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ΠΡΟΓΡΑΜΜΑ ΜΕΤΑΠΤΥΧΙΑΚΩΝ ΣΠΟΥΔΩΝ
«ΕΡΕΥΝΑ ΣΤΗ ΓΥΝΑΙΚΕΙΑ ΑΝΑΠΑΡΑΓΩΓΗ»

# Infertility factors, ART protocols and their relation to neonates and pregnancy complications: A narrative review of the literature.

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Αθήνα, 2020

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# Περίληψη


## Α) Σκοπός

Η τεχνολογία υποβοηθούμενης αναπαραγωγής (ART) έχει πλέον εισέλθει στην καθημερινή ρουτίνα της μαιευτικής πρακτικής, ενώ περισσότερα από 7 εκατομμύρια παιδιά εκτιμάται ότι έχουν γεννηθεί μέσω αυτής, τη στιγμή της μελέτης. Σκοπός αυτής της έρευνας είναι η εκτεταμένη καταγραφή των τεχνικών υποβοηθούμενης αναπαραγωγής, οι παράγοντες υπογονιμότητας που μπορούν να οδηγήσουν το ζευγάρι να υποβληθεί σε ART και οι συνέπειες αυτών των πρακτικών στους απογόνους. Επιπλέον, ερευνήσαμε τη δημοσιευμένη βιβλιογραφία για τα εμπορικά μέσα καλλιέργειας που χρησιμοποιούνται στις τεχνικές ART, και να προσπαθήσουμε να βρούμε τυχόν διαφορές, αν υπάρχουν. Για να αξιολογήσουμε όλες αυτές τις πτυχές, διενεργήσαμε μια ανασκόπηση της βιβλιογραφίας.

## Β) Μέθοδοι

Αυτή η ανασκόπηση πηγαίνει από την αρχή των τεχνολογιών υποβοηθούμενης αναπαραγωγής, από τα τέλη της δεκαετίας του '70 έως τα τέλη της δεκαετίας του '10. Μια ηλεκτρονική έρευνα πραγματοποιήθηκε στο PubMed σχετικά με την IVF (κλασσική εξωσωματική γονιμοποίηση) και την ICSI (Ενδοκυτταροπλασμική έγχυση σπέρματος), συμπεριλαμβανομένων των αποτελεσμάτων από το 1978 έως τον Αύγουστο του 2019. Οι λέξεις-κλειδιά και οι συνδυασμοί τους που χρησιμοποιήθηκαν περιελάμβαναν: IVF, ICSI, culture media, in vitro fertilization, ART conceived children, ART offspring, epigenetics, embryo culture, infertility, ART outcomes, infertility treatment, woman infertility. Συμπεριλήφθηκαν συνολικά 47 μελέτες, εκ των οποίων οι 9 ήταν προοπτικές και 12 αναδρομικές μελέτες κοόρτης, 4 ελεγχόμενες τυχαιοποιημένες μελέτες και 23 συστηματικές ανασκοπήσεις. Το κύριο αποτέλεσμα που μελετήθηκε ήταν παράγοντες αποτυχίας της εγκυμοσύνης κατά τη διάρκεια της ART. Τα δευτερογενή αποτελέσματα ήταν τα ποσοστά εμφύτευσης, η υγεία των νεογνών και οι επιπλοκές της εγκυμοσύνης.

## Γ) Αποτελέσματα

Η ανάλυση των δεδομένων δείχνει ότι οι εγκυμοσύνες ART έχουν φτωχότερο περιγεννητικό αποτέλεσμα από τις εγκυμοσύνες που προκύπτουν με φυσική σύλληψη. Επιπλοκές που μπορεί να προκύψουν επιβαρύνουν την έγκυο και / ή τον απόγονο. Ωστόσο, η βιβλιογραφία φαίνεται να συμφωνεί ότι η αιτία αυτών των επιπλοκών είναι κυρίως η ίδια η υπογονιμότητα και ο αιτιολογικός της παράγοντας, και όχι η διαδικασία της εξωσωματικής γονιμοποίησης. Ταυτόχρονα, όμως, η ART κατηγορήθηκε για αυξημένη συχνότητα εμφάνισης επιγενετικών συνδρόμων και διαταραχών, κάτι που επιβεβαιώνεται στην ανασκόπηση μας. Μία περιοχή που φαίνεται να είναι υπομελετημένη είναι τα μέσα καλλιέργειας που χρησιμοποιούνται στη διαδικασία της ART. Καθώς η παραγωγή εμπορικών μέσων καλλιέργειας είναι σχετικά νέα, βρήκαμε ένα μικρό δείγμα μελετών και δοκιμών πάνω σε αυτά,  καθιστώντας το ένα ακόμα ασαφές θέμα της επιστήμης της εξωσωματικής γονιμοποίησης.

## Δ) Συμπεράσματα

Όταν ένα ζευγάρι καταλήγει στην ART, θα πρέπει να ενημερωθεί ότι εάν έχουν επιτυχή εμφύτευση, η εγκυμοσύνη που θα προκύψει θα είναι υψηλού κινδύνου τόσο για τη γυναίκα όσο και για το έμβρυο. Ο λόγος γι 'αυτό είναι η ίδια η υπογονιμότητα ως επί το πλείστον, ενώ και οι ιατρογενείς χειρισμοί κατά τη διάρκεια της διαδικασίας διαδραματίζουν ένα δευτερεύοντα ρόλο. Οι παράγοντες υπογονιμότητας πρέπει πρώτα να εξαλειφθούν όσο το δυνατόν περισσότερο πριν ξεκινήσει ένας κύκλος εξωσωματικής γονιμοποίησης.

# Abstract

## A) **Purpose**

The purpose of this research is an extensive recording of the assisted reproduction techniques, the infertility factors that may lead the couple to undergo ART, and the effects of these practices on the offspring. In addition, we also searched the published literature about the commercial culture media used in ART techniques, to try and find any differences, if any exist.

## B) **Methods**

This review goes from the beginning of the assisted reproductive technologies. A computerized search was performed in PubMed and Embase. The keywords and their combinations used included: IVF, ICSI, culture media, in vitro fertilization, ART conceived children, ART offspring, epigenetics, embryo culture, infertility, ART outcomes, infertility treatment, maternal infertility.

## C) **Results**

Analysis of current data shows that ART pregnancies have poorer perinatal outcomes than pregnancies resulting from natural conception. Complications that may arise burden the pregnant and / or offspring. However, the literature seems to agree that the cause of these complications is mainly the infertility itself and its causative agent, rather than the process of assisted reproductive technology.

## D) **Conclusions**

When a couple ends up in ART, they should be informed that if they have a successful implantation, the pregnancy which will result is going to be a high risk for both the woman and the embryo.

# CONTENTS

# 1. Introduction

## A) Assisted reproductive technology

Dr. Robert G. Edwards and Dr. Patrick Steptoe opened a new era in medicine when they successfully fertilized a human egg, cultivated it in a Petri dish, and transferred it to the womb of a woman and nine months after, the first "baby of the tube" was born [Steptoe PC and Edwards RG. 1978]. This revolutionary achievement has allowed millions of infertile couples to conceive and women to have babies even after menopause.

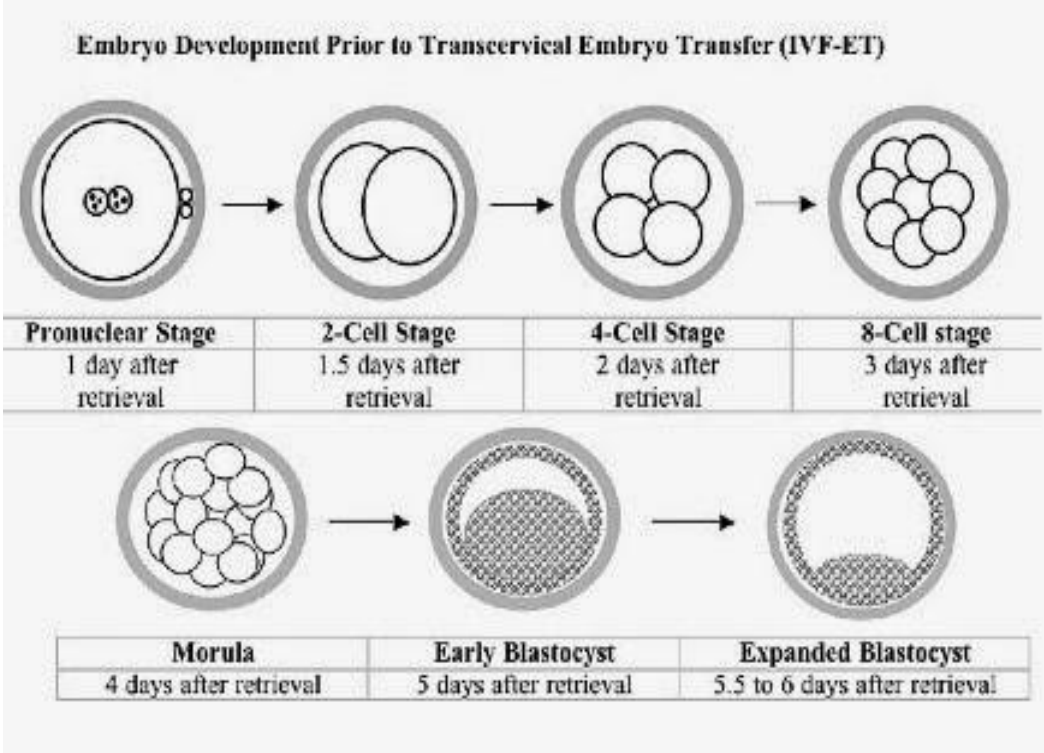
Initially, In vitro fertilization was discovered in order to study and further investigate the treatment of female infertility. More than 40 years have passed since then, however, the basic stages of In Vitro Fertilization (IVF) remain the same. These include ovarian stimulation, either with GnRH agonists (older mainly) or with GnRH antagonists and then administration of β-chorionic gonadotropin (β-HCG) to mature the ovarian follicles [N.S. Macklon et al. 2006]. Subsequently, transvaginal oocyte retrieval is performed with the help of ultrasound, usually 36 hours after administration of β-HCG [J. Cohen et al. 2005]. Fertilization of the oocytes is then carried out on culture medias that attempt to mimic the endometrial environment, which are different for each stage, from the zygote stage to the blastocyst stage [Lane M et al. 2007].

At this stage of in vitro fertilization, we find the two major differences between assisted reproductive techniques. In In Vitro Fertilization, mature oocytes fuse with sperm, followed by fertilization of the oocytes through cleavage of the zona pellucida by the spermatozoa itself [Edwards RG et al. 1969].

The other ART technique is intracytoplasmic sperm injection (ICSI) and is the latest breakthrough in the field of infertility. Thanks to Palermo [Palermo et al. 1992,2017], the first successful pregnancy with ICSI was achieved in 1992 and has since become the main technique of artificial fertilization, especially in recent years [de Mouzon j et al. 2010]. The main reason a couple will be referred to ICSI is male infertility and unexplained infertility. During ICSI, a spermatozoon is aspirated after its tail has been removed and then inserted into the oocyte’s cytoplasm by micropipette [Lanzendorf SE et al. 1988]. New

techniques have been researched and developed over the last 25 years, since the beginning of ICSI, which are still in the experimental stage to optimize the results, as shown by the systematic review of Patrizia Rubino [Patrizia Rubino et al. 2016].

After successful fertilization of the oocyte by either of the two methods, transcervical embryo transfer is followed, either in the zygote stage or in the blastocyst stage [Martins W et al. 2017].




Embryo transfer can be performed in the same menstrual cycle, otherwise the embryo is cryopreserved for the next menstrual cycle.

Cryopreservation plays an important role in reproductive medicine. The technique allows storing excess human oocytes or embryos to be used eventually in subsequent IVF cycles. In addition, this technique helped to solve a number of clinicals as well as technical problems of conventional IVF cycles. First, the transfer of multiple embryos to achieve a pregnancy is avoided and the related risk of multiple pregnancies is abolished or reduced. Second, cryopreservation reduces the number of ovarian stimulation cycles. Third, cryopreservation allows delaying the embryo transfer during an IVF cycle to prevent ovarian hyperstimulation syndrome and optimizing endometrial preparation . Fourth, embryo or oocyte cryopreservation preserves the reproductive capacity in women who are at risk of infertility because of gonadotoxic medical treatments or who wish to postpone their reproductive plans. [Levi-Setti PE et al. 2016].

Contemporary studies have shown that freezing of embryos in women who had a very good response to ovarian stimulation (> 15 oocytes per oocyte retrieval) and then had the embryo transfer to subsequent menstrual cycles, where hormone levels would have returned to normal, had the best implantation rates [Felipe C Dieamant et al. 2017].

Freezing of zygotes or embryos is done by two techniques. The first is freezing by the method of slow cooling and the second is freezing by the method of vitrification. In recent years, the technique of vitrification has been established, as it has been shown through studies that the in vitro cycles following this method have had the best perinatal results [Loutradi KE et al. 2008]. In recent years, ART clinics have added assisted hatching. This technique causes a small hole in the zona pellucida of the fetus, either by chemical erosion or by a special laser adapted to the microscope, to facilitate the implantation of the embryo into the endometrium. A recent meta-analysis by Zeng M [Zeng M et al. 2018], shows that cryopreserved embryos that have undergone assisted hatching had better implantation rates but not increased live birth rates and miscarriage rates.

## B) Methods

### i) Aims

The aim of this study was to further investigate the reason why conceived pregnancies and children born from ART had more adverse results, in comparison to naturally conceived pregnancies. The search was focused on the major parental infertility factors as well as the major steps involved in ART and the effects that both of them had on the offspring.

### ii) Search and inclusion criteria

A literature review was conducted, using Pubmed and Embase databases pretraining to studies from 1978 until 2019. No filters for the type of study or language were used, but only studies in human population were included. The following terms and keywords as well as combinations of them were used: IVF, ICSI, culture media, in vitro fertilization, ART conceived children, ART offspring, epigenetics, embryo culture, infertility, ART outcomes, infertility treatment, maternal infertility. 151 studies were picked out and a total of 48 studies were included, from which 9 were prospective and 12 retrospective cohort studies, 4 randomized controlled trials and 23 systematic reviews. The primary outcome studied was failure factors of pregnancy during ART. Secondary outcomes were implantation rates, health of neonates and pregnancy complications.

# 2. Failure Factors of ART

## A) Intrauterine Environment

One of the most important determinants of success in the ART process is the intrauterine environment, as this will host the fetus for the following months of pregnancy. Studies have shown that insufficient receptivity of the endometrium, and generally the endometrial environment, is responsible for one third of implantation failures, whereas the embryo is responsible for the other two thirds [Macklon NS et al. 2006].

After being fertilized in the fallopian tube, the zygote reaches the endometrium. Subsequently, the former penetrates the latter and together they form a unique structure, resulting in an embryo, through a series of complex orchestrated procedures [Zhang S et al. 2013]. The above processes occur naturally in most women, but in many cases, they are interrupted by disorders in the endometrial environment. These disorders can be anatomical, microbiological, hormonal, immunological or vascular. Initially, endometrial thickness measured by ultrasound, on the day of administration of β-chorionic gonadotropin, was used as an indicator of endometrial susceptibility. A meta-analysis of Momeni M, showed that there is indeed a difference in the endometrium of women who managed to become pregnant from those who failed, by the order of 0.4 mm. The same authors conclude that there may be some relationship between endometrial thickness and successful pregnancy, but implantation is particularly complicated to relate only to endometrial thickness. [Momeni M et al. 2011]

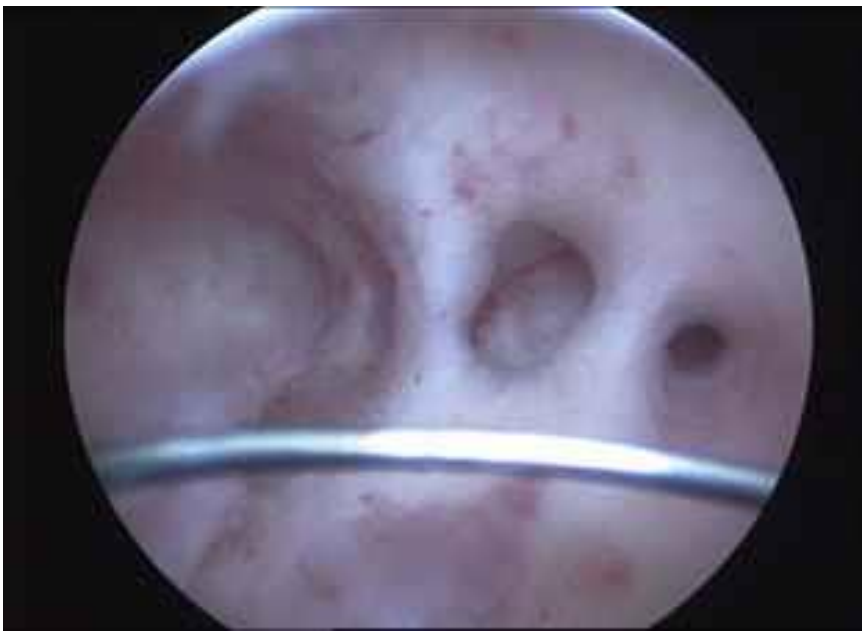
Another factor that has been studied is the endometrium itself. The triple layer of the endometrium when viewed by ultrasound appears to be superior to isoechogenic or hyperechogenic [Zhao J et al. 2012]. Endometrial volume and endometrial blood flow have also been studied by Yaman C [Yaman C et al. 2012], which conclude that the interaction of the embryo with the endometrium itself is more important than the two parameters examined. Concerning the thin endometrium and its quality, many drugs have been used to improve it. The most important of these are hormonal, estrogenic and GnRH agonists and vasomotor agents. The quality of the endometrium seems to be mainly influenced by the woman's age. However, the increase in successful implantations and pregnancies following the administration of the aforementioned agents is small and further research is needed [Lebovitz O et al. 2014].

*Triple layer endometrium*

In women with unexplained infertility, a common finding is intrauterine polyps (15.6-32%) [Hatasaka H. et al. 2011]. Polyps are benign endometrial tumors and are estrogen-dependent [Taylor LJ et al. 2003]. The relationship of polyps to infertility has long been known by Wallach [Wallach EE. 1972], and hysteroscopic polypectomy is the gold standard before an ART cycle [Kodaman PH. 2016], as it has been shown that removing them increases the chances of successful pregnancy.

Another infertility factor is endometrial adhesions and Asherman syndrome. They are usually of medical origin, with 90% of them being due to new pregnancies undergoing dilation and curettage, as their endometrium is very sensitive during the first months of pregnancy [Schenker JG et al. 1982]. However, any medical intervention in the endometrium, as well as tuberculosis of the uterus, can cause adhesions. Here again, the gold standard of treatment is hysteroscopic symphysiolysis, by inserting an intrauterine contraceptive device (without progesterone) or a Folley pediatric catheter into the uterus, to prevent recurrence of adhesions [Dreisler E et al. 2019].

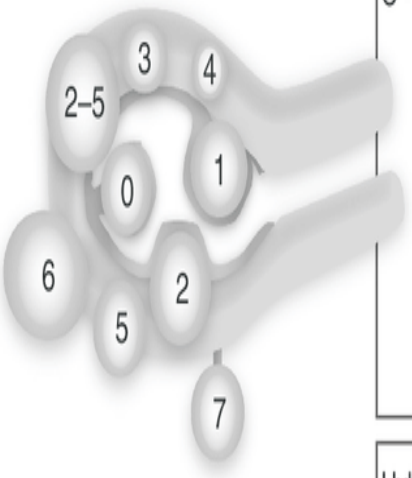
*Endometrial adhesions*



Other benign tumors of the uterus are the leiomyomas or fibroids, which originate from the myometrium. Uterine fibroids occur in up to 30% of reproductive age women and are more common in Afrocarribean women. Although most women affected with fibroids are fertile, fibroids may interfere with fertility secondary to anatomical distortion and alterations to the uterine environment , with the effect being dictated largely by the location and size of the fibroid [Sunkara SK et al. 2010] . They are subdivided into intramural, submucosal and subserosal, however, only the first two have been associated with infertility [Pritts EA et al. 2009, Erdinç Sarıdoğan and Ertan Sarıdoğan 2019]. In addition to their position, the size of the leiomyomas plays an important role in implantation, where leiomyomas over 3 cm appears to have a negative effect on pregnancy [Christopoulos G et al. 2017]. Surgical removal of intramural and submucosal leiomyomas, which influence endometrial morphology, have a positive effect on implantation [Fortin CN et al. 2019]. In addition, the size of the leiomyomas can be reduced by various procedures such as embolization of the uterine arteries, administration of GnRh agonists, danazol, mifepristone, raloxifene, ulipristal, aromatase inhibitors, and radiofrequency ablation [Galliano D et al. 2015]. However, none of these methods has been well studied in relation to infertility.

Federation of Gynecology and Obstetrics (FIGO) classified the fibromas by their location in the uterus. Below, it is demonstrated the classification of FIGO. As we already discussed, fibroids from 0 to 5 are linked to infertility while 6 and 7 seems to be harmless. Large fibroids are usually included in 2 or more categories and called hybrid. [Shannon et al. 2017]

*Classification of fibroids by FIGO, 2017*

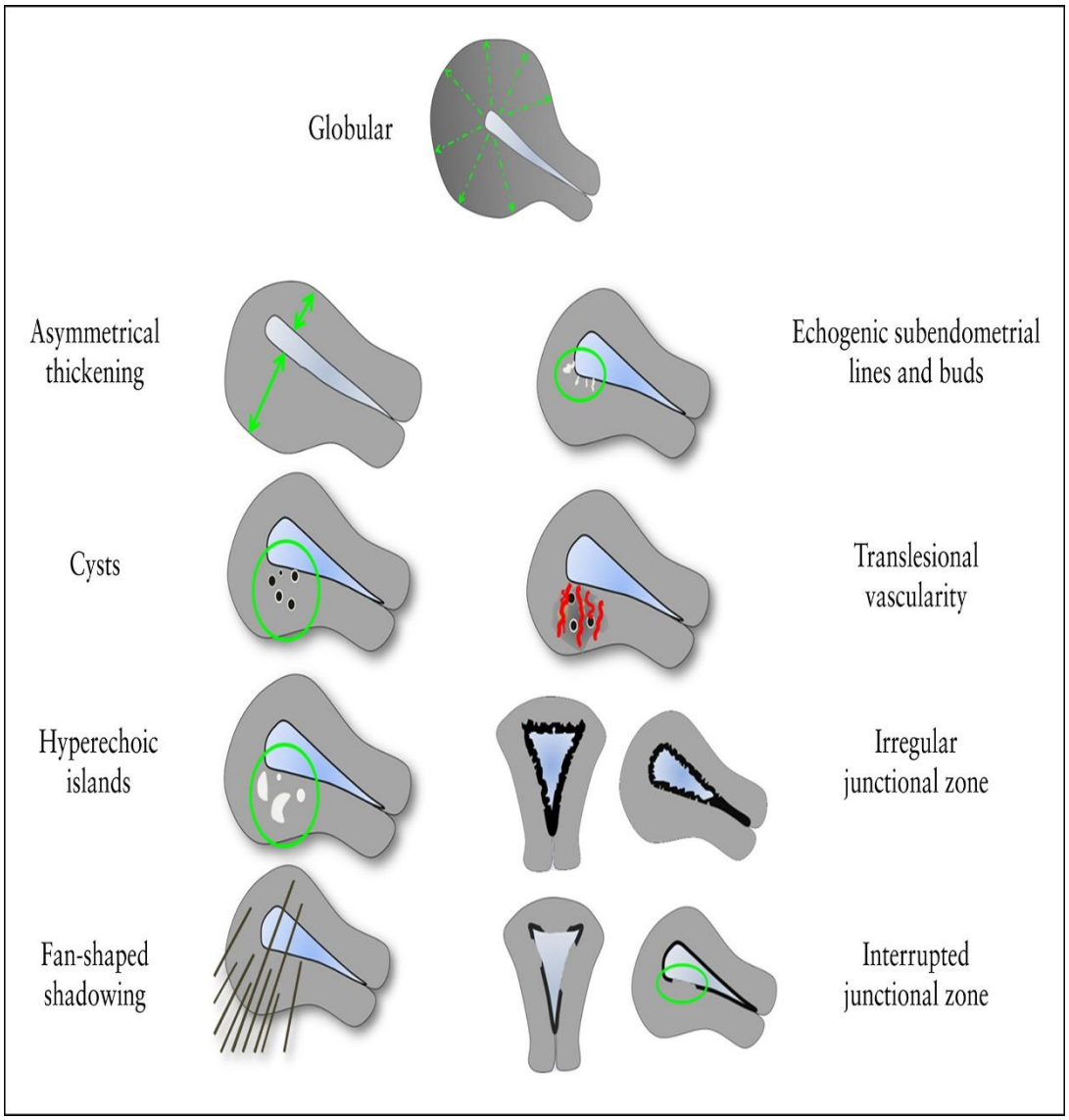



| | | |
|---|---|---|
| SM - Submucosal | 0 | Pedunculated intracavitary |
| | 1 | >50% intramural |
| | 2 | ≥50% intramural |
| O - Other | 3 | Contacts endometrium; 100% intramural |
| | 4 | Intramural |
| | 5 | Subserosal ≥50% intramural |
| | 6 | Subserosal <50% intramural |
| | 7 | Subserosal pedunculated |
| | 8 | Other (specify e.g., cervical, parasitic) |

| | | |
|---|---|---|
| Hybrid leiomyomas | 2-5 | Submucosal and subserosal, each with less than half the diameter in the endometrial and peritoneal cavities, respectively. |

Adenomyosis is yet another clinical entity that may cause infertility and failure in ART efforts. Adenomyosis is a heterogeneous gynaecologic condition with a range of clinical presentations, the most common being heavy menstrual bleeding and dysmenorrhea but patients can also be asymptomatic. [Dueholm M et al. 2017]. The most challenging aspect of adenomyosis is the differential diagnosis. Transvaginal ultrasound as well as MRI are the main tools for diagnosing adenomyosis, with MRI being superior [Tasuku Harada et al. 2016]. The most common imaging findings are listed in the picture below.

*Imaging findings in adenomyosis*



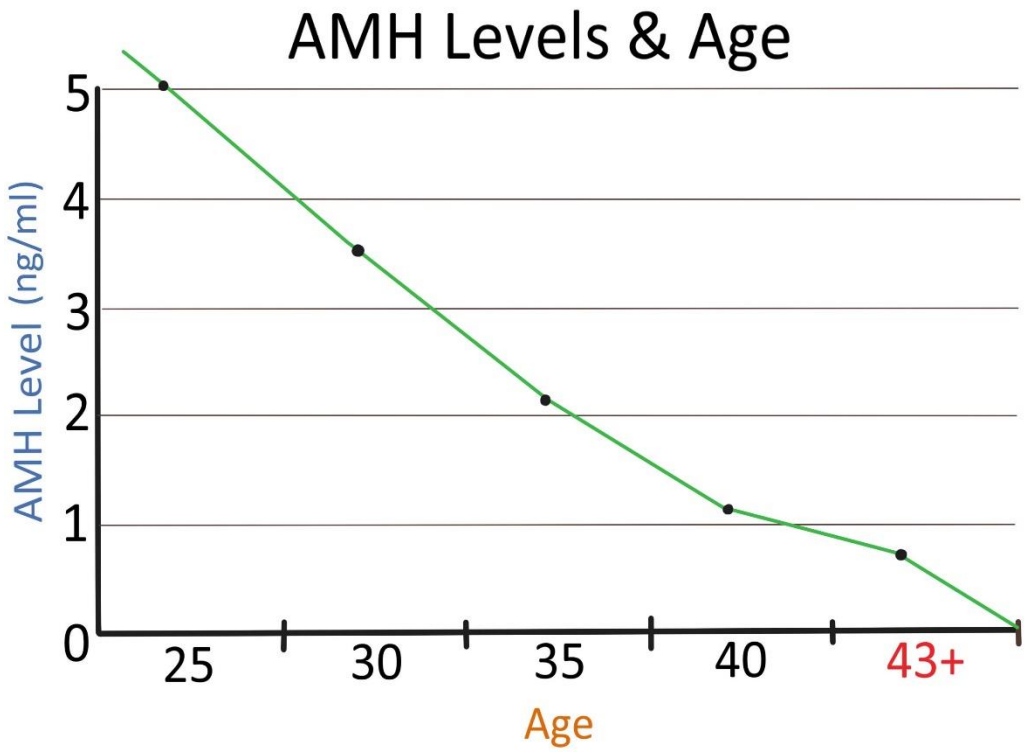
Adenomyosis can be diffuse or focal, which both affect the fertility of the patient. Treatment is the same for both occasions, which targets to reduce the size of the lesion. The golden standard treatment of adenomyosis is surgical excision. [Vannuccini S et al, 2019].

Younes G et al, in their meta-analysis of ART techniques and adenomyosis, conclude that adenomyosis has very negative effects on the process of implantation and generally, in pregnancy [Younes G et al. 2017]. They also recommend either surgical excision of the adenomyoma or administration of GnRh agonists to reduce the size of the lesion, as these two techniques appear to have a positive effect on the outcome of pregnancy.

## B) Ovarian function reserve

One reason that many couples resort to IVF, is the gradual trend of modern society to delay childbearing, especially in Western countries [Mills M et al. 2011]. More specifically, the average age at which a couple decides to have a child has increased, which may result in lower likelihood of achieving pregnancy naturally [Schmidt L et al. 2012]. Moreover, women have to face menopause which men do not [Ramasamy R et al. 2015]. Prior to this, however, it has been shown that their fertility declines exponentially after the age of about 35 [Ubaldi FM et al. 2019].

The main reason is the rapid decrease in ovarian function and reserve. We can measure this reduction today through the Anti-Müllerian Hormone - AMH [Gleicher N et al. 2011]. Values (ng/ml) below 1 mean low ovarian function, 1 to 7 is normal, and above 7 usually is connected to polycystic ovaries, with or without the syndrome. AMH is produced by the follicular granulosa cells and the major physiological role of AMH in the ovaries appears to be limited to inhibiting the early stages of follicular development [Visser JA et al. 2005].




AMH in ART is measured for two reasons. The first is to personalize ovarian stimulation, aiming to reduce the odds of ovarian hyperstimulation syndrome [Pilsgaard F et al. 2017]. Ovarian hyperstimulation syndrome (OHSS) is an iatrogenic condition characterized by abdominal pain and generalized edema due to the increased vascular permeability caused by the increased circulation of estrogens, prior to oocytes retrieval [Selma M et al. 2017]. The second reason is to examine the ovarian reserve before a cycle of ART. In women over 36 years AMH is a prognostic factor in pregnancy outcome, with AMH <1 meaning reduced chances of achieving a successful pregnancy [Gomez R et al. 2016]. However, a low hormone level, even at below the limit of detection, cannot rule out the success of an ART cycle. Also, in the same study, it was found that in women under 35, AMH had no predictive value, as all women had an increased chance of successful pregnancy. Through this result, they conclude that the age of a woman, and therefore her ovaries and oocytes, plays a decisive role in pregnancy and the perinatal outcome. Liu K et al, recommend oocyte donation in cases of increased ovarian age, as it is the only treatment for recurrent ART failures [Liu K et al. 2011].

## C) Infertility and pregnancy complications

Assisted reproductive technology was introduced into medical practice with little formal evaluation of its effects on maternal and fetal well-being [Ombelet W. et al. 2010]. Earlier reviews have suggested that ART pregnancies are associated with higher risks. However, there have been recent advances in the way ART is done, leading to some controversy as to whether ART singletons are associated with higher obstetric risks. In particular, along with the increasing implementation of infertility as the main reason of high-risk pregnancies, more and more researchers are interested in this topic.

The underlying mechanisms involved in the association between ART and poor outcomes in the singleton pregnancies are uncertain. One possible explanation is that ART procedures or maternal factors associated with infertility or a combination of these bring about increased risks of adverse outcomes in the ART pregnancies. Some studies have shown that factors associated with ART procedures themselves, such as the medications used to induce ovulation or to maintain the pregnancy in the early stages, the culture media composition, the length of time in culture, the freezing and thawing of embryos, the potential for polyspermic fertilization, the delayed fertilization of the oocyte, altered hormonal environment at the time of implantation, and the manipulation of gametes and embryos or a combination of these, may increase the risk of adverse outcomes [ Qin J et al. 2016]. Although, there have been a plenty of papers, linking preexisting infertility with increased pregnancy complications.

In the table below is a summary of the most common pregnancy complications, linked with infertility factors usually found in ART pregnancies [Table 1].

Zore T et al, in their literature review, focus on the complications of pregnancy in infertile women who gave birth through ART, concluding that the main cause of complications of ART-subjected pregnancies is infertility itself and the reasons that cause it [Zore T. et al. 2017].

Specifically, in a cohort study of 459,623 pregnant women, Luke B et al, divided these women into three groups, infertile, semi-fertile and fertile. The infertile group was treated with IVF, and the semi-infertile group by other methods. The offspring born to the infertile and semi-fertile groups had a much higher risk of low birth weight (LBW <2500gr) as well as a very low birth weight (VLBW <1500gr). The obstetric complications that were statistically increased for the 2 reported groups were postpartum hemorrhage (PPH), placental abnormalities, hospitalization during pregnancy, increased incidence of caesarean section, and premature birth (PTB) and very premature birth (VPTB), in relation to the fertile group [Luke B et al. 2017].

Sha T et al, performed a meta-analysis of pregnant women with a history of polycystic ovary syndrome (PCOS), a common infertility factor. These women underwent IVF, ovulation induction or conceived naturally. Women with PCOS had higher risks of miscarriage, ovarian hyperstimulation syndrome, gestational diabetes mellitus, pregnancy-induced hypertension, PTB and large-for-gestational-age babies (LGA). [Sha T et al. 2019].

Kawwass J. et al, showed that women undergoing IVF due to fallopian tube factor had an increased risk of miscarriage, PTB, and LBW compared to ICSI-treated male infertility couples [Kawwass et al. 2013].

Lacamara C. et al, carried out a meta-analysis in 104 articles, showing that children born with ICSI had an increased risk of congenital malformations compared to the general population [Lacamara C. et al. 2017]. The meta-analysis, however, could not establish whether ICSI was the cause of the malformations or infertility that led to ICSI.

Finally, Zullo F. et al, in their systematic review on endometriosis and pregnancy complications (women got pregnant either naturally or by ART), conclude that women with endometriosis had a statistically significantly higher risk of PTB, miscarriage placenta praevia, small for gestational age (SGA), and cesarean delivery compared with the healthy controls [Zullo F. et al. 2018].

*Table 1: Pregnancy complications related to infertility factor*

| Infertility factor | Number of pregnancies | Main pregnancy complications | Reference |
|---|---|---|---|
| Common infertility factors (endometriosis, ovulation disorders, tubal factors, diminished ovarian reserve, male factors, unexplained infertility) | 459,623 in total<br>441,420 Fertile<br>8,054 Subfertile<br>10,149 IVF | PPH, Placental abnormalities, Hospitalization during pregnancy, Higher incidence of caesarean section, PTB, VPTB. | Luke B et al, 2017 |
| Polycystic ovary syndrome | 29 studies of which the sample size varied between 9-631 pregnant women | Miscarriage, ovarian hyperstimulation syndrome, gestational diabetes mellitus, pregnancy-induced hypertension, PTB and LGA | Sha T. et al. 2019 |
| Fallopian tube | 40,046 IVF cycles | Miscarriage, PTB and LBW | Kawass J. et al, 2013 |
| Male and unexplained infertility | 1,959 ICSI cycles and 117,463 controls | Risk of congenital malformations is 7.1% in ICSI and 4.0% in the general population | Lacamara C. et al, 2017 |
| Endometriosis | 1,924,114 pregnant women, most of which underwent ART | PTB, miscarriage, placenta praevia, SGA, caesarean delivery | Zullo F., et al, 2017 |
| PPH = post-partum hemorrhage, PTB = preterm birth, VPTB = very preterm birth , LGA = large for gestational age, LBW = low birth weight, SGA = small for gestational age | | | |

# 3. ART culture media

From the first days of IVF to date, the success rate of clinical pregnancies and live birth ratio has increased dramatically. One of the most important factors was the research and development of the culture medias where zygotes and then the blastocysts are grown.

A remarkable scientific journey led to the development of the current complex IVF culture media. From the first tissue culture medium based on blood serum that was developed in University College London (UCL), England, UK and was able to support in vitro a beating frog heart [Ringer, 1882], we have reached an era where culture media contain up to 80 components including nutrients, vitamins and growth factors [Chronopoulou E et al. 2014]

The ideal steps that should be followed before the introduction of any new technique or culture media in the IVF laboratory have been described by Harper et al. In their review, they highlight the need for preliminary work on animal models, such as mice/rats/rabbits/larger mammals, followed by studies on human embryos donated for research and finally well-designed RCTs with a follow up of all children born from the procedure. It seems that new technology, such as new culture media, are being imported into the ART clinics, without being tested enough. It is possible that this technology, or these culture media to be specific, will bring no clinical benefit or will lead to adverse health outcomes in the children born by these practices. [Harper et al, 2012]

In comparison to culture media for mouse embryos, media for human embryos are not truly optimized and probably will never be. In order to optimize basic culture parameters for mice, Brinster used several thousand mouse embryos [Brinster, 1963, 1965]. To achieve similar experiments in humans, the number of human embryos would be very high and numerous ethical issues would be raised. Quality assessment is challenging since numerous parameters influence the outcome (manipulation of gametes and embryos, ovarian stimulation protocols, subfertility factors, genetic

background of parents) and there is no ‘gold standard’ quality assessment method.

There are currently two categories of culture media being used, monoculture and sequential culture medium. In the first case the zygote develops up to the blastocyst stage in the same medium, until embryo transfer. Sequential media consist of two different constituent media, one in which the zygote first grows and then transferred to a different medium, in the blastocyst stage until the embryo transfer [Sfontouris IA et al. 2016].

The results found in our literature review, regarding the ART culture media and their perinatal effects, appear to be contradictory, without being able to provide clear answers. John C. Dumoumlin et al, compared pregnancy rates and perinatal outcomes from single pregnancies resulting from a total of 826 first cycles of IVF treatment in which oocytes and embryos were randomly distributed for cultivation in one of two commercial culture media [John C. Dumumlin et al. 2010]. These were Vitrolife and Cook and the result of this study showed that the type of culture medium was significantly related to the birth weight of the newborn. Vitrolife was correlated with higher birth weight. Ewka C. Nelissen et al, compared the same culture media with the previous study and found that embryos cultured in Cook material produced lower average birth weight newborns compared to those born after Vitrolife [Ewka C. Nelissen et al. 2012]. Wunder D et al, conducted the same research and found that there were no significant differences in birth weight between these two commercially available culture media [Wunder D et al. 2014].

Gu F et al, compared two commercial culture media, SAGE and Vitrolife, and showed that embryos cultured in either medium did not affect neonatal weight [Gu F. et al, 2016]. Mantikou et al, conducted a systematic review that included 22 studies of 20 different media from 11 commercial companies, but was unable to find a superior medium from the aggregated data [Mantikou E et al. 2013]. Zandstra et al, conducted a literature review and found that of the 11 studies investigating the relationship between body weight after IVF and the type of culture medium used, 5 studies found

evidence of such a correlation, while the remaining 6 studies did not [Zandstra et al. 2015].

Sunde et al, also made a literature review on the environment in which the early embryo is exposed and showed that it can reprogram the embryonic development leading to alterations in the fetal development, birth weight, childhood development and long-term illnesses including type II diabetes as well as cardiovascular problems [Sunde A et al. 2016]. The reprogram process may involve epigenetic changes during the pre-implantation period, making the effect of culture media on the embryo clear. To illustrate this, Salvaing J et al, using rabbits as a model, showed that methylation and hydroxymethylation of DNA are both influenced by in vitro embryo culture and the effect observed depends on the culture medium used [Salvaing J et al. 2016].

Youssef MM et al, carried out a systematic review to determine which culture medium leads to the best success rates after IVF and ICSI and conclude that there is insufficient evidence to support or contradict the use of any particular culture medium [Youssef MM et al. 2015].

Eskild et al, conducted a cross-sectional study and found significant changes in birth weight and placenta weight in accordance with the media used in the ART process [Eskild A et al. 2013].

It is also important to emphasize that most of the work examined in this study did not investigate the potential long-term effects of the media on offspring, but mainly focused on pregnancy or implantation rates.

# 4. COMPLICATIONS IN OFFSPRINGS

## A) Children born with IVF and ICSI: risks and complications compared to children born with natural conception.

After 40 years of continuous cycles of IVF, many studies have focused on the risks that may arise, on the pregnant woman and her offspring. As ART is a rather new technology introduced to mankind, it is still not perfected. By perfection, it is implied that natural conceived pregnancies and ART pregnancies will have the same perinatal outcomes. But since infertility, is a major cause for adverse perinatal outcomes, it is quite difficult to understand the difference between the complications of the infertility factor and the complications of the ART technique.

However, the ART methods themselves, appear to be burdening the development of a pregnancy, regardless of the factor of infertility [Table 2].

Jackson et al, conducted a meta-analysis of fifteen studies including 12,283 IVF and 1.9 million natural conceived, single pregnancies. Compared to natural conceived pregnancies, IVF pregnancies were associated with significantly higher odds of: perinatal mortality, PTB, LBW, VLBW, and SGA. VPTB, placenta praevia, gestational diabetes, preeclampsia and admission to the neonatal intensive care unit were also associated with IVF [Jackson RA et al. 2004].

McDonald et al, conducted a systematic review and meta-analysis, showing that children born with ART have a significantly increased risk of PTB, LBW, and other adverse perinatal outcomes (e.g. Intrauterine Growth Restriction - IUGR) compared to natural conceived single pregnancies [McDonald SD et al. 2009].

Qin et al, performed a meta-analysis of fifty studies including 161,370 IVF pregnancies and 2,280,241 natural conceived single pregnancies, to identify the major risks in ART pregnancies. In their study, it was observed that single

pregnancies from ART had a significantly increased risk of gestational hypertension, gestational diabetes, placenta praevia, placental abruption, prepartum and postpartum hemorrhage, LBW, VLBW, SGA, perinatal mortality and congenital malformations [Qin J et al. 2016].

In addition, Zhao et al, with a systematic review and meta-analysis of 46 studies conducted, showed that children born after ART had an increased risk of birth defects compared to those born after natural conception. However, there was no difference in the risk of birth defects between the ART twins and the natural conception twins [Zhao J et al. 2018].

Specifically, Hargreave et al, performed a meta-analysis of twenty-five cohort and case control studies that included children born after ART, with history of cancer in one of the parents. The results showed a higher risk of childhood cancers, especially leukemia, neuroblastoma and retinoblastoma, for children born through IVF. They, however, question whether these results are due to ART techniques or infertility, since there is also the possibility of inheritance to these cancers [Hargreave M et al. 2013].

Also, Kissin et al, conducted a retrospective cohort study of all live births born of ART in California in 1997-2006 with a five-year observation period and evaluated the annual incidence of autism diagnosis. The results showed a higher impact on the diagnosis of autism in children born with ICSI compared to children born with IVF [Kissin DM et al. 2015].

Pinborg et al, carried out a systematic review and meta-analysis, showing that infertility is an important risk factor for a single pregnancy from IVF to present complications, however, even in the same mother, an ART pregnancy has a poorer perinatal outcome than a natural conception [Pinborg A et al. 2013].

Finally, Svahn MF et al, in their national retrospective study, included 2.412.721 children born in Denmark between 1969 and 2006 and found an increased risk of hospital admission or outpatient contact for mental disorders in children born to women with fertility problems [Svahn MF et al. 2015].

*Table 2: Complications in children born after ART*

| **Type of study /Number of studies** | **Number of ART children and controls** | **Complications in children born after ART** | **Reference** |
|---|---|---|---|
| Meta-analysis / 15 studies included | 12.283 ART children / 1,9 million controls | Perinatal mortality, PTB, LBW, VLBW, SGA. | Jackson R. et al, 2004 |
| Meta-analysis / 17 studies included | 31,032 ART children / 81,119 controls | PTB, LBW, IUGR | McDonald S. et al, 2009 |
| Meta-analysis / 50 studies included | 161,370 ART children / 2,280,241 controls | PTB, VPTB, LBW, VLBW, SGA, perinatal mortality, congenital malformation | Qin J. et al, 2016 |
| Meta-analysis / 46 studies included | 112,913 ART children / 4,471,368 controls | Birth defects | Zhao J. et al, 2020 |
| Meta-analysis / 25 studies included | Each study ranging between 28 – 40,330 ART children | Leukemia, neuroblastoma, retinoblastoma | Hargreave M. et al, 2013 |
| Retrospective cohort study | 42,383 ART children | Higher risk of autism | Kissin D. et al, 2015 |
| Meta-analysis / 65 studies included | 81,610 ART children / 4,919,619 controls (+16,464 ART children compared to the general population) | VPTB, LBW, VLBW, SGA and perinatal mortality | Pinbong A. et al, 2013 |
| Retrospective cohort study | 2,412,721 ART children | Increased risk of hospital admission or outpatient contact for mental disorders | Svahn M. et al, 2015 |
| PPH = Post-Partum Hemorrhage, PTB = Preterm Birth, VPTB = Very Preterm Birth , LGA = Large for Gestational Age, LBW = Low Birth Weight, SGA = Small for Gestational Age, IUGR = Intrauterine Growth Restriction | | | |

## B) ART and Epigenetic changes

One area of medical science, which seems to be strongly correlated with ART and the techniques used, are the epigenetics. Epigenetics is the study of inherited changes in the expression of the resulting genes without alterations in DNA [Wolffe AP et al. 1999]. Epigenetic changes of DNA include histone modification, DNA methylation (which is most common), nucleosome remodeling, chromatin rearrangement, and regulation of non-coding RNA [Laprise SL, 2009]. From the time of oocyte and sperm collection until embryo transfer, cells undergo epigenetic changes. Many of these are derived from techniques presented during ART techniques, but we are not fully aware of the impact these techniques have on future offspring [Chen M et al. 2017].

In their literature review, Manipalviratn et al, show that some human phenotypes associated with gene expression disorders have a higher incidence in children born of ART [Manipalviratn S et al. 2009]. The most common of these phenotypes reported in the literature are Beckwith-Wiedemann syndrome, Angelman syndrome and retinoblastoma. Similar results were found in their meta-analysis by Cortessis et al, where they examined the association between ART and epigenetic disorders. The results showed a clear link between ART conception and four epigenetic disorders-syndromes, namely Beckwith-Wiedemann, Angelman, Prader-Willi, and Silver-Russell syndromes [Cortessis V et al. 2018].

Canovas et al, analyzed DNA methylation in fetal development and the epigenetic effect of ART. Their review presents the epigenetic changes caused by culture media, ovarian stimulation and embryo transfer [Canovas S et al. 2017]. The authors conclude that while the mechanism of DNA expression is still not fully understood, the effects of iatrogenic techniques and manipulations during an ART cycle on embryonic gene expression are unquestionable.

Lazaraviciute et al, in their systematic review and meta-analysis, found an increased rate in epigenetic disorders in children born through IVF and ICSI [Lazaraviciute G et al. 2014]. However, there was insufficient evidence to correlate DNA methylation with specific genes, which are targeted for undergoing epigenetic changes during IVF.

Jiang et al, in their literature review, assess the genetic and epigenetic risks of ART techniques, in particular IVF, ICSI, cryopreservation and prenatal genetic diagnosis (PGD). During these procedures, embryos are shown to undergo epigenetic changes, and the authors wonder whether these processes could lead to later life-threatening diseases, such as neurological, metabolic and cardiovascular disorders [Jiang Z et al. 2017].

To better understand which part of the whole ART procedure is the main culprit behind imprinting disorders, we examined every embryo manipulation procedure separately. The major ART iatrogenic manipulations are preimplantation genetic diagnosis and screening (PGD/PGS), intracytoplasmic sperm injection (ICSI), embryo culture and cryopreservation.

PGD/PGS is the biopsy and analysis of the DNA of the soon-to-be embryo, to prevent the transfer of aneuploid embryos or genetic diseases and can be used to choose the sex, mainly for sex-linked mutations [Handyside AH et al, 1990]. Genetic analysis may be performed at various stages post fertilization, including the oocyte/zygote biopsied on the first day post-insemination (polar body analysis), on 1–2 blastomeres from cleavage-stage embryos biopsied on the third day post-insemination (blastomere biopsy) or on 5–10 trophectoderm cells biopsied from blastocysts on the fifth day post-insemination (blastocyst biopsy). Recent studies have shown that PGD/PGS doesn’t increase the risk of neonatal outcomes [Heijligers M et al. 2018, He H et al. 2019], although Zhang et al, found an increase in the odds of preeclampsia associated with trophectoderm biopsy [. Zhang WY et al. 2019]. Five-year-old children born after PGD/PGS, were shown to have normal cognitive and socio-emotional development, as well as normal growth, health and motor development, compared to natural conceived children [Heijligers M et al. 2018,2019]. From our research, although we came to find the lack of

well-organized studies investigating the correlation between PGD/PGS and epigenetic diseases, PGD/PGS doesn't seem to increase the odds of epigenetic syndromes.

Esteves et al, in their extensive literature review about ICSI and its consequences on offspring, conclude that epigenetic changes on embryo probably originate from the couple's infertility and the crucial question of whether imprinting and epigenetic diseases are more common after ICSI than conventional IVF remains unresolved [Esteves SC et al. 2018].

We have already discussed how culture media affect the neonatal outcome and some possible pathways [Youssef MM et al. 2015, Eskild A et al. 2013, Jackson RA 2004]. Mani et al, specifically focused on this target (mainly with animal models), showing that culture media can induce epigenetic changes on the zygote or blastocyst [Mani S et al. 2018]. Velker et al, agree with the previous study, suggesting that all culture media are suboptimal and will always lead to epigenetic changes, although most of them will be silent mutations [Velker et al. 2012].

Unfortunately, we found no studies connecting a culture medium or a culture method to a specific epigenetic syndrome or disease. Also, we didn't find any studies linking cryopreservation and epigenetic diseases.

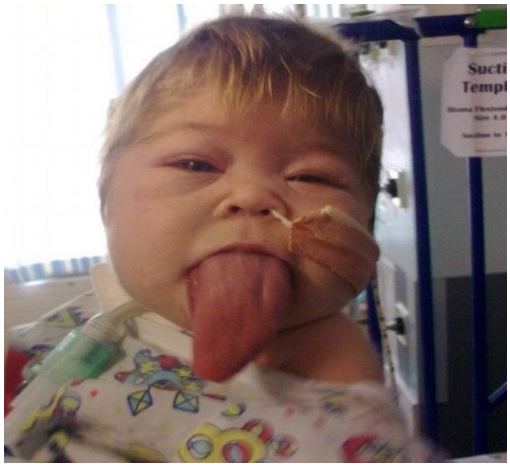
The negative impact on embryos associated with the slow-freezing technique has been confirmed in humans [Magli MC et al. 2009]. Al-Khtib et al, suggest that vitrification does not affect the methylation of the H19 domain in human oocytes, but don't have a follow up on the offspring [Al-Kthib et al. 2011]. The need for more focused and well-organized studies on cryopreservation and epigenetic changes is imperative, as there is also lack of research in the specific field.

DeAngelis et al, indicate the need for more focused studies on abnormalities of DNA imprinting and ART, as there is evidence that they appear to be higher in children born of ART than in children born of natural conception. These findings do not fully prove that there is no doubt as to the accuracy of the results [DeAngelis AM et al. 2018].

Finally, Litzky et al, in their study, they examined 108 imprinted genes in placental tissue from neonates born to parents with a history of infertility, neonates from natural conception, and neonates from ART. Their findings showed that epigenetic changes in placenta derived from ART, may be due to underlying infertility rather than to ART [Litzky J et al. 2017].

Below we demonstrate the major phenotypes of the most common imprinting disorders found in ART offspring.

## Beckwith-Wiedemann Syndrome



- Macrosomia
- Macroglossia
- Hemihypertrophy
- Omphalocele
- Embryonal tumor
- Visceromegaly

## Angelmann Syndrome

- Intellectual disability
- Movement / balance disorder
- Speech impairment
- Microcephaly
- Small and widely spaced teeth
- Blue eyes with a starry pattern

## Prader Willi Syndrome

- Hypotonia
- Obesity
- Almond shaped eyes
- Small hands and feet
- High pain threshold
- High vomiting threshold

## Silver-Russel syndrome

- Macrocephaly at birth
- Triangular shaped head
- Hypoglycemia
- Body asymmetry
- Clinodactyly
- Postnatal growth failure

# 5. Discussions

There continues to be conflicting literature regarding the association between ART and pregnancy complication, poor perinatal outcomes, offspring defects and imprinting disorders as well as a need for large, prospective studies to further delineate the possible risks and pathogenic mechanisms involved.

The focus of this review was on ART failure factors and complications that might occur on newborns. There is a rapidly growing body of knowledge that demonstrates that couples with infertility problems who choose ART to bear a child, are susceptible to a wide range of pregnancy complications. This review, demonstrates that most of these complications occur due to infertility. Although in the past, it was believed that IVF, and later ICSI, were responsible for high risk pregnancies, today it is shown that infertility plays a major role. It is still unclear if IVF or ICSI have a smaller chance of complications but it seems that ICSI, possibly because of more iatrogenic interventions, might have slightly higher odds of unfavorable results.

Newborns are also affected from infertility as well as from the ART procedure. Children born from ART have small odds of having health problems compared with naturally conceived children, where most of these health problems are linked to the infertility factor. It is also clear that IVF and ICSI interfere in the DNA activity and expression of the embryo. Epigenetic syndromes and mental issues on newborns are directly linked with ART techniques. The exact mechanism of these alterations is still under research and further studies are necessary to elucidate the stage of ART procedures where DNA expression is changed.

Another topic we focused was the culture media of zygotes and blastocysts. In our research, we found that there are very few well-designed studies concerning culture media. Also, the exact composition of commercial culture media is still unknown, making hard to make better culture media after constructive comparisons. There is a type of “secrecy” concerning culture

media and their composition as well as small heterogeneity in the findings of the studies discussed, making difficult to find the culture medium with the best results. It seems imperative for well-designed randomized controlled trials and large epidemiological studies to be conducted with synergy between commercial companies, fertility clinics and researchers, to produce better culture media.

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## Limitations

As with any literature review, the methodologic quality of the included studies was variable. Some of the studies included relatively small sample sizes. Some of the studies evaluated specific ethnic populations which may lack external validity. It is also very possible that not all relevant studies were included. This review included only studies published in English, possibly excluding other valuable studies.

## Conflicts of interest

The authors declare no conflicts of interest


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