

The Effect of Extended Embryo Culture on the Success of In Vitro Fertilization:

What is the impact of extended embryo culture on in vitro fertilization?

Biology

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Introduction

A continuous debate in the world of artificial reproductive technology surrounds the harms and benefits of extended embryo culture. The most commonly practiced technique for many years had been to transfer the embryo 3 days after fertilization. However, a newer method of embryo transfer after 5/6 days has become increasingly more popular. In vitro fertilization is a complex process, with many factors playing into a successful pregnancy. Researchers are trying to optimize the way in vitro fertilization is performed, and ultimately find a way to increase the rate of success. The duration of embryo culture is significant, due to its new-found effects on life before and after birth. To determine the most successful culture duration of an embryo, retrospective-studies, meta-analyses and small-scale studies were considered and examined. This research question is worthy of investigation because of the increasing popularity of in vitro fertilization. This investigation challenges the practice of extended embryo culture to justify that it should be more commonly practiced.

Section 1: Why IVF?

A couple may opt to perform in vitro fertilization for many different reasons. IVF is commonly performed due to malfunctions in the male and female reproductive systems. Concerning the ovaries, there are many factors that could reduce the amount of eggs a woman can produce. IVF becomes more common in women of greater age, due to the decrease in ovarian function after age 40. When the amount of eggs entering the uterus decreases, the ability to conceive decreases dramatically [Mayo Clinic, 2018].

If the fallopian tubes are damaged or blocked, the egg cannot reach the uterus. If a woman's egg cannot travel to the uterus she cannot conceive naturally. Additionally, in vitro fertilization can allow a woman who underwent tube ligation, having tubes tied or cut, to conceive [Mayo Clinic, 2018].

Complications within the uterus can also prohibit natural conception. With age, women can have uterine fibroids. Uterine fibroids are benign tumors growing in the uterine wall, impairing the ability for a fertilized egg to implant. Endometriosis, uterine tissue growing outside the uterus, also creates difficulty when conceiving [Mayo Clinic, 2018].

Within the male reproductive system, impaired sperm production makes it difficult to conceive. Furthermore, the function of the sperm is impaired, such as abnormalities in the shape and size, IVF can assist with pregnancy [Mayo Clinic, 2018].

Beyond issues within the reproductive systems, there are other reasons why IVF is performed. If the mother or father is high risk for a condition, they can utilize IVF to insure their offspring will not be affected. An in vitro fertilized egg can be screened for specific genetic disorders and chromosomal defects before transfer. Additionally if one of the parents is awaiting cancer treatment, gametes can be removed to be protected for use at a later date. The eggs can be frozen, and IVF can be performed after the cancer treatment [American Pregnancy Association, 2018].

Section 2: Risks and Considerations

IVF can make it possible for many couples to have children, but it does not come without risk. In vitro fertilization does not guarantee pregnancy and there is potential for

failure throughout the process. A main concern when considering IVF is the risk of a high-order pregnancy [American Pregnancy Association, 2016]. Fertility drugs can increase this chance, along with doctors choosing to transfer multiple embryos into the uterus for a higher chance of implantation. However, there is potential that every one of the embryos will implant, causing a high-order pregnancy. One of the most infamous high-order pregnancies occurred in 2009 when Nadya Suleman gave birth to octuplets. Suleman, also known as Octomom, gave birth to 8 children after a dozen embryos had been transferred into her uterus [Duke, 2011]. High-order pregnancies have higher risk for complications concerning the mother and each child.

There is concern that in vitro fertilization pregnancies are associated with preterm birth. Preterm birth may not only affect the development of the fetus, but also can cause low birth weight and various health complications [Mayo Clinic, 2018]. Whether it be a natural pregnancy or an in vitro pregnancy, there is always a chance of miscarriage. However, it is believed that IVF can increase the chance of a miscarriage [American Pregnancy Association, 2018].

Beyond the pregnancy, in vitro fertilization also presents risks for the family. In vitro fertilization may cause ovarian cancer in the mother, due to hyperstimulation of the ovaries during egg retrieval [American Pregnancy Association, 2018]. Besides the health risks, the cost of IVF can burden a family. IVF is not covered by most health insurance plans. In vitro fertilization may not be an option for some, being a costly procedure with a single cycle success rate of less than 50% [Dangcil, 2008]. With more and more cycles of IVF completed, the cost continues to rise, without guarantee of a pregnancy.

Section 3: The Process and Embryological Development

When a couple decides to proceed with in vitro fertilization, there are many steps leading up to the embryo transfer. Generally the process of in vitro fertilization is ovulation induction, egg retrieval, sperm retrieval, fertilization and embryo transfer [Mayo Clinic, 2018].

The process of invitro fertilization begins when eggs are retrieved from the ovarian follicles after ovarian stimulation. The eggs are examined to ensure they are mature and fit for transfer. Simultaneously the sperm is collected and prepped for combination of the gametes, which will occur 4-6 hours after egg retrieval [Meyer, 2018].

There are two methods used to combine the egg with the sperm. The first and most common method is insemination; mixing the eggs and sperm together and leaving them overnight. The second method, intracytoplasmic sperm injection, is performed when sperm count is low. A single sperm is injected into the cytoplasm of the egg. This method insures that the limited number of sperm have a better chance of successfully fertilizing the egg [Mayo Clinic, 2018].

About 16-18 hours after insemination, the eggs are examined to detect if fertilization has occurred. Two pronuclei, one from the egg and one from the sperm, are a sign of normal fertilization. If the pronuclei count is not two, the eggs are not fit for transfer. The normally fertilized eggs will be moved to a media that replicates the body's tubal fluid [Meyer, 2018].

Two days after fertilization, the embryos are assessed for cell division. Embryos must have between 2 and 4 cells to be viable at this stage. Embryos that have not undergone cell division are non-viable. After two days, the quality of the embryo's cell divisions outlines its culture duration. If the cell divisions of the embryo are poor quality it cannot culture for an extended time and must be transferred on day 3. If the cell divisions are good quality, the embryo can be transferred after extended culture [Meyer, 2018].

On the third day after fertilization the embryos have undergone at least 2 more cell divisions and are 6-8 cells. If the embryo has not undergone further cell divisions, it is no longer viable. On day 3 a biopsy of the embryo is taken for pre-implantation genetic screening, ensuring that the transferred embryos do not have chromosomal defects or genetic conditions. Viable embryos will be transferred or frozen. The viable embryos undergoing extended culture are moved to a media that replicates the human body's uterine fluid [Meyer, 2018].

As the embryos undergo more cell divisions, they begin to compact. When the embryo becomes 16 cells it is now considered a morula. If embryos have not compacted by the fourth day they will not survive until blastocysts, and will not be transferred [Meyer, 2018].

The embryos become blastocysts on day five. Now, it is possible to grade the inner cell mass, the fetal component, the trophectoderm cells and the placental component of the blastocyst. Based on these findings, the chance of successful pregnancy of each blastocyst can be measured. Developed blastocysts may be frozen or transferred on this day. The remaining viable blastocysts that are not fully develop

will continue to culture. On the final day of in vitro fertilization, day six, the viable fully developed blastocysts must be transferred or frozen. Blastocysts that were not viable or not used are discarded [Meyer, 2018].

If a couple plans to become pregnant immediately after the culture, their embryos are kept fresh and transferred. However, in the instance where the eggs are fertilized for later use, they are frozen. The choice to keep the fertilized eggs fresh or frozen, is usually dependent on the timeline of the couple. However, research is being furthered on whether or not preservation has effects on the success of the pregnancy [Columbia University Fertility Center, 2018].

Section 4: Analysis

Extended embryo culture is performed to ensure that the eggs being transferred are high quality and have the greatest chance of clinical pregnancy. A culture of 5/6 days is thought to be more synchronized with the events of natural conception. A blastocyst's culture more closely mimics the endometrium development [Maheshwari, 2015]. Additionally, after this extended culture a comprehensive screening can be performed to ensure the healthiest blastocysts are transferred [Mangalraj, 2009]. Extended culture transfer is becoming more practiced, and as its popularity increases, researchers must further understand its effects.

4.1: Implantation

In a single tertiary hospital, a randomized controlled study was completed to examine the implantation and pregnancy rates between cleavage-stage embryos and blastocyst-stage embryos. The study examined 388 women, 194 for each method. The

results of the study can be examined in Figure 1. The implantation rate after a blastocyst transfer was $28.37\% \pm 39.00\%$. The implantation rate after a cleavage-stage embryo transfer was $25.67\% \pm 34.96\%$ [Levi-Setti, 2018]. The blastocyst transfer showed a slightly higher chance of implantation. However both measurements exhibit large margins of error, so the difference lacks much significance. The researchers concluded that the implantation rate for blastocyst-stage transfer versus cleavage-stage transfer was not significant. The implantation rate for any IVF method is low, however the culture duration did not affect it in this study.

Variable	All	Blastocyst	Cleavage
Implantation rate (%)	26.99 ± 36.96	28.37 ± 39.00	25.67 ± 34.96
Pregnancies per retrieval (%)	146 (37.63%)	71 (36.60%)	75 (38.66%)
Pregnancies per transfer (%)	146 (39.89%)	71 (39.66%)	75 (40.11%)
Intrauterine pregnancies with embryonic heart rate			
Single pregnancies	87 (64.93%)	39 (60.00%)	48 (69.57%)
Twin pregnancies	47 (35.07%)	26 (40.00%)	21 (30.43%)

Figure 1: Implantation Rate [Levi-Setti 2018]

4.2: Clinical Pregnancy

A study was conducted to examine the clinical pregnancy outcomes between elective single blastocyst transfer and elective single cleavage stage transfer. This Korean study examined 37-year-old women, 164 who had single blastocyst transfer and 450 who had single cleavage-stage embryo transfer. The results of the study can be seen in Figure 2. The number of clinical pregnancies resulting from a single blastocyst transfer was 84, and the number of clinical pregnancies resulting from a single cleavage-stage embryo transfer was 210. The results correlate to 51.2% of the blastocyst transfers resulting in pregnancy, and 46.7% of the cleavage-stage transfers

resulting in pregnancy [Kang, 2011]. The blastocyst transfer yielded a higher percentage of clinical pregnancies compared to cleavage-stage embryo transfer. It should be noted that the sample of single cleavage-stage embryo transfer is almost three times larger than the sample of single blastocyst transfer.

Variables	eSBET (n = 164)	eSCET (n = 450)	p-value
Clinical pregnancies	84 (51.2)	210 (46.7)	0.318
Extrauterine pregnancies	2 (1.2)	6 (1.3)	0.912
Gestational sacs	85 (51.8)	211 (46.9)	0.278
Twin pregnancies	1 (1.2)	1 (0.5)	0.501
On-going pregnancies	74 (45.1)	180 (40.0)	0.254
Miscarriage > 10 wk	1 (1.4)	12 (6.7)	0.080
Lost to follow-up	1 (1.4)	3 (1.7)	0.855
Live birth	72 (43.9)	165 (36.7)	0.103

Figure 2: Clinical Pregnancy [Kang, 2011]

4.3: Live Birth

In the same Korean study, live birth rate was examined between blastocyst transfer and cleavage-stage embryo. The study's live birth analysis can be seen in Figure 2. The number of live births resulting from a single blastocyst transfer was 74, and the number of live births resulting from a single cleavage-stage embryo transfer was 165. These results correlate to 43.9% of blastocyst transfers, and 36.7% of cleavage-stage embryo transfers that resulted in live birth. In the study 84 blastocyst transfer clinical pregnancies were recorded, and 72 survived to birth. These results show that 14.3% of blastocyst-transfer clinical pregnancies did not result in a live birth. Also in the study, 210 cleavage-stage transfer clinical pregnancies were recorded, and 165 survived to birth. These results show that 21.4% of cleavage-stage transfer clinical pregnancies did not result in a live birth [Kang, 2011]. This study results show that

blastocyst transfer is associated with a higher rate of live birth, along with a lower rate of miscarriage.

In another study, a meta-analysis, live birth rate between blastocyst or cleavage-stage embryo was examined. The study examined six studies completed in the early 2000s. The results can be seen in Figure 3. Each study shows the number of live births recorded and the number of pregnancies examined. The study Reinzi et al. (2002), showed a live birth rate of 48.0% with blastocyst transfer, and a live birth rate of 50.0% with cleavage-stage transfer. The Van der Auwera et al. (2002) study showed a live birth rate of 34.3% with blastocyst transfer, and a live birth rate of 25.8% for cleavage-stage transfer. The study completed by Hreinsson et al. (2004) showed a live birth rate of 28.1% for blastocyst transfer, and a live birth rate of 28.8% for cleavage-stage transfer. The Kolibianakies et al. (2004) study showed a live birth rate of 32.3% with blastocyst transfer, and a live birth rate of 28.6% with cleavage-stage transfer. Papanikolaou et al. conducted two studies, one in 2005 and one in 2006. The 2006 study showed a live birth rate of 47.5% with blastocyst transfer, and a live birth rate of 27.4% with cleavage-stage transfer. The 2006 study showed a live birth rate of 32% with blastocyst transfer, and a live birth rate of 21.5% with cleavage-stage transfer [Papanikolaou, 2008].

This retrospective analysis is valuable because the variability between studies can be seen. The effect of extended embryo culture is so undecided due to variability such as this. The analysis however, primarily showed higher live birth rates with a blastocyst-transfer, supporting the findings of the more recent study conducted in Korea.

Study	Blastocyst n/N	Cleavage stage n/N
Rienzi <i>et al.</i> (2002)	24/50	24/48
Van der Auwera <i>et al.</i> (2002)	24/70	17/66
Hreinsson <i>et al.</i> (2004)	18/64	23/80
Kolibianakis <i>et al.</i> (2004)	73/226	67/234
Papanikolaou <i>et al.</i> (2005)	38/80	23/84
Papanikolaou <i>et al.</i> (2006)	56/175	38/176

Figure 3: Live Birth [Papanikolaou, 2008]

4.4: Birthweight

In a recent study completed in the summer of 2018, the birth weight was examined between fresh embryo and fresh blastocyst transfer pregnancies. The researchers examined 1092 singleton pregnancies in the retrospective study. The results of the study can be examined in Figure 4. The mean birthweight of a singleton resulting from a day 3 culture was $3422\text{g} \pm 547\text{g}$. The mean birthweight of a singleton resulting from a day 5/6 culture was $3433\text{g} \pm 559\text{g}$ [Fang, 2018]. The day 5/6 culture had a slightly higher mean birthweight compared to day 3 culture. However, being that the mean birthweights are in such close proximity, the data cannot conclude whether the culture duration affects birthweight. With such low significance, it is assumed culture duration does not affect birthweight.

	Day 3	Day 5–6	<i>P</i> value
Gestational age (weeks)	38.8 ± 1.6	38.8 ± 1.9	0.905
Normal, 37+ weeks	512 (92.4) ^a	490 (91.1)	0.486
Preterm, < 37 weeks	37 (6.7)	39 (7.2)	
Very preterm, < 32 weeks	5 (0.9)	9 (1.7)	
Infant gender			
Male	303 (54.7)	305 (56.7)	0.506
Female	251 (45.3)	233 (43.3)	
Mean birthweight (g)	3422 ± 547	3433 ± 559	0.732
Normal weight, 2500–4000 g	477 (86.1)	453 (84.2)	0.859
LBW (< 2500 g)	17 (3.1)	20 (3.7)	
VLBW (< 1500 g)	4 (0.7)	5 (0.9)	
HBW (> 4000 g)	45 (8.1)	51 (9.5)	
VHBW (> 4500 g)	11 (2.0)	9 (1.7)	

Figure 4: Birthweight [Fang, 2018]

4.5: Size at Gestational Age

In the same study, the researchers examined the relationship between culture duration and size at gestational age. The results are shown in Figure 5. Of the 54 singletons that were small for gestational age, 31 had a 3-day culture and 23 had a 5/6-day culture. 5.6% of the total number of day 3 singletons were small for gestational age, and 4.3% of the total number of day 5/6 singletons were small for gestational age [Fang, 2018]. Transferring on day 5/6 resulted in a lower amount of singletons that were small for gestational age. However, it should be noted there was a small amount of small for gestational age singletons from the study.

	SGA	AGA	LGA
All	54 (5.0) ^a	792 (72.5)	246 (22.5)
Age			
< 35	44 (4.8)	667 (72.5)	209 (22.7)
> 35	10 (5.8)	125 (72.7)	37 (21.5)
BMI			
< 20	12 (5.7)	172 (81.5)	27 (12.8) ^b
20–25	37 (5.1)	516 (71.7)	167 (23.2) ^b
> 25	5 (3.1)	104 (64.6)	52 (32.3) ^b
Pattern of infertility			
Primary	29 (4.7)	442 (71.9)	144 (23.4)
Secondary	25 (5.2)	350 (73.4)	102 (21.4)
Insemination method in fresh cycles			
IVF	41 (4.7)	637 (73.1)	194 (22.2)
ICSI	13 (5.9)	155 (70.5)	52 (23.6)
Stimulation protocol in FET cycle			
hCG-induced natural cycles	11 (3.7)	229 (77.9)	54 (18.4)
HRT cycles	43 (5.4)	563 (70.6)	192 (24.1)
Culture duration			
Day 3	31 (5.6)	399 (72.0)	124 (22.4)
Day 5–6	23 (4.3)	393 (73.0)	122 (22.7)

Figure 5: Size at Gestational Age [Fang, 2018]

4.6: Preterm Birth and Very Preterm Birth Rate

A Canadian retrospective study was completed to examine how culture duration may affect the rate of preterm birth. The study examined 9,442 singleton pregnancies that were cultured for 3 days, and 3,194 singleton pregnancies that were cultured for 5/6 days. In Canada, day 3 embryo transfer is much more common than day 5/6 blastocyst transfer. Although, the amount of day 5/6 transfers has increased over the years. The results of the study are shown in Figure 6. Of the 9,442 pregnancies with a culture of 3 days, 1335 singletons were born prematurely. Of the 3,194 pregnancies with a culture of 5/6 days, 548 were born prematurely. These results correlate to 14.1% of the embryo transfers and 17.1% of the blastocyst transfers [Dar, 2013]. This data shows a significantly higher risk of preterm birth with extended embryo culture.

In addition to examining preterm birth, the study also examines very preterm birth. These results can also be seen in Figure 6. Of the 9,442 pregnancies with a culture of 3 days, 251 singletons were born very prematurely. Of the 3,194 pregnancies with a culture of 5/6 days, 95 singletons were born very prematurely. These results correlate to 2.7% of the embryo transfers, and 3% of the blastocyst transfers [Dar, 2013]. Although these results are not as significant as those of preterm birth, embryo transfer still has lower risk.

Outcome	Day 3		Day 5/6	
	Instances/deliveries with data	Percentage	Instances/deliveries with data	Percentage
Preterm birth (<37 weeks)	1335/9442	14.1	548/3194	17.2
Very preterm birth (<32 weeks)	251/9442	2.7	95/3194	3

Figure 6: Preterm and Very Preterm Birth [Dar, 2013]

4.7: Congenital Abnormalities

A register study in Sweden was conducted to examine if neonatal outcomes are affected by extended embryo culture. The study examined many neonatal characteristics, including abnormalities of the infants. The sample size when examining abnormalities of infants who cultured for 5/6 days was 1311. The sample size when examining abnormalities of infants who culture for 3 days was 12,562. The study was performed on all Swedish in vitro pregnancies, so we can assume that cleavage-stage embryo transfer is the normal. The results of the study are shown in Figure 7. Of the 1,311 blastocyst transfers that were examined, 90 infants had a congenital malformation. Of the 12,562 cleavage-stage transfers that were examined, 645 infants

had a congenital malformation. These results correlate to 6.9% of blastocyst transfers having a congenital malformation and 5.1% of embryo transfers having a congenital malformation [Kallen, 2010]. These results show an increase in risk of congenital malformation when practicing extended embryo culture.

In addition to examining congenital malformations, the study recorded when the malformation was relatively severe. These results can also be seen in Figure 4. Of the 1,311 blastocyst transfers that were examined, 61 infants had a relatively severe congenital malformation. Of the 12,562 cleavage-stage transfers that were examined, 509 infants had a relatively severe congenital malformation. These results correlate to 4.7% of blastocyst transfers having a relatively severe malformation and 4% of embryo transfers having a relatively severe malformation [Kallen, 2010]. Although these results are not as significant as those of congenital malformation, it is still valid that cleavage-stage embryo transfer is associated with lower risk.

Neonatal characteristic	Blastocyst transfer		Cleavage-stage transfer		OR	95% CI
	No. with outcome	Total no.	No. with outcome	Total no.		
<32 w ^a	18	1071	142	10513	1.44	0.87–2.40
<37 w ^a	97	1071	757	10513	1.35	1.07–1.71
<1500 g ^a birth weight	16	1071	126	10493	1.41	0.83–2.41
<2500 g ^a birth weight	55	1071	532	10493	1.13	0.84–1.54
SGA ^a	25	1071	338	10488	0.82	0.54–1.26
Low APGAR score	25	1303	240	12469	1.06	0.71–1.60
Intracranial hemorrhage	6	1311	36	12562	1.46	0.64–3.34
Respiratory diagnoses	94	1311	774	12562	1.15	0.90–1.47
CPAP treatment	23	1311	203	12562	1.15	0.71–1.85
Any congenital malformation	90	1311	645	12562	1.43	1.14–1.81
Relatively severe malformation ^b	61	1311	509	12562	1.33	1.01–1.75
Cardiovascular malformation	20	1311	177	12562	1.18	0.94–1.90

Figure 7: Congenital Abnormalities [Kallen, 2010]

In addition to the Swedish study, the previous Canadian study also examined the prevalence of congenital abnormalities within extended embryo culture. The results can

be seen in Figure 8. Of the 9,506 pregnancies with 3-day culture, 215 infants were born with congenital abnormalities. Of the 3,206 pregnancies with 5/6-day culture, 78 infants were born with congenital abnormalities. These results correlate to 2.3% of embryo transfers having congenital abnormalities and 2.4% of the blastocyst transfers having congenital abnormalities [Dar, 2013]. The Canadian results are much less significant than the results of the Swedish study. Although embryo transfer is still associated with a lower risk, it is not significant enough in this particular sample. However, from what is known of the Swedish sample, increased risk of abnormalities can be connected to extended embryo culture.

Outcome	Day 3		Day 5/6		P-value
	Instances/deliveries with data	Percentage	Instances/deliveries with data	Percentage	
Preterm birth (<37 weeks)	1335/9442	14.1	548/3194	17.2	<0.001
Very preterm birth (<32 weeks)	251/9442	2.7	95/3194	3	0.34
Low birthweight (<2500 g)	897/9109	9.8	274/2985	9.2	0.28
Very low birthweight (<1500 g)	155/9109	1.7	50/2985	1.7	0.92
Congenital anomalies	215/9506	2.3	78/3206	2.4	0.58
Stillbirth	98/9506	1	33/3206	1	0.99
Neonatal death	39/9506	0.4	13/3206	0.4	0.97

Figure 8: Congenital Abnormalities [Dar, 2013]

Section 5: Conclusion

The debate of extended embryo culture is continuously prevalent in the world of artificial reproductive technology. There are many factors that affect the success of pregnancy, and it is very difficult to examine the effects that result from extended embryo culture. It is difficult to control an experiment examining in vitro fertilization, because it is difficult to control pregnancy in general. Variability and insignificance in data, is the reason both culture durations are still practiced.

To analyze the harms and benefits of extended embryo culture, many factors must be considered. When considering the rate of implantation between cleavage-stage embryo transfer and blastocysts transfer, there was no significant difference. The implantation rate after a blastocyst transfer was $28.37\% \pm 39.00\%$, and the implantation rate after a cleavage-stage embryo transfer was $25.67\% \pm 34.96\%$ [Levi-Setti, 2018]. There was a slight increase in implantation rate for blastocyst transfers, however, the large margins of error make the data insignificant. As for the rate of clinical pregnancy, blastocyst transfer was associated with a significantly higher rate than cleavage-stage embryo transfer. The results correlate to 51.2% of the blastocyst transfers resulting in pregnancy, and 46.7% of the cleavage-stage transfers resulting in pregnancy [Kang, 2011].

In the same study, the researchers examined the live birth rate, as well as the rate of clinical pregnancies that did not survive until birth. The study found that 43.9% of blastocyst transfers, and 36.7% of cleavage-stage embryo transfers that resulted in live birth [Kang, 2011]. Of the embryos that developed a clinical pregnancy, 14.3% of blastocyst transfers and 21.4% of cleavage-stage embryo transfers did not survive until birth [Kang, 2011]. These results show that blastocyst transfers are associated with a much higher rate of surviving throughout pregnancy. To further examine the effect of extended culture duration on live birth rate, a meta-analysis of 6 studies was analyzed. Of the 6 studies, all completed in the early 2000s, four supported a higher birth rate in blastocyst transfers rather than cleavage-stage embryo transfers. The remaining 2 studies supported a higher live birth rate in cleavage-stage embryo transfers. Therefore,

this meta-analysis supported blastocyst transfer as the better transfer method to support a higher chance of live birth [Papanikolaou, 2008].

Low birth weight is associated with underdevelopment as well as preterm delivery. In a study examining the effect of extended culture on birth weight, the difference between blastocyst transfers and cleavage-stage embryo transfers was insignificant. The mean birthweight of a singleton resulting from a day 3 culture was $3422\text{g} \pm 547\text{g}$ and the mean birthweight of a singleton resulting from a day 5/6 culture was $3433\text{g} \pm 559\text{g}$. There is little difference between the mean birthweight, including a large margin for error. This study's results show that transferring a blastocyst versus a cleavage-stage embryo does not affect birthweight. In the same study, weight at gestational age was recorded. If a fetus is small for gestational age, it may be under developing or developing slowly. 5.6% of the total number of day 3 singletons were small for gestational age, and 4.3% of the total number of day 5/6 singletons were small for gestational age. Here, cleavage-stage embryos support a healthier weight at gestational age [Fang, 2018].

Preterm birth is dangerous for infants and can lead to difficulties in the infant's early life. The effect of extended embryo culture on preterm birth rate, and very preterm birth rate, to see if there is a correlation. The results show that 14.1% of the embryo transfers and 17.1% of the blastocyst transfers were born prematurely. Of the same sample, 2.7% of the embryo transfers, and 3% of the blastocyst transfers were born very prematurely. The study supports cleavage-stage embryo transfer to reduce the risk of preterm birth [Dar, 2013].

Not only does extended embryo culture affect pregnancy, it can have effects on the child's life after birth. When examining the effect of extended embryo culture on the rate of congenital abnormalities, the results showed 6.9% of blastocyst transfers having a congenital malformation and 5.1% of embryo transfers having a congenital malformation [Kallen, 2010]. In the same sample, 4.7% of blastocyst transfers and 4% of embryo transfers had a relatively severe malformation [Kallen, 2010]. This study's findings show a correlation between blastocyst transfer and a higher risk for congenital abnormalities. An alternate study found 2.3% of embryo transfers and 2.4% of the blastocyst transfers having congenital abnormalities [Dar, 2013]. Although this study does not show a significant difference, blastocyst transfers had a slightly higher rate of congenital abnormalities.

Of the factors examined, the traditional cleavage-stage embryo transfer is supported by lower rates of congenital abnormalities, lower rates of preterm and very preterm birth and lower rates of small for gestational age. Blastocyst transfer was supported by higher rates of clinical pregnancy, higher rates of live birth and lower rates of miscarriage. Rightfully so, blastocyst transfer is becoming more and more popularized. Extended embryo culture has a positive impact on in vitro fertilization.

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