sustitutivas para el endometrio podría disminuir la incidencia de este síndrome.

CONCLUSIONES:

- La OD está asociada a una mayor incidencia de PE que la FIV, incluso ajustando por edad materna y embarazo múltiple.
- Estos datos sugieren que en OD, el origen de la PE está relacionada con una mala adaptación de la madre a antígenos embrionarios.
- En OD, la transferencia de embriones previamente vitrificados no confiere más riesgo de desarrollar PE que la transferencia de embriones frescos.
- El ambiente hormonal en el endometrio probablemente tiene un papel en la patogénesis de la PE en TRA.
- Los tratamientos de doble donación de gametos están incrementando, con resultados positivos para pacientes seleccionadas
- Las pacientes embarazadas tras doble donación de gametos tienen múltiples factores de riesgo para desarrollar PE.
- La DD está asociada a una mayor incidencia de PE pretérmino que la OD, pero no de PE a término o hipertensión gestacional, incluso ajustando por edad materna, primigravidad y embarazo múltiple.
- En reproducción asistida, cuantos más gametos donados se reciben, más riesgo de desarrollar PE.

