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Risks in Assisted Reproductive Technology

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Abstract

The first *In Vitro* Fertilization (IVF) was in 1978. Since then, evolution of assisted reproductive technology (ART) took place ultimately aiding couples to have a healthy child. This literature review describes potential risks associated with *in vitro* creation and culture. Intra cytoplasmic sperm injection, IVF, embryo stage of transfer, embryo culture (media) and frozen or fresh embryo may not result in adverse perinatal outcomes, as maternal age, previous medical history or obstetrical complications are the principle risk factors. Furthermore, Diethylstilbestrol has been withdrawn from medical use due to harmful effects to children born from mothers using such medication. It is unknown weather children born from ovarian stimulation protocols may suffer from detrimental consequences in the future. All of these considerations leads us to the conclusion that the current ART practice is diverse and evolving with many outcomes yet to discover. The use of pre implantation genetic diagnosis (PGD) in order to eliminate adverse effects of ART could be the upcoming future. Yet further research remains vital for guidance to the best medical practice, as prevention is better than treatment.

Keywords: IVF; Culture Media; Infertility

Introduction

Louise Brown was born on the 25th of July 1978, she was the first *in vitro* fertilization (IVF) baby in the world. Some IVF centers celebrate this unforgettable date, as arguably it is a moment of happiness when we see an infertile couple achieve such a result. It has been almost four decades since this major discovery. But, is it possible that assisted reproductive technology (ART) is leading to adverse consequences rather than benefits?

The journey of IVF (Figure 1) starts by ovarian stimulation followed by oocyte retrieval. Good quality eggs are fertilized by two main modes: one is by putting the egg with a motile sperm in a culture dish (IVF), the other is by intra cytoplasmic sperm injection (ICSI). The latter is usually reserved for cases of male factor infertility. The journey then continues with various culture media being used to try and mimic in vivo conditions of embryo growth. Transfer of the embryo to the uterus is either at cleavage stage (3-4 days) or blastocyst stage (5-6 days). Quality excess embryos can be frozen [1].

This literature review will attempt to describe potential risks associated with *in vitro* creation and culture. Firstly, does IVF and embryo culture (media) result in adverse perinatal outcomes? Furthermore, is it possible that it could lead to detrimental consequences in adult life in comparison to natural conception? Secondly, do outcomes differ between embryo transfers at blastocyst stage versus cleavage stage? The role of epigenetics will take place next. Followed by discussion of other ART modalities (fresh and frozen embryo transfer, IVF and ICSI) that may potentiate adverse outcomes. All of these considerations will lead to the conclusion that the current ART practice is diverse and in order to confirm or deny effects of ART, Prospective randomized controlled trials should take place.

After ovarian stimulation, egg retrieval under ultrasound guidance (C); A mature human egg recovered from aspirated follicular fluid (D); Recovered eggs can be fertilized via culturing them with many motile sperms (E); or by intra cytoplasmic sperm injection (F); Embryos are cultured for three days (eight cell embryo) (G); or five days (blastocyst embryo) (H); Selected embryo is transferred back in the uterus (I) [1]

Does IVF potentiate adverse perinatal outcomes?

Infertility treatments have an effect for mothers and children. Mothers have a higher tendency of multiple pregnancy [2], ovarian hyper stimulation syndrome (OHSS) [3], ectopic pregnancy (Klemetti *et al.*, 2005), bleeding and infection[4]. Although OHSS is a rare complication of controlled ovarian stimulation yet its prevention is highly important. Various IVF centers have mastered to some extent a way to avoid OHSS by identifying patients at an increased risk with the implementation of individualized controlled ovarian stimulation[5], cancelation of HCG trigger [6] and cryopreservation of oocytes and embryos [7]. In 2000, more than two third of IVF centers in the United States used to transfer

more than two embryos. This practice has resulted into a higher probability of multiple births. Moreover, The ASRM recent guidelines in 2009 to minimize the number of embryos transfer resulted in a decline of multiple embryo transfer with ultimately fewer triplet births [8]. Therefore, multiple pregnancies have been found directly correlated with the number of embryos transferred [9]. Though the United States embryo transfer guidelines decreased the risk of triplet births yet the chance of multiple births remains steady which could be due to the rise of double embryo transfer (DET). An Australian leading IVF center looked at multiple pregnancy and live birth rates by comparing DET and elective single embryo transfer (eSET). The authors found that multiple pregnancies rates were significantly higher in double embryo transfer (34%) in comparison to eSET (7%) yet live birth rates were non statically significant [10,11] conducted a meta- analysis including 8 randomized controlled trials looking at the clinical effectiveness of eSET versus DET. In the meta-analysis, eSET had a much lower multiple pregnancy rates compared to DET with an odds ratio of 0.04 (CI 0.01 – 0.12) [11]. Various societies emphasized the importance of a single healthy baby [12,13]. The United Kingdom leading country supporting eSET with its policies and legislation, The Human fertility and embryology authority (HFEA) is an independent checkpoint for fertility clinics in the UK as they monitor and inspect IVF clinics. They have set a national target of multiple births of 10%. Other countries might or might not have a clear legislation or an independent authority. Variation in percentage of eSET across countries worldwide has been reported ranging from 2.8% up to 69.4% [14]. Although these differences could be multifactorial yet the presence or absence of legislation may play a role. Thus, it is vital to implement a clear legislation to have approximately similar IVF standards across centers to maximize live birth rates and minimize multiple birth rates.

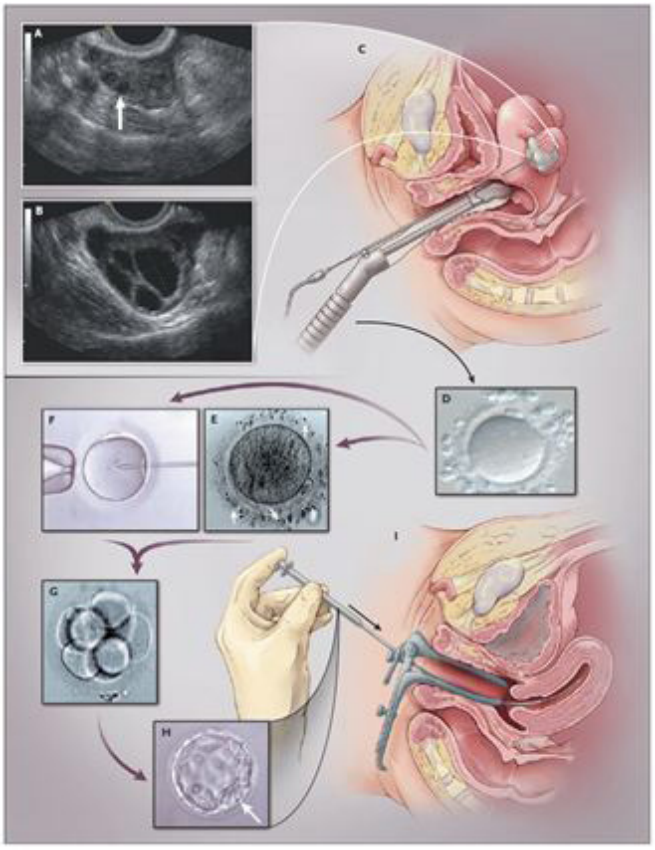

Figure 1: Describes the journey of IVF process
Ultrasonography of unstimulated ovary (A); Followed by an ultrasonography of stimulated ovary (B); After ovarian stimulation, egg retrieval under ultrasound guidance (C); A mature human egg recovered from aspirated follicular fluid (D); Recovered eggs can be fertilized via culturing them with many motile sperms (E); or by intra cytoplasmic sperm injection (F); Embryos are cultured for three days (eight cell embryo) (G); or five days (blastocyst embryo) (H); Selected embryo is transferred back in the uterus (I) [1]

One reproductive cycle results in a conception rate of up to 25% [11]. Pregnancy can be difficult to achieve in women older than 40 years old due to a higher tendency of abortions and lower fertility rates. [15]. IVF singleton pregnancies were found to have a higher tendency of worse perinatal outcomes in comparison to natural conception [17-21]. A study that was conducted in Sweden found higher tendency of preterm birth after blastocyst transfer (OR 1.35; 95% CI, 1.07–1.71). Conversely, two years later a study found that the risk does not increase. (Definitions are listed in Table 1). The studies by Kallen *et al.* (2010) and Fernando *et al.* (2012) found no difference in regards to very preterm birth, very low birth weight (VLBW), large birth weight (LBW) and small gestational age (SGA) [22,23]. Perhaps the risk increased in the Kallen *et al.* (2010) study was due to the inclusion of a variety of centers in Sweden, the unknown medical status of patients (previous preterm delivery or preeclampsia) or the use of different stimulation protocols or culture media. On the other hand, the Fernando *et al.* (2012) study had an advantage of using a single cook medium and the exclusion of uncommon stimulation protocols [22,23]. However the study had some limitations, including: smoking was only noted at time of embryo transfer, body mass index in 1524 women that were included in the study was unknown and previous medical history was not included. Perhaps there could be no distinction between ART and natural conception toward adverse perinatal outcomes, it could be instead related to maternal age, obstetrics complications or previous medical history rather than ART itself.

Terminology	Definition
Preterm	Less than 37 weeks of gestation
Very Preterm	Less than 32 weeks of gestation
Small Gestational Age (SGA)	Less than 10th percentile on intrauterine growth chart
Large Gestational Age (LGA)	More than 90th percentile on intrauterine growth chart
Low Birth Weight (LBW)	Less than 2.5 kilograms at birth
Very Low Birth Weight (VLBW)	Less than 1.5 kilograms at birth

Table 1: Definitions of some aspects that may lead to adverse perinatal outcomes Adapted from)[22]

Could IVF lead to cardiac malformations and long-term effects in adulthood?

Barker *et al.* (2009) mentioned that there is a correlation between being born SGA and chronic diseases [24]. Also, Dietz, (1994) states that obesity and LGA are correlated [25]. Two studies looked at development in children, the first study noted that children born after ART are similar to children born after natural conception [26]. The second study noted that cognitive development in IVF children was not affected [27]. Perhaps those findings mean that ART does not affect mental development. Although Mains *et al.* (2010) study came four years later; it may be considered to support the findings of the Ludwig *et al.* (2006) study upon development [26,27].

What about the risk of cardiac malformations? Cancer development? Is it due to ART modalities?

Congenital cardiac defects (CHD) have been linked to chromosomal abnormalities such as Down syndrome and Di George syndrome. Other heart defects occurrences are not linked to known cause. Giorgione *et al.* (2018) reviewed the effects of ART modalities and CHD. This systematic review and meta-analysis included 41 studies including 35 cohorts and 6 case control studies leading to 8 studies fulfilling their criteria for meta-analysis. Children conceived via IVF/ICSI had a significantly higher risk of CHD compared to spontaneously conceived children with an OR, 1.45; (95% CI, (1.20-177). Additionally, they looked at the risk of CHD in singleton pregnancies and they found that the risk remains statistically significant with an OR 1,55; (95% CI, (1.21-1.99). Various types of cardiac defects have been reported with highest percentage in ventricular septal defects (VSD) in IVF/ICSI conceptions while rates of teratology of fallot (TOF) and transposition of great arteries (TGA) were higher in spontaneous conception. Conversely, a population-based study found that ART led to a significantly higher risk of TOF compared with natural conception with an OR 2.4 (95%CI, (1.5-3.7) [28]. The discrepancy into the results shows us the complexity of the situation to reach a solid conclusion whether if ART modalities itself is the factor of CHD or other factors such as maternal age, poor eggs quality or gestational diabetes. Thus, the importance of second trimester ultrasound remains vital for all pregnancies naturally conceived or by ART modalities.

Childhood cancer has been hypothesized to develop during fetal stages [29]. Diethylstilbestrol (DES) was given to pregnant women to prevent pregnancy complications. It was noticed that there was a structural similarity among DES and anti-estrogens that are used for ovarian stimulation [30]. In fact, DES was withdrawn by the Food and Drug Administration (FDA) due to its detrimental long-term effects [31,32]. It might be probable that the medications currently used, lead to similar consequences as DES that are as of yet unknown. Many reports stated a correlation between offspring conceived by ART and cancer development at a young age [33-35]. Others found no association [36-40]. It is difficult to link ART with cancer development, as it is difficult to isolate the cause of cancers. Arguably it is more likely that cancer risks decrease with the advances in ART and the implementation of pre implantation genetic diagnosis (PGD) modalities.

Since infertile couples sought infertility treatments, are their future children going to have the same consequences? Gonadal development has been reported to be normal in IVF/ICSI children in various studies. For example, testicular and penile size was measured in 88 boys at around 8 years old and results were found comparable to reference values [41]. Similarly, pubertal assessment in girls conceived by ICSI at the age of 14 years old. It was found that menarche; genital development and pubic hair development were comparable to spontaneously conceived girls [42]. Thus, it appears that children born to infertile parents may not undergo the same circumstances as their parents as sexual development tends to be similar to non-IVF children. However, further research at older ages is needed to confirm these findings.

Do embryo culture (media) affect the embryo?

Some studies have noticed a correlation between *in vitro* creation and birth weight [43,44]. Others denied it [45-47]. The single center study of Fernando *et al.* (2012) found no association between culture and SGA or LGA [23]. Whereas another study conducted a year later found a correlation in LGA in the blastocyst stage (OR 2.23; 95% CI, 1.17–4.26), which was statistically significant on the culture period (P value = 0.007 on multiple linear logistic regression analysis) [48]. The culture media were different in both studies (Table 2). Fernando *et al.* (2012) [23] used Cook MediumTM whereas Makinen *et al.* (2013) [48] applied MediculTM, which could lead to the conclusion that the culture medium used could have an effect. However a randomized prospective study, conducted in the same year, that compared Vitrolife and Cook media found no association to birth weight [49]. Theoretically the embryo could have the ability to adapt to the culture environment to some extent, alternatively culture media may not play a role on embryo growth after all. In order to determine the role culture media plays more prospective trials are needed.

Type of Medium			
Essential Amino Acids	Cook MediumTM	MedicultTM	Methionine only
	Methionine	Methionine	
	Isoleucine	Isoleucine	
	Leucine	Leucine	
	Threonine	Threonine	
	Tryptophan	Tryptophan	
	Tyrosine	Tyrosine	
	Valine	Valine	

Table 2: Essential Amino Acids of different types of media Adapted from [49]

Do outcomes differ between embryo transfers at blastocyst stage versus cleavage stage?

An intriguing question is whether there is any discrepancy between singleton babies born after blastocyst versus cleavage stage transfer. Kallen *et al.* (2010) observed an increased risk of prematurity and congenital malformation in blastocyst stage [22]. On the other hand, a retrospective study that was conducted at McGill University reproductive unit found no association [50]. This is supported by earlier studies by Papanikolaou [51,52]. In addition, Oron *et al.* (2014) [50] found that the blastocyst stage had a higher live birth rate in comparison to cleavage stage. The Canadian study at McGill University was a retrospective study and therefore had the potential for bias. However, the reliability is greater than that of the earlier Swedish study due to meticulous records in the areas of: patient's previous medical history, grading of the embryos, single media use and comparability of sample size. It could be suggested that blastocyst and cleavage transfer are not associated with adverse perinatal outcomes.

What about the role of epigenetics in ART? Do they have an effect?

Mechanisms of epigenetics are critical. A reset system starts early in gametogenesis [53]. This process tries to eliminate unwanted genes either in maternal or paternal gametes (Figure 2). It has been noted that paternal imprints may be less likely affected than maternal imprints due to earlier establishment of male gamete [54,55]. Ovarian stimulation may predispose the female allele to alteration. Beckwith Wiedemann syndrome and Angelman's syndrome (Figure 3) are examples of various syndromes that are attributed by defects in imprintation. These are reported to be more common among children born after IVF [56-60]. It is believed that the epigenetic reset system is established before implantation, thus making it more susceptible to changes by ART tactics [49]. It was stated that there is a link between these syndromes and ART. Surprisingly, three recent studies found no imprinting defects in children after ART [61-63]. Leading to the conclusion that more extensive clinical trials are needed to extract further information on maternal and paternal defects.

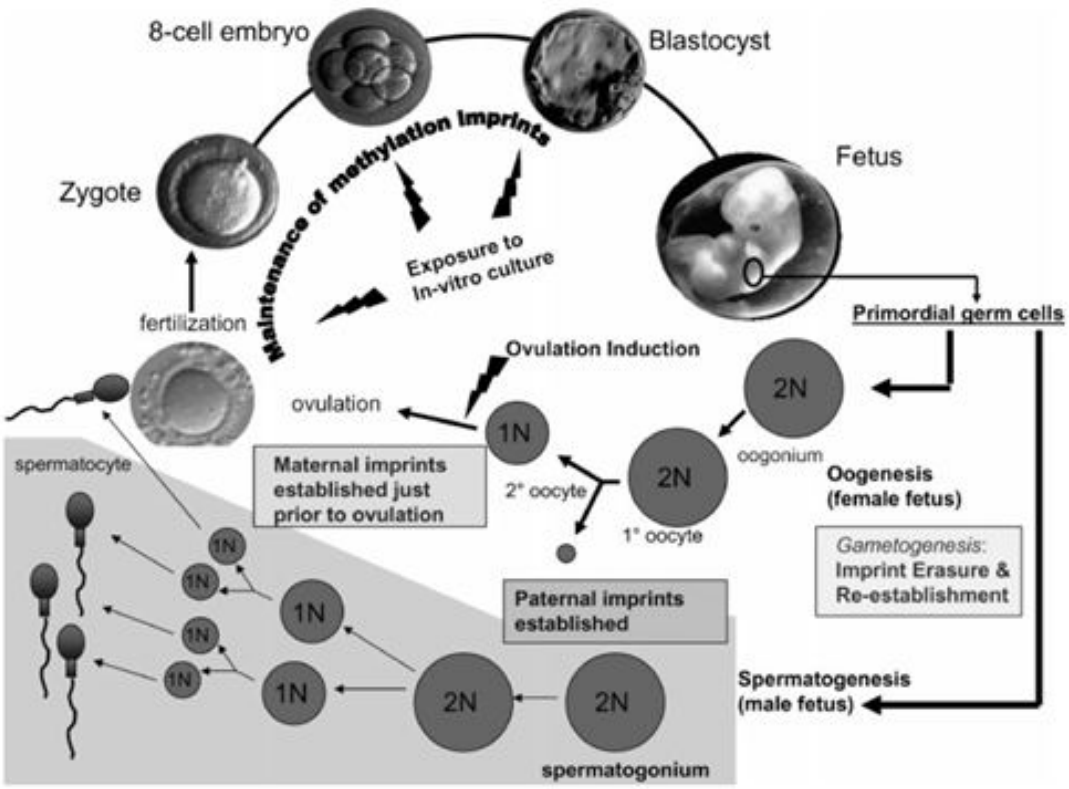

Figure 2: Illustrates the timeline of epigenetic imprintation from[64]

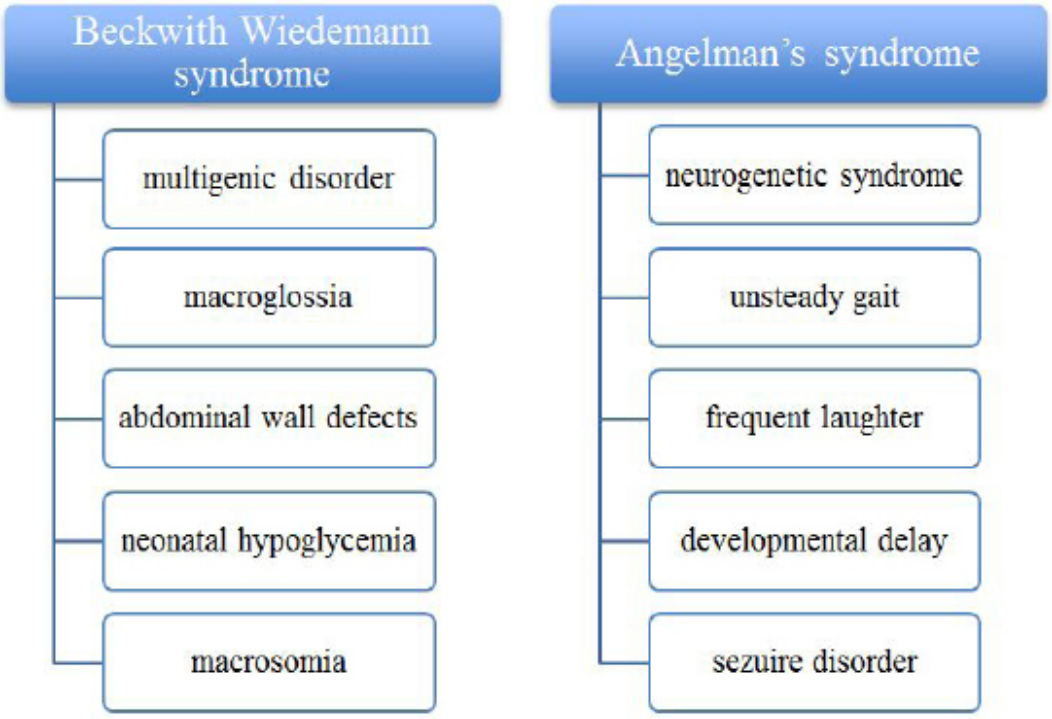

Figure 3: Features of Beckwith Wiedemann and Angelmann's syndrome. Adapted from [65,66]

Is it possible that other ART methods (IVF and ICSI, fresh and frozen embryo transfer) may potentiate adverse outcomes?

Two meta-analyses compared IVF and ICSI and the results in relation to congenital malformations were similar leading to the conclusion that both methods are comparable in their risk to congenital malformations [67,68]. Furthermore, studies have noted that children born by ICSI are not at risk of neonatal complications or developmental impairments [67-69]. Perhaps no difference was noted because usually couples undergo ICSI due to male factor infertility, thus neonatal complications and developmental defects might maternally derived rather than paternal.

Is cryopreservation a better solution over fresh transfer in terms of adverse effects? Some studies found that fresh embryo transfer was linked to higher risk of prematurity and lower birth weight than cryopreservation [70,69,71]. Moreover, the number of premature births from frozen embryo transfer follows a similar trend to that of natural conception rather than fresh embryo transfer [72-75] calculated the odds of low birth weight infants (odds ratio 1.35; 95 CI 1.20–1.51). As well as individual calculations for having a low birth weight infant at term (1.73; 1.37–2.03) and having a low birth weight preterm infant (1.49; 1.24-1.78) It was found in all calculations that embryo transfers in comparison to frozen embryo transfer had a higher likelihood of infants having a low birth weight [75]. Shapiro *et al*. (2013) compared fresh and frozen single embryo transfer in a matched cohort study, pregnancy and ongoing pregnancy rates were significantly higher in frozen embryo transfer (77.4% versus 40.9%) and (55.9% versus 26.9%) respectively [76,77]. It is possible; cryopreservation may become a strong alternative in embryo transfer with a higher ongoing pregnancy rates and lower risk of maternal OHSS.

Final Thoughts

It is worth noting that ART has changed over the past decades. The explanations for these changes are numerous. The use of different culture media and attempts to mimic in vivo environment are contributory factors. In addition, the introduction of pre implantation genetic diagnosis (PGD) to maximize the chances of having a healthy child might be a contributor as well. From this review, various studies stated different or parallel findings to others. Adverse perinatal outcomes and risk of cancer development were debated and it was not feasible to reach a solid conclusion to lean on. In addition, it is suggested that using different media for culture has yield no connection in embryo growth as shown in a recent prospective study [49]. Arguments about transferring embryos in blastocyst or cleavage stage are numerous. However, A recent Canadian study noticed a greater chance of live birth in blastocyst stage with no variance in risk of congenital malformation or prematurity [50]. The utilization of other factors (ICSI and IVF, fresh and frozen embryo transfer) showed that no distinction was noted between ICSI and IVF while cryopreservation was found to be more satisfactory than fresh transfer. With regards to epigenetics and its effect on ART, findings were inconclusive. We have enhanced the probability of pregnancy; nevertheless, we have not yet accomplished the final objective, which is (A term healthy baby!). The number of embryos transferred has played an important role in obtaining such results. We have increased the risk of multiple pregnancies, perinatal mortality and an overall cost to the patient, by just elevating the number of embryos transferred. With optimum legislation, culture conditions and efficient vitrification/slow cooling, eSET in the following cycle is the current principal approach to minimize these adverse outcomes of assisted reproduction, ultimately leading to happy families.

So far, a final answer about whether ART is hazardous in comparison to natural conception requires large prospective trials to confirm or deny.

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